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REVIEW ARTICLE

Exercise-induced hypoalgesia: potential mechanisms in animal models of neuropathic pain

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Abstract Physical exercise, such as forced treadmill running and swimming, can sufficiently improve mechanical allodynia and heat hyperalgesia in animal models of neuropathic pain (NPP), including partial sciatic nerve ligation, chronic constriction injury, and spinal nerve ligation models. Thus, physical exercise has been established as a low-cost, safe, and effective way to manage NPP conditions, but the exact mechanisms underlying such exercise-induced hypoalgesia (EIH) are not fully understood. A growing body of evidence has identified several factors that work at different levels of the nervous system as playing important roles in producing EIH in animal models of NPP. The objective of this review is to provide an overview of key players associated with EIH, and then to discuss our current understanding of the mechanisms underlying EIH. Relevant studies have demonstrated that physical exercise can dramatically alter the levels of inflammatory cytokines, neurotrophins, neurotransmitters, endogenous opioids, and histone acetylation at various sites in the nervous system, such as injured peripheral nerves, dorsal root ganglia, and spinal dorsal horn in animal models of NPP, thereby contributing to the production of EIH. These results suggest that EIH is produced through multiple cellular and molecular events in the pain pathway.

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Introduction

Neuropathic pain (NPP) is an intractable form of chronic pain that is produced by damage to and pressure on the peripheral and central nervous systems, and is the most difficult type of pain to treat among chronic pain diseases because of its complex pathophysiology (Jain 2008; Almeida et al. 2015). Pharmacological management of NPP has been challenged by clinicians, whereas nonpharmacological approaches have been proven to significantly attenuate chronic pain. One of those approaches is physical exercise, such as running or swimming. Relevant studies demonstrated that physical exercise in animal models of NPP can significantly improve pain-related behaviors, such as mechanical allodynia and heat hyperalgesia (exercise-induced hypoalgesia, EIH) (Kuphal et al. 2007; Shen et al. 2013). However, the underlying mechanisms of how exercise attenuates NPP are not yet well understood. In addition, it is known that physical exercise in clinical patients also attenuates their pain symptoms, and can appreciably improve their activities of daily living (ADL) (Simons et al. 2012; Koltyn et al. 2014; Toth et al. 2014; Ambrose and Golightly 2015). However, exercise therapy is not actively encouraged in patients with chronic pain because of the uncertainty about the mechanisms underlying EIH. Therefore, elucidating the mechanisms underlying EIH will allow a compelling argument to be made for exercise therapy with the goal of improving chronic pain.

Urban et al. (2011) reported that mice models of chronic constriction injury (CCI) and spared nerve injury (SNI)

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developed prolonged neuropathic pain, but the quality of life of the mice did not significantly alter. In addition, von Frey and plantar tests, which are frequently used to assess pain behaviors in animal models, simply evaluate the reactions of spinal reflex to pain. They thus reflect only one aspect of pain, whereas clinical pain is more complex and influenced by emotional, cognitive, and many psychosocial factors. Therefore, the results of animal studies cannot be directly applied to clinical treatment. However, animal models of NPP are essential to elucidate the mechanisms underlying EIH, and much of our knowledge of the mechanisms of EIH is based on data obtained from animal models of NPP. Therefore, although animal studies have limitations that warrant caution when interpreting results, NPP animal models, including those of partial sciatic nerve ligation (PSL) and CCI, are indispensable for elucidating the mechanisms underlying EIH.

Emerging evidence from animal studies has identified several factors that work at different levels of the nervous system as playing critical roles in the production of EIH in NPP animal models (Cobianchi et al. 2010, 2013; Bobinski et al. 2011, 2015; Shankarappa et al. 2011; Stagg et al. 2011; Chen et al. 2012; Almeida et al. 2015; López-Álvarez et al. 2015; Kami et al. 2016a, b). One line of research has demonstrated that EIH is a hypoalgesia composed of multiple events, including marked alterations in inflammatory cytokines, neurotrophins, neurotransmitters, endogenous opioids, and histone acetylation in injured peripheral nerves, dorsal root ganglia (DRG), and spinal dorsal horns of NPP animal models following physical exercise (Fig. 1). In this review, we provide an overview of the key factors associated with EIH, and then discuss our current understanding of the mechanisms underlying EIH in NPP animal models.

Injured peripheral nerves

Some studies have investigated the relationship between injured peripheral nerves and EIH (Fig. 1). A previous study reported that both treadmill running and swimming in CCI rat models induced significant attenuation of pain behaviors and lowered the levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in injured sciatic nerves compared with those of animals that did not perform the stated exercises (Chen et al. 2012). Moreover, Bobinski et al. (2011) demonstrated that treadmill running in mice with crushed sciatic nerves not only reduced pain behaviors but also TNF-α and IL-1β levels in the injured sciatic nerves. These findings suggest that the downregulation of pro-inflammatory cytokines in injured peripheral nerves by physical exercise may play a role in producing EIH.

It is known that macrophages play an important role in the peripheral nervous system in regard to the management of pain symptoms. Activated macrophages are categorized as either M1 (classically activated) or M2 (alternatively activated) (Jones and Ricardo 2013), and several studies have demonstrated that M1 macrophages are associated with the development of pain as they release pro-inflammatory cytokines such as IL-1β, TNF-α and interleukin-6 (IL-6), while M2 macrophages are involved in pain relief as they produce anti-inflammatory cytokines such as interleukin-10 (IL-10) and interleukin-4 (IL-4) (Hasegawa-Moriyama et al. 2013; Grace et al. 2014). These results suggest that M2 macrophages contribute to EIH. Taguchi et al. (2015) demonstrated that PSL sedentary mice develop notable mechanical allodynia and thermal hyperalgesia, but pain behaviors were significantly attenuated in PSL runner mice (Fig. 2). After PSL, the number of $CD68^{+}$M1 macrophages increased in injured sciatic nerves, but after treadmill running, the number of M1 macrophages decreased significantly (Fig. 3a–c, g). On the other hand, the number of $CD206^{+}$M2 macrophages was significantly increased in the proximal parts of injured sciatic nerves in PSL runner mice than in PSL sedentary mice (Fig. 3d–f, h). In addition, changes in the proportions of macrophage subtypes (intermediate, M1, and M2) indicated that running exercise increases both intermediate and M2 subtypes within the proximal parts of injured sciatic nerves. These results indicated that treadmill running attenuated NPP (at least partly) by changing the ratio of M1 to M2 macrophages in injured sciatic nerves, and they suggested that activation of M2 macrophages contribute to the production of EIH in NPP. In addition, these results suggest that treadmill running generates signals that switch the polarization of activated microglia from M1 to M2 in the superficial dorsal horn. Therefore, further studies will be necessary to elucidate such treadmill-running-derived signals.

The spinal dorsal horn and DRG

Following peripheral nerve injury, microglia and astrocytes in the ipsilateral superficial dorsal horn of the spinal cord are strongly activated, thus producing pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which are critical factors in the development and maintenance of NPP (Calvo and Bennett 2012; Mika et al. 2013). Therefore, many EIH studies have focused on changes in pro-inflammatory cytokines in the spinal dorsal horn brought about by physical exercise in order to probe the underlying mechanism for EIH (Fig. 1). In addition, neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are upregulated in the spinal dorsal horn as well as the DRG in NPP animal models. These play critical roles in the initiation and maintenance of NPP (López-Álvarez et al. 2015). Interestingly,

Factors that work at different levels of the nervous system to produce EIH in NPP model animals

	Nerve injury	Exercise	Ref.
1. Peripheral nerve			
IL-1β	↑	↓	Bobinski(a), Chen
TNF-α	↑	↓	Bobinski(a), Chen
Hsp72	/	↑	Bobinski(a)
CD68(M1)	↑	↓	Taguch
CD206(M2)	↑	↑	Taguch
2. DRG			
BDNF	↑	↓	Cobianchi(b), Almeida
NGF	↑	↓	Cobianchi(b),Almeida, López-Álvarez
GDNF	↑	↓	Cobianchi(b)
3. Spinal dorsal horn			
Iba-1	↑	↓	Almeida, López-Álvarez
CD11b	↑	↓	Cobianchi(a)
GFAP	↑	↓	Cobianchi(a), Almeida
IL-1β	↑	↓	Bobinski(a)
IL-6R	↑	↓	Bobinski(a)
BDNF/Iba-1	↑	↓	López-Álvarez
GABA	↓	↑	Kami(b)
GAD65/67	↓	↑	Kami(b)
HDAC1/CD11b	↑	↓	Kami(a)
H3K9ace/CD11b	–	↑	Kami(a)
4. Brainstem			
β-endorphin	/	↑	Stagg*
met-enkephalin	/	↑	Stagg*
Serotonin	↓	↑	Bobinski(b)
Serotonin transporter	↑	↓	Bobinski(b))#
IL-1β	↑	↓	Bobinski(b)
TNF-α	↑	↓	Bobinski(b)

*; PAG, RVM, #; Rob, RMg, Rpa

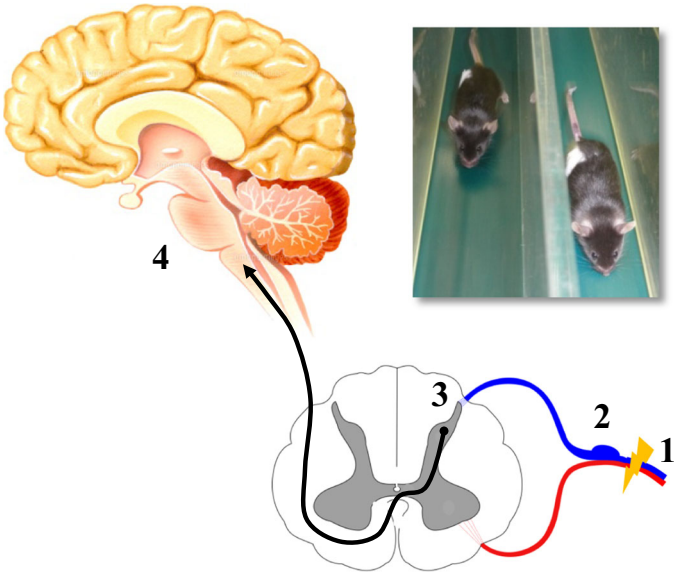


Fig. 1 EIH is produced through multiple cellular and molecular events that occur at different levels of the nervous system following physical exercise in NPP animal models. *1* Peripheral nerves, *2* dorsal root ganglia, *3* spinal dorsal horn, *4* brainstem

treadmill running and swimming in NPP animal models markedly reversed the levels of IL-1β, IL-6 receptor, and BDNF in the dorsal horn (Bobinski et al. 2011; López-Álvarez et al. 2015) and the levels of NGF and BDNF in the DRG (Cobianchi et al. 2013; Almeida et al. 2015; López-Álvarez et al. 2015) (Fig. 1). Thus, the downregulation of pro-inflammatory cytokines and neurotrophins in the dorsal horn and DRG by physical exercise may be the predominant mechanisms involved in EIH.

On the other hand, some studies have shown that treadmill running and swimming in NPP animal models can significantly reduce the expression levels of CD11b, Iba-1, and glial fibrillary acidic protein (GFAP), which are reliable markers of microglia and astrocytes in the ipsilateral superficial dorsal horn (Cobianchi et al. 2010; Almeida et al. 2015; López-Álvarez et al. 2015) (Fig. 1). These results suggest that the inactivation of glial cells by physical exercise plays a role in EIH. However, a recent study showed that PSL runner mice maintained a markedly increased number of microglia (microgliosis) in spite of attenuated pain behaviors (Kami et al. 2016a). This discrepancy may be attributed to the different treadmill running protocols used in these studies, suggesting that the duration and intensity of treadmill running are particularly important influences on the analgesic levels of EIH. These results also suggest that the physical-exercise-induced attenuation of microgliosis in the spinal dorsal horn is not a prerequisite for EIH.

Gamma-aminobutyric acid (GABA) is the principal inhibitory transmitter in the central nervous system, including the spinal dorsal horn. Previous studies have shown a loss of GABA-immunoreactive cells and fibers in the spinal dorsal horns of NPP animal models, and the impairment of GABAergic inhibition in the spinal dorsal horn is assumed to play an important role in producing NPP (Castro-Lopes et al. 1993; Ibuki et al. 1997; Eaton et al. 1998). GABA is synthesized from glutamate by glutamic acid decarboxylase (GAD). Two distinct isoforms of GAD, GAD65 and GAD67, have been identified, with each isoform being encoded by separate genes, namely

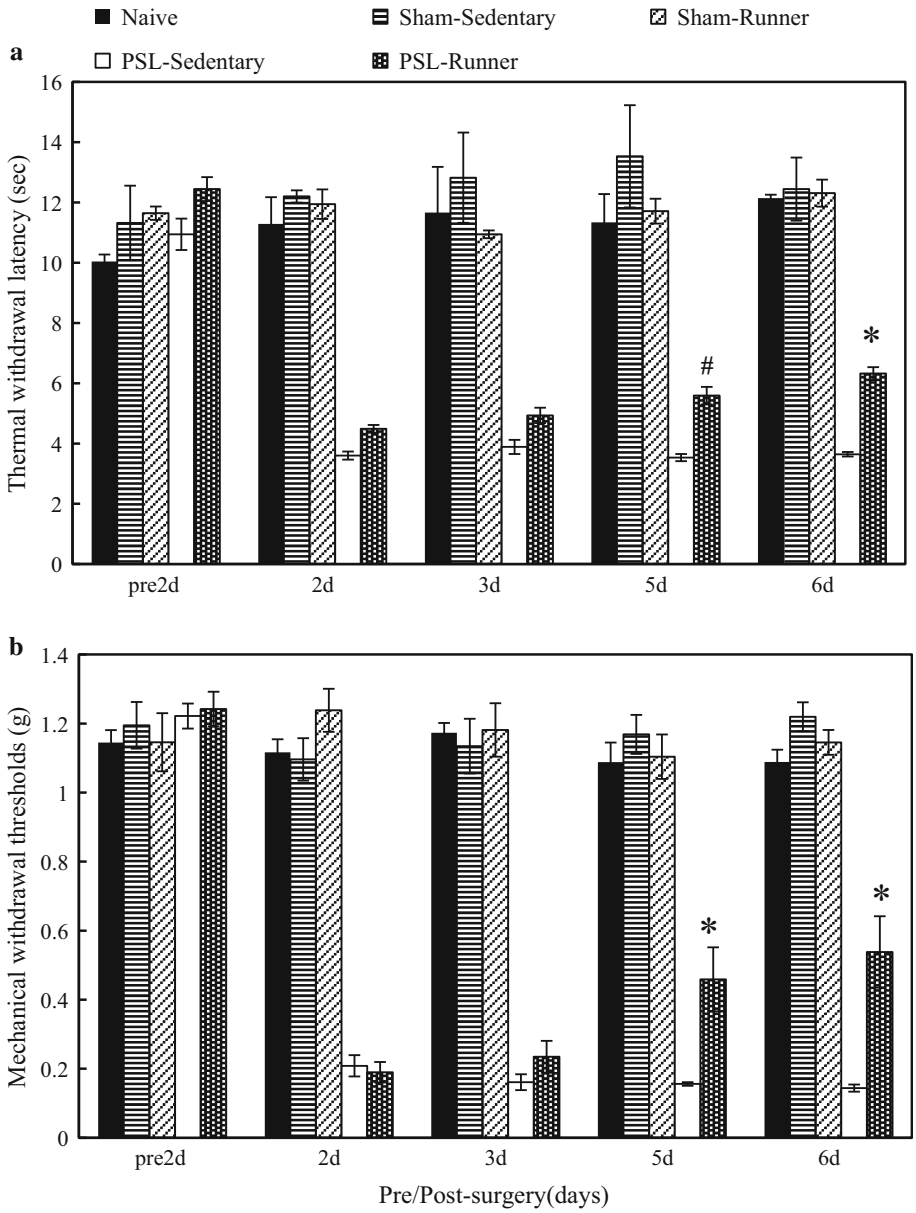


Fig. 2 Changes in pain behaviors in NPP mice models following treadmill running. **a** Plantar and **b** von Frey tests were performed in naive ($n = 6$), sham-sedentary ($n = 6$), sham-runner ($n = 6$), PSL-sedentary ($n = 6$), and PSL-runner ($n = 6$) mice 2 days before and 2, 3, 5, and 6 days after surgery. **a** Thermal withdrawal latencies were significantly higher in PSL-runner mice than in PSL-sedentary mice 5 and 6 days after PSL (#$p < 0.05$, *$p < 0.01$). **b** Mechanical withdrawal thresholds were significantly higher in PSL-runner mice than in PSL-sedentary mice 5 and 6 days after PSL (*$p < 0.01$). Differences among groups were checked for statistical significance by performing a repeated-measures ANOVA followed by Tukey's post hoc test

Gad2 and *Gad1* (Erlander et al. 1991). A number of studies have indicated that the functional loss of GABA and/or GADs in the spinal dorsal horn contributes to the development of NPP via reductions in the GABA inhibitory tone (Vaysse et al. 2011; Zhang et al. 2011; Lorenzo et al. 2014). A recent study showed that exacerbated pain behaviors following PSL surgery were significantly reduced by treadmill running (Fig. 2), and PSL-induced reductions in GAD65/67 production in the superficial dorsal horn were prevented by treadmill running after PSL surgery (Fig. 4l–o, q), leading to the retention of GABA in interneurons and neuropils (Fig. 4a–k, p). Positive correlations were also observed between the thresholds of pain behaviors and GABA and GAD65/67 levels or GABAergic interneuron numbers in the ipsilateral dorsal horn of PSL sedentary and runner mice (Kami et al. 2016b). Therefore, these results demonstrated that EIH is achieved, at least in part, by the retention of GABAergic inhibition in the spinal dorsal horn. On the other hand, GADs at the protein and mRNA levels are present in the rostroventral medulla (RVM), and these GABAergic RVM neurons massively project into the spinal dorsal horn (Winkler et al. 2006; Morgan et al. 2008; Pedersen et al. 2011; Aicher et al. 2012; Hossaini et al. 2012). These GABAergic RVM neurons were also shown to participate in pain inhibition (Zhang et al. 2011). Therefore, GABAergic neurons in the RVM may be involved in the generation of EIH.

Recent studies have shown that pharmacological inhibition of histone deacetylases (HDACs) in the spinal cord of an NPP animal model improves pain-related behaviors

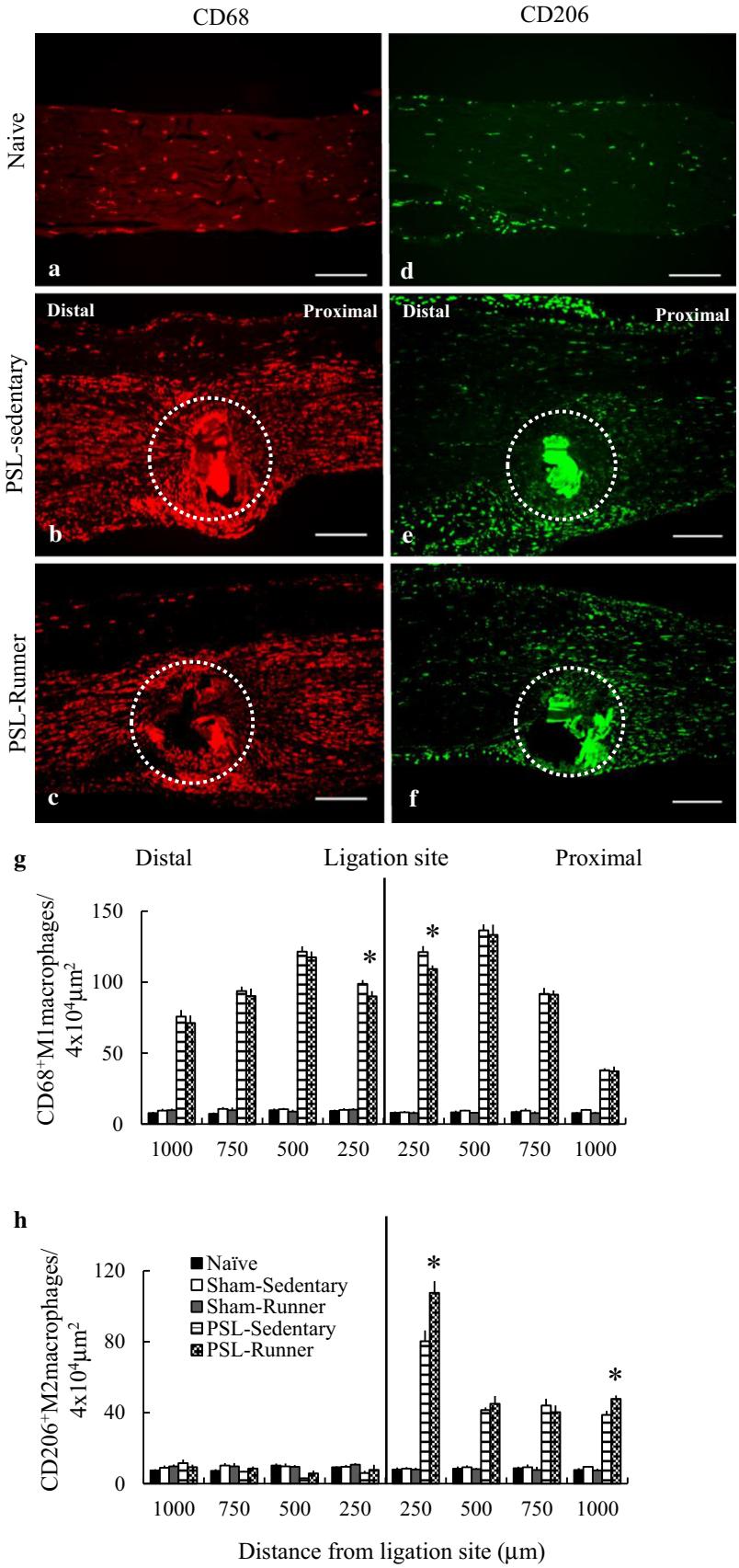
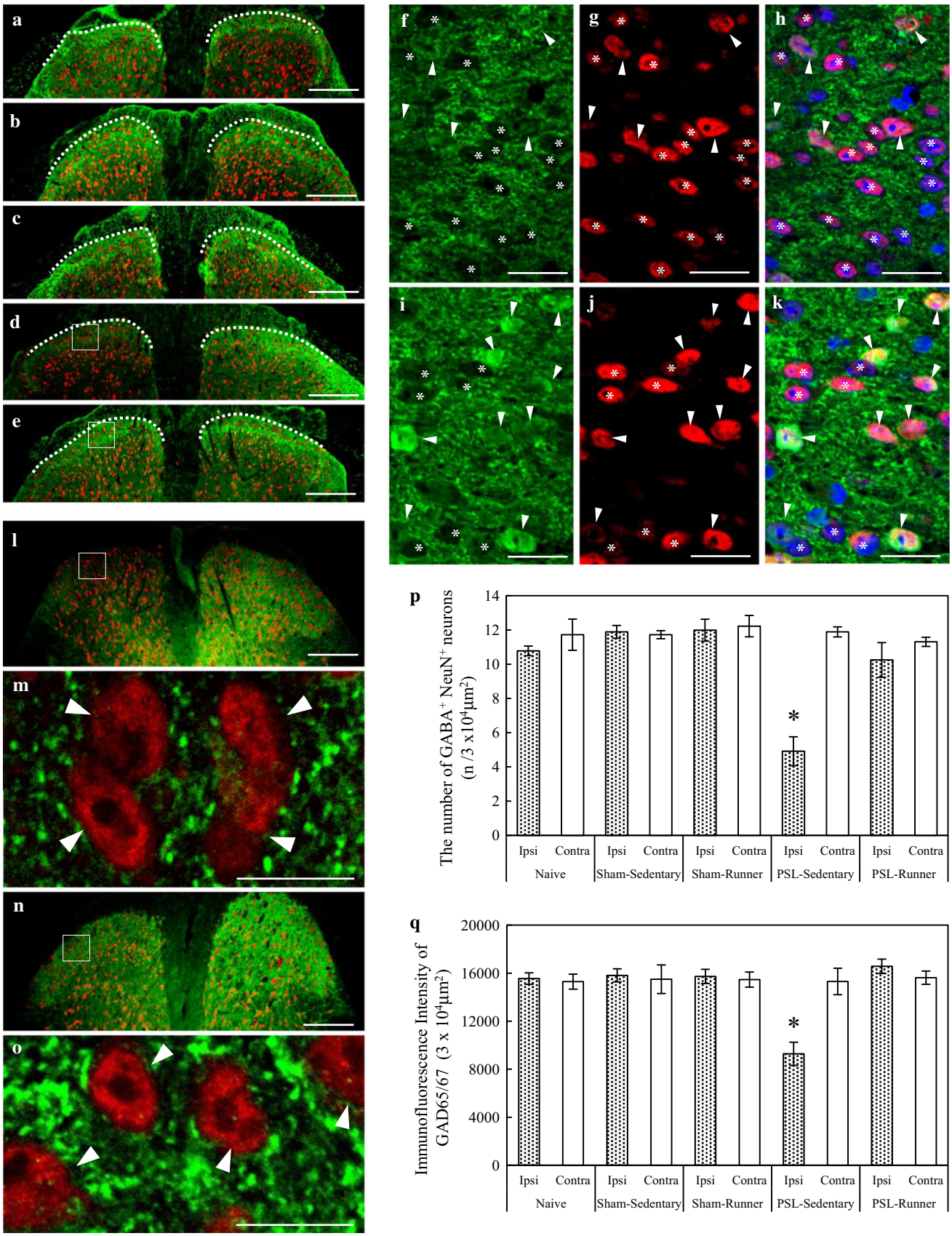


Fig. 3 Treadmill running decreased CD68$^+$M1 macrophages and increased CD206$^+$M2 macrophages in injured sciatic nerves of NPP mice models. **a** CD68$^+$ and **d** CD206$^+$ macrophages were scattered in the normal sciatic nerve. After PSL, **b** the number of CD68$^+$M1 macrophages was increased in injured sciatic nerves, but **c** treadmill running significantly decreased the number of M1 macrophages. In contrast, **f** the number of CD206$^+$M2 macrophages was significantly increased in the proximal parts of injured sciatic nerve in PSL-runner mice compared with **e** PSL-sedentary mice. The *dotted circles* indicate ligation sites. *Bars* (**a**–**f**) = 200 µm. The numbers of **g** CD68$^+$M1 and **h** CD206$^+$M2 macrophages were quantified in eight different areas of injured sciatic nerve. Data are presented as the mean ± SEM. $n = 4$. *$p < 0.01$: PSL-sedentary vs PSL-runner. Differences among groups were checked for statistical significance using a two-way ANOVA and Bonferroni's post hoc test


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
The number of GABA+ NeuN+ neurons (n /3 x10⁴μm²)
14
12
10
8
6
4
2
0
Ipsi
Contra
Naive
Sham-Sedentary
Sham-Runner
PSL-Sedentary
PSL-Runner
q
Immunofluorescence Intensity of GAD65/67 (3 x 10⁴μm²)
20000
16000
12000
8000
4000
0

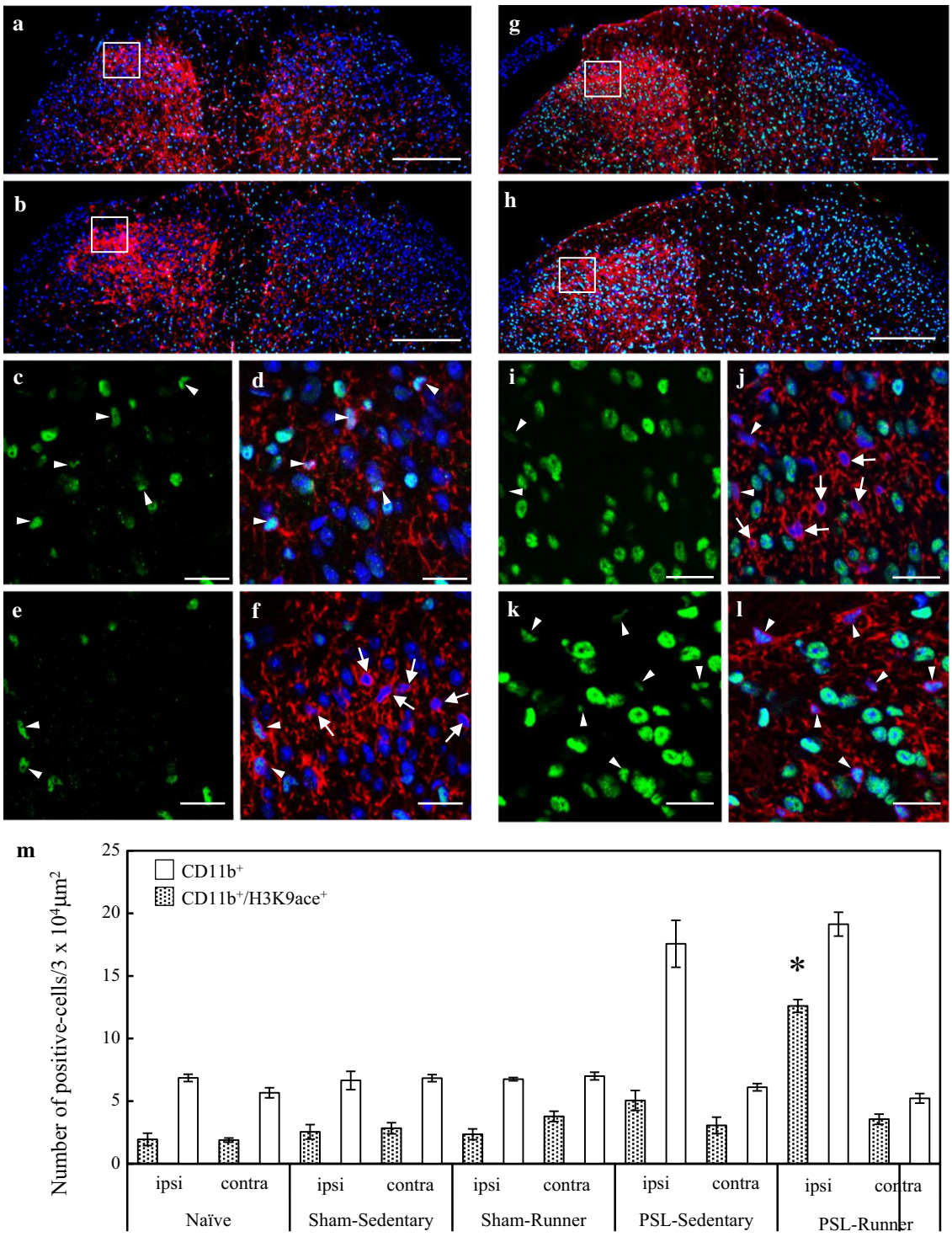
◀**Fig. 4** Changes in GABA and GAD65/67 immunoreactivities in the superficial dorsal horn of NPP mice models following treadmill running (Kami et al. 2016b). Lumbar spinal cord (L4–5) sections in **a** naive, **b** sham-sedentary, **c** sham-runner, **d** PSL-sedentary, and **e** PSL-runner mice were immunostained with GABA (*green*) and NeuN (*red*) antibodies. The right and left sides of the dorsal horns indicate the contralateral and ipsilateral sides, respectively. The *dotted lines* drawn in each photomicrograph indicate the boundary between the dorsal horn and white matter. *Bars* (**a**–**e**) = 200 μm. Ipsilateral superficial dorsal horn areas indicated by *squares* in **d**, **e** are enlarged in **f**–**h** (PSL-sedentary) and **i**–**k** (PSL-runner), respectively. **f**, **i** and **g**, **j** indicate the immunoreactivities of GABA (*green*) and NeuN (*red*) in the superficial dorsal horn, respectively, and in **h**, **k** three images including DAPI are merged. *Arrowheads* and *asterisks* indicate $GABA^+$ $NeuN^+$, and $GABA^-$ $NeuN^+$ neurons, respectively. *Bars* (**f**–**k**) = 20 μm. **p** As shown in **d**, squares of size 10^4 μm^2 were placed on the lateral, central, and medial parts, respectively, of the superficial dorsal horn in microscope images, and the number of $GABA^+$ $NeuN^+$ neurons within it was counted. The number of $GABA^+$ $NeuN^+$ neurons in the ipsilateral dorsal horn was significantly lower in PSL-sedentary mice than in the other groups ($n = 6$, $^*p < 0.01$). Lumbar spinal cord (L4–5) sections in **l** PSL-sedentary and **n** PSL-runner mice were immunostained with GAD65/67 (*green*) and NeuN (*red*) antibodies. The *right* and *left* sides of the dorsal horns correspond to the contralateral and ipsilateral sides, respectively. The *square* in **l** is enlarged in **m** GAD65/67 (*green*) and NeuN (*red*). The *square* in **n** is enlarged in **o** GAD65/67 (*green*) and NeuN (*red*). Intense GAD65/67 immunoreactivities were detected in neuropils but not in neurons. *Bars* (**l**, **n**) = 200 μm, (**m**, **o**) = 10 μm. **q** As shown in **l**, *squares* 10^4 μm^2 in size were placed on the lateral, central, and medial parts, respectively, of the superficial dorsal horn in microscope images, and the immunofluorescence intensity of GAD65/67 within it was measured using ImageJ software. GAD65/67 immunoreactivity levels were significantly lower in PSL-sedentary mice than in the other groups ($n = 6$, $^*p < 0.01$). Differences among groups were checked for statistical significance using a one-way ANOVA and Tukey's post hoc test (color figure online)

by reducing HDAC1 and enhancing histone acetylation (Denk et al. 2013; Kukkar et al. 2013; Cherng et al. 2014), and they have also suggested that epigenetic modification plays an important role in producing and attenuating NPP (Descalzi et al. 2015). Interestingly, intrathecal administration of rat IL-10 protein or intrathecal lentiviral-mediated transfer of IL-10 can reverse the enhanced pain behaviors in CCI model rats (Milligan et al. 2005; He et al. 2013). A line of evidence has shown that epigenetic modification, such as phosphorylation, acetylation, and methylation of histone H3 at specific regions in the IL-10 promoter is an important regulatory step for IL-10 production in myeloid cells, including macrophages (Shoemaker et al. 2006; Zhang et al. 2006; Leghmari et al. 2008; Leng and Denkers 2009; Zhou et al. 2011; Lee et al. 2012; Hill et al. 2015). These results suggest that epigenetic modifications in activated microglia in the spinal dorsal horn participate in producing EIH, perhaps via the upregulation of analgesic factors, including IL-10. Kami et al. (2016a) showed that PSL surgery markedly increased the number of $HDAC1^+/CD11b^+$ microglia in the ipsilateral superficial dorsal horn, while the number significantly decreased with treadmill running (Fig. 5a–f). Moreover, the number of microglia with nuclear expression of acetylated histone H3K9 (H3K9ace) in the ipsilateral superficial dorsal horn remained at low levels in PSL sedentary mice, but running exercise significantly increased it (Kami et al. 2016a) (Fig. 5g–m). Thus, these results indicate that the epigenetic modification that causes hyperacetylation of histone H3K9 in activated microglia plays a role in producing EIH. A reasonable explanation for the results may involve the upregulation of analgesic factors, perhaps IL-10, in the activated microglia, since a recent study showed that IL-10 inhibits the production of LPS-induced pro-inflammatory cytokines in activated microglia (Cianciulli et al. 2015). Therefore, the increase of H3K9ace in activated microglia may promote the production of IL-10, and thereby downregulate pro-inflammatory cytokines, consequently contributing to the development of EIH. The underlying mechanisms of EIH are summarized in Fig. 6.

Brainstem

Pain information is conveyed via the primary afferent system to the spinal dorsal horn, and then transmitted to the brain via a number of ascending pain pathways. The primary afferent nociceptive signals are modulated by a number of descending pathways from supraspinal areas. A particularly well-characterized descending pathway projects via the midbrain periaqueductal gray (PAG) and RVM to inhibit ascending nociceptive transmission at the spinal dorsal horn (Basbaum and Fields 1984; Lau and Vaughan 2014). It has been proposed that opioids can activate the PAG–RVM descending pathway by indirectly suppressing the inhibitory influence of local GABAergic interneurons, thereby inhibiting nociceptive transmission at the spinal dorsal horn level, which can endogenously modulate perception of pain (Lau and Vaughan 2014). Stagg et al. (2011) investigated whether treadmill running in spinal nerve ligation (SNL) rat models can alter endogenous opioid content in brainstem regions. The results showed that regular moderate aerobic exercise reversed pain behaviors and increased β-endorphin and met-enkephalin content in RVM and PAG (Fig. 1). In addition, these effects of exercise were significantly reversed by the administration of opioid antagonists. Thus, their results suggest that EIH results from the upregulation of endogenous opioids in the brainstem.

It has been established that the descending 5-hydroxytryptamine (5-HT, serotonin) pathway has an inhibitory or facilitatory effect on the spinal processing of nociceptive information. The descending inhibitory 5-HT pathway, in particular, may play a functionally more important role in


a
g
b
h
c
d
i
j
e
f
k
l
m
Number of positive-cells/3 x 10⁴μm²
CD11b⁺
CD11b⁺/H3K9ace⁺
25
20
15
10
5
0
*
ipsi
contra
Naïve
Sham-Sedentary
Sham-Runner
PSL-Sedentary
PSL-Runner

◀**Fig. 5** Changes in HAC1$^+$/CD11b$^+$ and H3K9ace$^+$/CD11b$^+$ microglia in the superficial dorsal horns of NPP mice models following treadmill running (Kami et al. 2016a). Lumbar spinal cord (L4–5) sections in **a** PSL-sedentary and **b** PSL-runner mice were immunostained with HDAC1 (*green*) and CD11b (*red*) antibodies, and nuclei were labeled with DAPI (*blue*). The *right* and *left* sides of the dorsal horn correspond to the contralateral and ipsilateral sides in PSL surgery, respectively. The *square* in **a** is enlarged in **c** HDAC1 (*green*) and **d** HDAC1 (*green*), CD11b (*red*), and DAPI (*blue*). The *square* in **b** is enlarged in **e** HDAC1 (*green*) and **f** HDAC1 (*green*), CD11b (*red*), and DAPI (*blue*). *Arrowheads* indicate HDAC1$^+$/CD11b$^+$ microglia, while *arrows* indicate HDAC1$^-$/CD11b$^+$ microglia. Lumbar spinal cord (L4–5) sections in **g** PSL-sedentary and **h** PSL-runner mice were immunostained with H3K9ace (*green*) and CD11b (*red*) antibodies, and nuclei were labeled with DAPI (*blue*). The *right* and *left* sides of the dorsal horn correspond to the contralateral and ipsilateral sides in PSL surgery, respectively. The *square* in **g** is enlarged in **i** H3K9ace (*green*) and **j** H3K9ace (*green*), CD11b (*red*), and DAPI (*blue*). The *square* in **h** is enlarged in **k** H3K9ace (*green*) and **l**: H3K9ace (*green*), CD11b (*red*), and DAPI (*blue*). *Arrows* and *arrowheads* in **i–l** indicate H3K9ace$^-$/CD11b$^+$ microglia or H3K9ace$^+$/CD11b$^+$ microglia, respectively. *Bars* (**a**, **b**, **g**, **h**) = 200 μm, (**c–f**, **i–l**) = 20 μm. **m** As shown in **a**, *squares* 10^4 μm^2 in size were placed on the lateral, central, and medial parts, respectively, of the superficial dorsal horn in microscope images, and the number of H3K9ace$^+$ NeuN$^+$ neurons within it was counted. The number of H3K9ace$^+$/CD11b$^+$ microglia in the ipsilateral superficial dorsal horn was significantly increased in PSL-runner mice compared with those in other groups. In addition, the number of CD11b$^+$ microglia in the ipsilateral dorsal horns of both PSL-sedentary and PSL-runner mice was also significantly increased in comparison with those of other groups except for each other ($n = 6$, $^*p < 0.01$). Differences among groups were checked for statistical significance by a one-way ANOVA and Tukey–Kramer's post hoc test (color figure online)

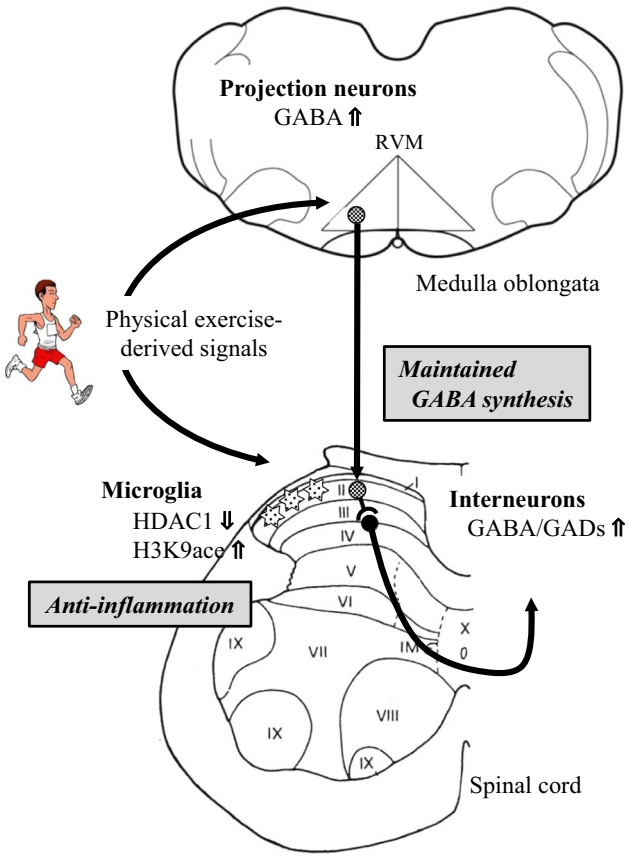


Fig. 6 Underlying mechanisms of EIH (summary of our findings). Physical exercise prevents decreased GABA synthesis in the RVM and dorsal horn, which maintains GABAergic inhibition in PSL mice. Physical exercise decreases HDAC1 in the activated microglia in the dorsal horn, and increased acetylation of H3K9 in the nuclei of these cells may increase the transcription of anti-inflammatory cytokines such as IL-10. Such alterations following physical exercise in NPP animal models may contribute to EIH

governing the sensitivity of the dorsal horn neurons, as well as in pain transmission (Viguier et al. 2013). Intrathecal transplantation of serotonergic precursor cells, which secrete 5-HT, not only alleviates pain behaviors but also reduces bilateral hyperexcitability in dorsal horn neurons following spinal hemisection injury in rats (Hains et al. 2002, 2003). Recently, Bobinski et al. (2015) showed that low-intensity treadmill running in sciatic nerve crushed mice increases the levels of 5-HT and its receptors (5HT-1B, 2A, 2C), and decreases the expression of serotonin transporter in the brainstem (Fig. 1). These results suggest that the activation of the descending inhibitory 5-HT system in the brainstem via treadmill running plays a role in producing EIH.

Conclusion

This review shows that EIH is accomplished through multiple cellular and molecular events produced at different levels of the nervous system following physical exercise (Figs. 1, 6), but further studies will be required to elucidate several matters. For instance, the analgesic level of EIH may be influenced by the style of exercise, i.e., forced or voluntary exercise (Sheahan et al. 2015). Kami et al. (2015) showed that voluntary exercise on a running wheel attenuates pain behaviors in NPP mice models, and that its analgesic effects were greater than those of forced exercise such as treadmill running. It has been demonstrated that the mesolimbic reward system, including the nucleus accumbens (NAc) and ventral tegmental area (VTA), is involved in pain perception and modulation (Mitsui and Zachariou 2016). Dopamine deficiency in these regions results in excessive pain (Saadé et al. 1997). On the other hand, Greenwood et al. (2011) showed that following voluntary wheel running for 6 weeks in rats, tyrosine hydroxylase mRNA levels in the VTA were increased, and increased delta FosB/FosB immunoreactivities were also detected in the NAc, indicating that voluntary running is a strong natural reward that activates mesolimbic reward neurocircuitry (Werme et al. 2002; Brené et al. 2002). Therefore, activations of the mesolimbic reward pathway,

including the VAT and NAc, by voluntary running may be considered a potential mechanism for EIH in the higher brain regions, because a growing body of evidence from human neuroimaging and experimental animal studies suggests that relief from pain is reflected in the activation of dopaminergic neurons in the mesolimbic reward system (Navratilova et al. 2016). Therefore, it will be of interest to investigate the effects of voluntary wheel running on VTA dopaminergic neurons in NPP animal models. It is also well known that exercise activates the medial prefrontal cortex (mPFC), and subsequent corticostriatal projection to the NAc may relieve chronic pain (Lee et al. 2015; Ellingson et al. 2016).

In addition, skeletal muscle is an active endocrine organ, releasing myokines that may in part be responsible for the beneficial effect of exercise. Myokines such as hepatocyte growth factor, insulin-like growth factor, fibroblast growth factor, leukemia inhibitor factor, IL-4, IL-6, and BDNF have been identified to date (Raschke and Eckel 2013; Muñoz-Cánoves et al. 2013). In particular, IL-4 is able to induce the activation of M2 macrophages, which contribute to pain relief via the production of anti-inflammatory cytokines. Interestingly, IL-4 protein levels are enhanced in the contracting muscles during running exercise (Rosa Neto et al. 2011; Pedersen and Febbraio 2012), and thereby may increase the production of anti-inflammatory cytokines in injured peripheral nerves. Therefore, increased IL-4 production in contracting muscles may be considered a potential mechanism for EIH in terms of exercise-associated peripheral metabolism.

Physical exercise-derived signals that produce EIH in NPP animal models remain obscure. It is known that the levels of serotonin, dopamine, and noradrenaline in the spinal dorsal horn of rats change following treadmill running (Gerin et al. 2008, 2011). Therefore, neurotransmitters in the spinal dorsal horn may influence neurons and glial cells as a physical-exercise-derived signal. Also, neurons located in the spinal dorsal horn are activated during treadmill running by afferent feedback input from the exercising limb muscles via group Ia–IV muscle afferents that project into distinct laminae (Maxwell and Riddell 1999; Watson and Bazzaz 2001; Ling et al. 2003; Dai et al. 2005; Jankowski et al. 2013). These results suggest that an increase of neural input into dorsal horn neurons with treadmill running may be involved in the physical-exercise-derived signals that trigger EIH. Furthermore, it is still unknown if each kind of exercise protocol has its own characteristic mechanism for producing EIH. Uncovering the answer to this question would expand our knowledge and understanding of the underlying mechanisms of EIH. In addition, more research would aid the development of novel exercise therapies for NPP and help to expand the availability of exercise therapy for chronic pain patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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