Analgesic effect of voluntary exercise in a rat model of persistent pain via suppression of microglial activation in the spinal cord

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ABSTRACT

In this study, we employed a rodent model for persistent allodynia and hyperalgesia to determine whether voluntary exercise could exert analgesic effects on these pain symptoms. Rats were subcutaneously injected with formalin into the plantar surface of the right hind paw to induce mechanical allodynia and hyperalgesia. We assessed the analgesic effects of a voluntary wheel running (VWR) using the von Frey test and investigated microglial proliferation in the dorsal horn of the spinal cord. We also determined the effect of formalin and VWR on the protein expression levels of brain-derived neurotrophic factor (BDNF), its receptor TrkB, and K+–Cl− cotransporter 2 (KCC2), which play a key role in inducing allodynia and hyperalgesia. Rats with access to the running wheels showed beneficial effects on persistent formalin-induced mechanical allodynia and hyperalgesia. The effects of VWR were elicited through the suppression of formalin-induced microglial proliferation, TrkB up-regulation, and KCC2 down-regulation in the spinal cord. BDNF, however, might not contribute to the beneficial effects of VWR. Our results show an analgesic effect of voluntary physical exercise in a rodent model with persistent pain, possibly through the regulation of microglial proliferation and TrkB and KCC2 expression in the spinal cord.

INTRODUCTION

Chronic pain affects patients’ quality of life and incurs economic losses and is therefore a growing problem for many countries (Kurita et al. 2012). Whereas acute pain has biological importance in sensing injuries and protecting wounded tissues, chronic pain appears to have no biological benefits for humans and other animals. Chronic and persistent pain is therefore one of the most important challenges that are yet to be overcome with regards to human health.

Chronic pain is characterized by long-lasting enhanced pain perception and includes allodynia (a response to a normally non-noxious stimuli) and hyperalgesia (an exaggerated response to noxious stimuli) (Ji et al. 2003). Synaptic potentiation of the neural circuits involved in pain perception can explain the cause of chronic pain (Hucho et al. 2007; Basbaum et al. 2009; Ossipov et al. 2010; Woolf 2011). Neuronal plasticity-dependent central sensitization in the spinal cord and brain (Ossipov et al. 2010; Woolf 2011) and peripheral sensitization in the dorsal root ganglia and trigeminal ganglia (Hucho et al. 2007; Basbaum et al. 2009) have been reported to be involved in the symptoms. Therefore, suppressing synaptic potentiation in the dorsal horn to stop the onset of central sensitization caused by intense peripheral noxious stimuli is known to be effective for preventing the transition from acute to chronic pain (Ji et al. 2003; Chapman et al. 2017).
Microglia, macrophage-like cells in the central nervous system (CNS), can be activated to generate central sensitization by releasing proinflammatory cytokines. Iba1, a 17-kDa microglia/macrophage-specific calcium-binding protein, has actin-bundling activity and plays a role in membrane ruffling and phagocytosis (Ohsawa et al. 2000). It is well known that Iba1 is up-regulated in the spinal cord in parallel with neuropathic pain-related microglial activation (Jones et al. 2018). Microglial activation and enhanced proliferation are observed after neuronal injury and inflammation in the brain and spinal cord (Banati et al. 1993; Barone et al. 1997; Kawasaki et al. 2008). Previous studies using animal models of persistent pain have reported that hyperalgesia and allodynia were attenuated by inhibiting microglial activation in the dorsal horn, suggesting an essential role for spinal microglia in long-lasting neuropathic pain (Jin et al. 2003; Tsuda et al. 2004; Hua et al. 2005). Blocking microglial activation in the spinal cord could therefore be a key target for treating symptoms in patients with chronic pain.

It has been well established that the neuropathic pain-induced microglial activation triggers the production and release of brain-derived neurotrophic factor (BDNF) in microglia. The BDNF released from microglia binds to and activates its specific receptor TrkB that is expressed in spinal neurons, which in turn reduces KCC2 expression levels in the dorsal horn of the spinal cord (Coull et al. 2003). The reduced expression of KCC2 induces an intracellular accumulation of chloride ions that increases the excitability of spinal cord layer I neurons by switching the effect of gamma-aminobutyric acid from inhibitory mode to excitatory mode (Coull et al. 2005). This enhanced excitability of the neurons is considered to underlie the development of chronic pain. Because blocking BDNF-TrkB signaling pathway in an animal model of inflammatory pain successfully suppressed pain-related behavior (Thompson et al. 1999), the regulation of BDNF-TrkB signals is one of the key targets for pain control.

Subcutaneous injections of formalin have been widely used as an animal model of inflammatory pain (Dubuisson et al. 1997). The injection of formalin into the plantar surface of rodent paws induces acute nociceptive responses, such as lifting, licking, and flinching of the paw, and generates long-lasting mechanical allodynia and hyperalgesia (Ambriz-Tututi et al. 2009, 2011). Although the mechanisms underlying formalin-induced persistent mechanical allodynia and hyperalgesia still elude satisfactory explanations, the possible involvement of spinal microglial activation after the formalin injection has been reported (Fu et al. 1999; Tan et al. 2012).

Moderate physical exercise is known to be beneficial in reducing the risk of cardiovascular, metabolic, and neurodegenerative diseases (Singh 2002; Rovio et al. 2005). Physical exercise also reduces the pain perception of patients with low back pain, osteoarthritis, and fibromyalgia (Bergman 2007). Studies have investigated the anti-inflammatory and analgesic effects of physical exercise in human and rodent models (Kim et al. 2015; Polaski et al. 2019), and a number of reports have indicated that reduced microglial activation in the brain and spinal cord could contribute to the anti-inflammatory and analgesic effects (Kami et al. 2016).

In this study, we investigated whether formalin-induced persistent mechanical allodynia and hyperalgesia in rats are attenuated by voluntary wheel running (VWR). We further examined the effects of voluntary exercise on microglial activation and BDNF/TrkB and KCC2 expression levels in the dorsal horn of the spinal cord. This study could help contribute to the development of new treatments for chronic pain by providing a mechanism for the generation of persistent pain and for the analgesic effects of voluntary exercise.

MATERIALS AND METHODS

Animals and experimental design. Wistar rats (7–8-week-old males, purchased from Nippon Bio-Supp. Center, Tokyo, Japan) were housed in standard plastic cages in our animal facilities at 25 ± 2°C with 55% ± 5% humidity under a light/dark cycle of 12 h/12 h. The rats were provided water and food (CE-2; CLEA Japan, Tokyo, Japan) ad libitum throughout the duration of the study. Animals were assigned to the following three groups: 1) Control group, kept in a cage without access to a running wheel after a saline injection; 2) Formalin + sedentary group, kept in a cage without access to a running wheel after a formalin injection; and 3) Formalin + VWR, kept in a cage with access to a running wheel after a formalin injection. VWR was started immediately after the formalin injection on Day 0 (Fig. 1).

This study was approved by the Ethics Committees of Showa University School of Medicine (the chairperson: Masahiko Izumizaki MD, Ph.D., certificate number: 09048, approved on April 1st 2019). All procedures of the study were approved by the Committee of Animal Care and Welfare of Showa University and were performed according to the
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We employed the von Frey test that uses small nylon fibers of varying diameters to test rodent sensitivity to mechanical stimuli. Studies have assessed rodent responses to von Frey filaments of varying diameters to determine the thresholds for noxious stimuli for the body part of interest (Ambriz-Tututi et al. 2009, 2011; Tan et al. 2012). The formalin-induced allodynia and hyperalgesia were assessed at baseline (Day 0) and on Day 1, 6, and 11 after the formalin injection. The rats were placed in test cages with a wire mesh bottom for 40 min before the test. The surface of the injected hind paw was pushed against each von Frey filament (Stoelting Co, Wood Dale, IL) 10 times with 10-second intervals, and the paw withdrawal responses were considered as pain perception. We selected the 1 g-force for von Frey filaments to test the formalin-induced allodynia because this was the maximum force that did not induce a paw withdrawal response in the control rats on Day 0. Similarly, we employed the 4 g-force to check hyperalgesia because it induced few responses out of 10 trials in the control rats on Day 0. The number of withdrawal responses out of the 10 trials in each test was compared among the groups to determine the formalin-induced mechanical allodynia and hyperalgesia and the effect of VWR.

Immunohistochemistry. On Day 7, samples of the fifth lumbar spinal cords (L5) were collected for immunostaining. The rats were deeply anesthetized with an intraperitoneal administration of a combination of three anesthetics: medetomidine hydrochloride, 0.3 mg/kg (Domitor; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan); midazolam, 4.0 mg/kg (Sandoz; Sandoz K.K. Tokyo, Japan); and butorphanol, 5.0 mg/kg (Vetorphale; Meiji Seika Pharma Co., Ltd., Tokyo, Japan). The rats were intracardially perfused with the anesthetic agents and phosphate buffered saline (PBS) at pH 7.4 and then with 4% paraformaldehyde in 0.1 M PBS. The spinal cords (L5) were removed and stored overnight in 4% paraformaldehyde solution. The tissues were immersed in 20% sucrose solution for 48 h. The tissues were then subsequently embedded and frozen in Tissue-Tek optimum cutting temperature (OCT) compound (Tissue-Tek; Sakura Finetek, Tokyo Japan) and stored at −80°C until use. The frozen spinal cords in OCT compound were cut into 20-μm sections using a cryostat (CM1860; Leica Biosystems, Nussloch, Germany). The sections were rinsed with PBS three times and incubated with 10% goat serum containing 0.5% triton-X (Sigma-Aldrich Japan).
pan Co., Tokyo, Japan) for 2 h for blocking and permeabilization. The sections were then incubated with rabbit anti-ionized calcium-binding adapter molecule 1 (Iba1) antibody (1 : 250, #019-19741; FUJIFILM Wako Pure Chemical, Osaka, Japan), rabbit KCC2 antibody (1 : 500, #07-432; MilliporeSigma, Burlington, MA, USA), and mouse anti-NeuN antibody (1 : 1000, #MAB377; MilliporeSigma) overnight at 4°C, and then incubated for 2 h with fluorescence-tagged secondary antibody, anti-rabbit Alexa Fluor 555 (1 : 1000, #A31572; Thermo Fisher Scientific, Waltham, MA), anti-rabbit Alexa Fluor 488 (1 : 1000, #A21206; Thermo Fisher Scientific), and anti-mouse Alexa Fluor 555 (1 : 1000, #A31570; Thermo Fisher Scientific). After washing with PBS 3 times, we stained the nuclei with DAPI (4’, 6-diamidino-2-phenylindole, 1 : 1000; Thermo Fisher Scientific) for 10 min. Fluorescent images of the sections were obtained using a confocal laser scanning fluorescence microscope (FV1000D; Olympus, Tokyo, Japan), and the optical densities of Iba1 and KCC2 immunoreactivities in the right dorsal horn were measured with FV10-AW software (Olympus). The mean optical density was calculated with three sequential sections in each rat. The number of Iba1-positive cells in the ipsilateral dorsal horn was manually counted.

Western blot analysis. After deeply anesthetizing the rats with an intraperitoneal combination of the three anesthetics, they were euthanized and a spinal cord segment (a right dorsal part of L4–L5) was immediately removed and frozen in liquid nitrogen. The tissues were homogenized with lysis buffer containing 1% sodium dodecyl sulfate (SDS), 20 mM Tris–HCl (pH 7.4), 5 mM ethylene-diamine-tetraacetic acid (EDTA) (pH 8.0), 10 mM sodium fluoride, 2 mM sodium orthovanadate, 0.5 mM phenylarsine oxide, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was then centrifuged at 15,000 rpm for 60 min at room temperature, and the supernatant was collected. We determined the protein concentration using the BCA protein assay kit (Thermo Fisher Scientific) to standardize the concentration of all samples. The samples (10 μg each) containing the same amount of proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10% SDS) and transferred onto a polyvinylidene difluoride membrane. The membranes were blocked with 5% (w/v) BSA (#011-21271; FUJIFILM Wako Pure Chemical) for 1 h at room temperature and then incubated with the following primary antibodies overnight at 4°C: anti-Iba1 antibody (1 : 1000, #019-19741; FUJIFILM Wako Pure Chemical), anti-BDNF antibody (1 : 1000, #B5050; Sigma-Aldrich Japan Co.), anti-TrkB antibody (1 : 500, #610102; BD Biosciences, San Jose, CA), and beta-actin (1 : 1000, #4970; Cell Signaling Technology, Billerica, MA). The membrane was washed with tris-buffered saline buffer with Tween 20 (Sigma-Aldrich Japan Co.) and incubated with the goat anti-rabbit secondary antibody conjugated with horseradish peroxidase (1 : 1000, #611-1302; Rockland Immunochemicals, Gilbertsville, PA) for 1 h at room temperature. β-actin was used as a loading control for total protein. Chemiluminescence images were obtained with Pierce™ ECL western blotting substrate (Thermo Fisher Scientific) and captured by a charged-coupled device camera system (Ez-Capture MG; Atto Co., Tokyo, Japan). The immunoreactivity of each band was quantified using Lane & Spot Analyzer software (Atto Co.).

Statistical analysis. All experimental data are presented as mean ± standard deviation (SD). Statistical significance of differences in the data was evaluated using one-way analysis of variance test in SPSS version 18 (SPSS Japan, Tokyo, Japan). The groups were compared using the post-hoc Tukey test. P values < 0.05 were considered statistically significant.

RESULTS

VWR inhibited to generate the allostynia and hyperalgesia induced by formalin

Mechanical allodynia (Fig. 2A) and hyperalgesia (Fig. 2B) were evaluated by the von Frey test with 1 g and 4 g filament, respectively, on Day 0, 1, 6, and 11. As previously reported (Ambriz-Tututi et al. 2011), mechanical allodynia and hyperalgesia were confirmed from Day 1 to Day 11, and VWR significantly reduced both allostynia and hyperalgesia on Day 1, 6, and 11 (Allodynia: Day 1 F(2,15) = 28.53, P < 0.001; Day 6 F(2,15) = 30.11, P < 0.001; Day 11 F(2,15) = 47.40, P < 0.001. Hyperalgesia: Day 0 F(2,15) = 0.33, P > 0.05; Day 1 F(2,15) = 15.00, P < 0.001; Day 6 F(2,15) = 18.46, P < 0.001; Day 11 F(2,15) = 19.89, P < 0.001) (Fig. 2A, B). The mean distance traveled by the VWR group was 13,300 ± 2,700 m over 11 days (approximately 1,200 m per day). Furthermore, no significant correlation was observed between the distance traveled by the rats in VWR and the degree of analgesic effects [Pearson’s correlation coefficient R = 0.675, P = 0.141 (allostynia); R = 0.517, P = 0.294 (hyperalgesia)].
VWR reduced formalin-induced microglial proliferation in the spinal cord
We determined microglial activation in the dorsal horn of the spinal cord on Day 7. The immunoreactivity of Iba1, a marker for microglia, increased in the right dorsal horn of the spinal cord as a result of formalin injection, which was suppressed in the VWR group (Fig. 3A). However, we could not observe the increased immunoreactivity of Iba1 in the spinal cord anterior horn and intermediate substance. In parallel with the changes observed in the levels of mechanical allodynia and hyperalgesia, VWR significantly suppressed the formalin-induced augmentation of Iba1 immunoreactivity (F(2,12) = 6.51, P < 0.05) (Fig. 3A, B). Furthermore, we counted the number of Iba1-positive cells with DAPI signal (blue) in the spinal cord dorsal horn (Fig. 3B). The number of Iba1-positive cells in the right dorsal horn also increased after formalin injection and was inhibited by VWR (F(2,12) = 36.53, P < 0.001) (Fig. 3C). The western blot analysis also revealed that formalin increased the Iba1 expression in the dorsal horn, which was blocked by VWR (F(2,9) = 9.48, P < 0.01) (Fig. 3D, E). These data indicate that formalin-induced mechanical allodynia and hyperalgesia were caused by the stimulation of microglial proliferation and that VWR reduced the allodynia and hyperalgesia levels by suppressing microglial proliferation.

Formalin and VWR regulated expression levels of TrkB in the spinal cord
Formalin injection increased the expression of TrkB in the dorsal horn (F(2,9) = 7.44, P < 0.05) (Fig. 4A, B). This up-regulation of TrkB returned to the level observed in the Control group by VWR. In contrast, BDNF expression levels were not affected by formalin injection and VWR (F(2,9) = 0.50, P > 0.05) (Fig. 4A).

VWR rescued the down-regulation of KCC2 in the dorsal horn induced by formalin
We further determined whether KCC2 expression was regulated by VWR. KCC2 immunoreactivity in the right dorsal horn was significantly reduced by formalin injection and VWR reversed the down-regulation (F(2,9) = 5.57, P < 0.05) (Fig. 5A). When magnified, as can be seen from the Fig. 5A, KCC2 immunoreactivity aligned along cell membrane of neurons in the Control group and Formalin + Sedentary group (yellow arrowheads) (Fig. 5A, lower panels).

DISCUSSION
In this study, the injection of formalin into the plantar surface of the right hind paw of rats resulted in persistent mechanical allodynia and hyperalgesia that lasted for 11 days as shown in previous reports (Ambriz-Tututi et al. 2011). Immunohistochemical and western blot analysis revealed that formalin injection induced microglial proliferation in the right dorsal horn of the spinal cord. VWR after the injection significantly inhibited the generation of both formalin-induced mechanical allodynia and hyperal-
Activated microglia have been hypothesized to be an important mediator in generating chronic and persistent neuropathic pain by modulating synaptic functions and connectivity in the spinal cord (Taves et al. 2013; Tsuda 2016). Microglia are activated and proliferate in response to peripheral nerve injury and inflammation and release proinflammatory factors such as tumor necrosis factor-α and interleukin-1β which increase the activity of excitatory neu-

Fig. 3 Immunohistochemical and western blot analysis of Iba1 in the dorsal horn of the spinal cord on Day 7. Images of Iba1 immunoreactivity (red) in the whole slice of the spinal cord are shown (A, upper; the images were taken by 4× objective). White bars = 500 μm. Enlarged images of the right dorsal horn indicated by white square in the upper panels (A, lower; the images were taken by 10× objective). White bars = 50 μm. White arrows point at Iba1-positive cells. Quantified Iba1 immunoreactivity (B) and the number of Iba1-positive cells (white arrow) (C) in the right dorsal horn (400 μm × 400 μm) are shown. n = 5, *P < 0.05, **P < 0.01 (vs. Control); #P < 0.05, ##P < 0.01 (vs. Formalin + Sedentary). Immunoblot images of Iba1 and beta-actin (internal control) in the spinal cord sampled on Day 7 (D) and quantified data of the immunoblot for Iba1 (E). n = 5, *P < 0.05 (vs. Control); ##P < 0.01 (vs. Formalin + Sedentary).

gesia. In parallel with this VWR effect, we confirmed that microglial proliferation induced by formalin injection in the spinal cord was suppressed. Interestingly, BDNF receptor TrkB expression levels were positively and negatively regulated by formalin injection and VWR, respectively. To the best of our knowledge, our results show for the first time that voluntary exercise can suppress the formalin-induced persistent pain and regulate TrkB and KCC2 levels in the spinal cord as a possible mechanism by which voluntary exercise alleviates pain.

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Activated microglia show increased proliferation and changes in morphology and cytosolic/cell surface protein expression through several intracellular signaling pathways (Butovsky et al. 2018; Hickman et al. 2017). Consistent with this process, numerous reports have shown that mechanical allodynia and hyperalgesia accompany the activation of microglia in the spinal cord and could be suppressed by inhibiting the inflammatory activation (Fu et al. 1999; Jin et al. 2003).

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Fig. 4 The protein expression levels of TrkB and BDNF in the spinal cord on Day 7. Immunoblot bands of TrkB, BDNF and beta-actin (internal control) are shown (A). Quantified TrkB levels are shown in (B). n = 4, *P < 0.05 (vs Control); #P < 0.05 (vs. Formalin + Sedentary).

Fig. 5 Immunohistochemical analysis of KCC2 expression in the right dorsal horn of the spinal cord on Day 7. Images for KCC2 (green), NeuN (a neural maker, red), and DAPI (nuclei, blue) are shown (A, upper; the images were taken by 100× objective). White bars = 30 μm. Neurons pointed by arrows in the upper panels are enlarged in the lower panels (A, lower; the images were taken by 100× objective). White bars = 5 μm. Note that KCC2 immunoreactivity expressed along cell membrane of neurons in the Control group and Formalin + VWR group (white arrowheads) was reduced in the Formalin + sedentary group (yellow arrowheads). Quantified data for immunoreactivity of KCC2 in the right dorsal horn (125 μm × 125 μm) (B). n = 4, *P < 0.05 (vs Control); #P < 0.05 (vs. Formalin + Sedentary).
et al. 2019). We confirmed that Iba1 immunoreactivity and the number of Iba1-positive cells in the right dorsal horn were increased in the formalin-injected rats, and VWR clearly suppressed them. These results suggest that the formalin injection into the hind paw stimulated microglial proliferation in the spinal cord, which could be inhibited by VWR. Iba1 protein expression levels revealed by western blot analysis also supported that VWR could suppress formalin-induced microglial proliferation in the spinal cord. Considering that the Iba1 expression levels corresponded to the mechanical allodynia and hyperalgesia levels, the reduced microglial proliferation would contribute to the analgesic effects of VWR.

Activated microglia can produce and release BDNF (Inoue and Tsuda 2018), which in turn increases the excitability of spinal cord layer I neurons via TrkB receptor activation and subsequent KCC2 down-regulation (Coull et al. 2003, 2005; Trang et al. 2009). In the formalin-induced hyperalgesia/allodynia rodent models, the blockade of BDNF-TrkB signaling by TrkB-IgG, a BDNF-scavenging protein, inhibited behavioral nociceptive responses induced by intraplantar injection of formalin (Kerr et al. 1999; Thompson et al. 1999). Subarachnoid injection of K252a, a kinase inhibitor for Trk receptors, suppressed the KCC2 down-regulation caused by plantar injection of formalin (Tsuruga et al. 2016). Similar to the findings of previous studies which showed increased TrkB levels in the spinal cord in mice models of neuropathic pain generated by the ligation of the sciatic nerve (Narita et al. 2000; Yajima et al. 2002), this study revealed that formalin injection increased the expression of TrkB receptor in the ipsilateral side of spinal cord. Interestingly, we also found that VWR suppressed the up-regulation of TrkB to the baseline. TrkB is predominantly expressed in neurons (Zhou et al. 1993; Garraway et al. 2003) in the spinal cord, although some studies reported that microglia can express TrkB receptor (Spencer-Segal et al. 2011). Considering that the formalin-induced microglia proliferation and KCC2 down-regulation, both of which were inhibited by VWR, were limited to the right dorsal horn, the changes in TrkB expression levels in the spinal cord observed in this study would at least partly reflect the changes in the dorsal horn.

Unexpectedly, we found that the expression of BDNF protein levels in the spinal cord neither changed in the Formalin + sedentary group nor in the Formalin + VWR group, whereas microglial activation was affected. As the inflammatory stimulation caused by lipopolysaccharide (LPS) could decrease intracellular BDNF and increase extracellular (released) BDNF in cultured microglia (Gomes et al. 2013), it is possible that the formalin injection may have enhanced BDNF release from microglia without affecting the apparent total amount of BDNF. It is still controversial whether physical exercise changes BDNF expression levels in the spinal cord or dorsal root ganglion. Using different types of exercises, duration of the experiment, and animal pain models, some studies reported increased (Gómez-Pinilla et al. 2001; Ying et al. 2005; Bernardes et al. 2013) and decreased (Cobianchi et al. 2013; Almeida et al. 2015) expression of BDNF mRNA or protein, whereas others showed unchanged levels of BDNF after exercise (Engesser-Cesar et al. 2007), as shown in the present study. Altogether, our findings indicate that VWR controls allodynia and hyperalgesia by influencing microglial activation and at least partly by regulating TrkB expression levels that determine the sensitivity of neurons to BDNF.

It is interesting that there was no significant correlation between the distance traveled in the VWR group and the degree of analgesic effects. Cobianchi et al. (2010) showed that the analgesic effect of short runs was not significantly different from that of long runs. The degree of analgesic effect of VWR obtained in this study should correlate to the strength of the physical stimuli if the effect of VWR could be attributed to the exercise-dependent activation of the descending pain inhibitory system. In this study, however, VWR nearly completely inhibited formalin-induced allodynia and hyperalgesia regardless of the distance traveled by the rats in the VWR group. These data suggest that a certain strength and duration of physical stimuli might be enough to produce microglial quiescence and analgesic effects, although further studies are required to determine the mechanisms in detail. Our results indicate the necessity for considering the appropriate strength of physical stimuli for the patient in addition to the beneficial effects of voluntary exercise for chronic pain.

This study provided evidence of an analgesic effect of voluntary physical exercise on persistent pain in a rat model of formalin-induced inflammatory pain. Microglial quiescence in the dorsal horn of the spinal cord would be a key factor in this effect. Voluntary physical exercise could ameliorate persistent pain by regulating microglial proliferation and TrkB and KCC2 expression levels in the spinal cord. This study provides evidence of the beneficial effect of voluntary exercise in the treatment against persistent pain.
CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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