Exercise-Induced Hypoalgesia Profile in a Rat Neuropathic Pain Model Predicts Pain Severity Following Infraorbital Nerve Injury and Is Associated with Local Cytokine Levels, Systemic Endocannabinoids, and Endogenous Opioids

Junad Khan, DDS, MSD, MPH, PhD
Qian Wang, PhD
Orofacial Pain and TMJ Disorders
Eastman Institute for Oral Health
University of Rochester Medical Center
Rochester, New York, USA

Olga A. Korczeniewska, PhD
Rutgers School of Dental Medicine
Department of Diagnostic Sciences
Rutgers, The State University of New Jersey
Newark, New Jersey, USA

Rotem Eliav, MD
Rabin Medical Center
Petah Tikva, Israel

Yanfang Ren, DDS, PhD
Eli Eliav, DMD, PhD
Orofacial Pain and TMJ Disorders
Eastman Institute for Oral Health
University of Rochester Medical Center
Rochester, New York, USA

Correspondence to:
Junad Khan
625 Elmwood Ave
Rochester, NY 14620, USA
Email: junad_khan@URMC.rochester.edu
Fax: +1 (585) 256-3154

Submitted May 4, 2021; accepted May 25, 2021.

Aims: To investigate the role of exercise-induced hypoalgesia (EIH) in the development of neuropathic pain (NP) following infraorbital nerve (ION) injury and to explore possible underlying mechanisms defining the differences between rats with high and low EIH. **Methods:** EIH was evaluated by measuring the percentage of withdrawal responses to a series of 30 mechanical stimuli applied to the hind paw before and after 180 seconds of exercise on a rotating rod. The rats were assigned to low- and high-EIH groups based on reduction in the percent of withdrawal responses following exercise. NP was induced in high- and low-EIH rats via ION constriction injury. Rats were tested with graded nylon monofilaments to establish the withdrawal threshold. Increasingly stiff monofilaments were applied to the ION territory until there was a clear withdrawal by the rat. This was repeated a total of three times. A decreased withdrawal threshold indicates allodynia. Testing was performed at baseline and at 3, 10, and 17 days following the injury. On day 17 postinjury, IONs were harvested for the assessment of interleukin (IL)-6, IL-1β, and IL-10 levels. Samples from high-EIH and low-EIH surgically naïve rats served as control for the cytokines study. In this second part of the study, the effects of cannabinoid 1 (CB1) and cannabinoid 2 (CB2) antagonists and naltrexone on EIH profiles and on the withdrawal thresholds to mechanical stimulation were measured. EIH and withdrawal thresholds in high- and low-EIH rats were measured before and after administration of antagonists. **Results:** Low-EIH rats developed significantly more pronounced allodynia in the ION territory following injury compared to high-EIH rats. At 17 days postinjury, ION IL-1β levels were higher in low-EIH rats, and IL-10 levels were higher in high-EIH rats. CB1 antagonist blocked the analgesic effect induced by exercise in high- but not in low-EIH rats. The CB2 antagonist had no significant effect on high- or low-EIH rats. Naltrexone blocked the effects of EIH in both high- and low-EIH rats. Exercise induced a significant analgesic effect in high-EIH but not in low-EIH rats. CB1 or CB2 antagonist administration had no effect on pre-exercise responses to mechanical stimulation, while naltrexone administration resulted in significant allodynia in both low- and high-EIH rats. **Conclusion:** This study demonstrated substantial differences between rats with high and low EIH. The results suggest that following ION injury, high-EIH rats may have a more prominent or activated endocannabinoids system and that their inflammatory response is moderated, with higher levels of IL-10 and lower levels of IL-1β. *J Oral Facial Pain Headache 2021;35:230–240. doi: 10.11607/ofph.3003*

Keywords: *exercise-induced hypoalgesia, nerve injury, orofacial neuropathic pain, pain modulation, trigeminal*

Neuropathic pain in the orofacial region shares some mechanisms and features with neuropathic pain in spinal nerves, though it also demonstrates inimitable characteristics. The trigeminal system tends to be more resistant to developing neuropathic pain (NP) following trauma; it has been shown that injury to the infraorbital nerve (ION) is followed by lower ectopic activity compared to spinal nerve injury and that sympathetic sprouting is absent in the trigeminal ganglion (TG).[1,2] A recent study also suggested that the dorsal root ganglion

(DRG) and the TG present subtle, distinct transcriptional variations and are differently regulated following peripheral nerve injury.[3]

Faulty pain modulation mechanisms have been linked to various chronic pain conditions, such as fibromyalgia,[4–7] tension-type headache, musculoskeletal pain,[8,9] migraine,[10] chronic low back pain,[11] irritable bowel syndrome,[12] and neuropathic pain, as well as to chronic NP in the orofacial region, such as trigeminal posttraumatic neuropathies[13] and burning mouth syndrome (BMS).[14,15]

Exercise is a known trigger of the pain modulation system and has been used in various studies to evaluate pain modulation efficacy. Its palliative effect on pain sensitivity is commonly termed exercise-induced hypoalgesia (EIH).[16]

The exact mechanisms underlying EIH are not fully understood; however, the endogenous opioid and cannabinoid systems are recognized as playing a major role in this phenomenon.[17–19] Other suggested mechanisms include involvement of neurotransmitters such as norepinephrine and 5HT,[20,21] involvement of the adenosinergic system,[22] and interactions with the cardiovascular system.[23]

It has been previously demonstrated that, following sciatic nerve damage, the severity of pain is associated with the EIH profile.[24] In a previous study, it was demonstrated that rats classified as low EIH developed significantly more severe neuropathic pain following nerve injury compared to high-EIH rats. This finding may have significant clinical importance for identifying individuals that are at higher risk for developing postsurgery chronic pain.[24]

The objectives of the current study were to assess whether EIH profile can predict pain in the orofacial region following damage to the rat ION. Additionally, the potential roles of the endocannabinoid system, the opioid system, and pro- and anti-inflammatory cytokines in the development of neuropathic pain were examined. The study hypotheses were that rats with less efficient EIH would develop more severe pain following ION injury, that cannabinoid and opioid antagonists would reduce EIH (mainly in rats with efficient EIH), and that rats with less efficient EIH would have higher levels of proinflammatory cytokines and lower levels of anti-inflammatory cytokines.

Materials and Methods

All experimental protocols were in compliance with the guidelines of the International Association for the Study of Pain[25] and approved by the Rutgers Institutional Animal Care and Use Committee (protocol no. 10077E1113). Adult male Sprague Dawley rats weighing 250 to 300 g were used. Rats were habituated preoperatively by spending a daily 10–20 minutes in the sensory testing apparatus for 5 consecutive days. During this period, the rats were tested for mechanical allodynia in the region of the ION. During the entire study period, the rats were maintained on standard feed, reverse osmosis–treated water, and a 12-hour day and night cycle.

Study Overview

The rats were assessed for EIH levels following daily habituation in the testing apparatus for 3 days. Rats that demonstrated response reduction to mechanical stimuli of $\geq$ 70% following exercise were considered to have high EIH, while rats with a reduction of $\leq$ 30% were considered to have low EIH. Only high- and low-EIH rats were included in the study. Rats were randomly allocated into the experimental and control groups. The investigator performing the pain behavior or surgery was blinded to the EIH profiling and intervention.

Experiment 1. Fourteen high-EIH and 12 low-EIH rats underwent ION constriction injury. All rats were tested for mechanical allodynia at baseline and at 3, 10, and 17 days following the surgery.

On day 17, under deep anesthesia and prior to euthanasia, each rat's injured and contralateral IONs were harvested for assessment of the interleukin (IL)-6, IL-1β, and IL-10 levels. ION samples from 6 high-EIH and 6 low-EIH naïve rats served as controls for the cytokine study.

Experiment 2. In a different group of low- and high-EIH rats, EIH and mechanical allodynia at the ION territory were assessed before and following administration of the CB1 antagonist (high-EIH n = 15, low-EIH n = 14), CB2 antagonist (high-EIH n = 15, low-EIH n = 14), and naltrexone (high-EIH n = 14, low-EIH n = 15).

Exercise-Induced Hypoalgesia

EIH levels were assessed employing a method previously described by Khan et al.[24] In brief, 30 repetitive stimuli with a 60-g von Frey filament were applied to the rat's hind paw before and immediately after 180 seconds on a rotating rod (RotaRod, Stoelting), where they accelerated from 8 to 16 rpm over 100 seconds, and then maintained the 16 rpm for an additional 80 seconds. EIH score was calculated as: (% responses at baseline – % responses following exercise) / % responses at baseline $\times$ 100.

Rats that demonstrated an EIH reduction of $\geq$ 70% were considered to have high EIH, while rats with EIH reduction of $\leq$ 30% were considered to have low EIH. In experiment 1, EIH was recorded twice (1 week apart) at baseline only. This was done to validate that the EIH profiling did not change. However, in experiment 2, EIH was measured at baseline

and then before and 10 minutes after administration of the antagonist.

ION Injury

Fourteen high-EIH and 12 low-EIH rats underwent left ION constriction injury under deep ketamine (50 mg/kg) and xylazine (7.5 mg/kg) intraperitoneal anesthesic solution. Chronic constriction injury (CCI) of the ION was performed based on the original model described by Imamura et al.[26] In brief, an incision approximately 1 cm long was made along the maxillary left gingivobuccal margin, proximal to the first molar. About 0.5 cm of the ION was freed of the adhering tissue, and two ligatures (4.0 chromic gut) were loosely tied around the nerve. A single investigator (J.K.) performed all of the surgeries.

Pain Behavior Assays

The tests were performed with the rat placed inside a chamber located on an elevated, perforated floor.

In experiment 1, the rats' responses to mechanical stimulation were assessed prior to the surgery (baseline) and then at 3, 10, and 17 days following the surgery. In experiment 2, responses for mechanical allodynia (MA) were recorded before and 10 minutes after the antagonist administration.

MA was tested with Semmes-Weinstein monofilaments sorted by ranks expressing the log base 10 of the force, applied in grams, to bend the filament (Stoelting). The rats' withdrawal threshold was established by applying an increasing amount of force to the ION territory with the calibrated monofilament. The monofilaments were applied in an ascending order from a low to a high force; the lowest force to induce withdrawal response was considered as the detection threshold. Typically, rats in pain respond to a lower force.

Cytokine Levels

Under deep anesthesia with ketamine (50 mg/kg) and xylazine (7.5 mg/kg) and prior to euthanasia, the ION samples were harvested from high-EIH ION-CCI (n = 6) and low-EIH ION-CCI (n = 6) rats. A 1-cm piece of the ION including the area of nerve damage was collected. Approximately 0.25 cm of the proximal and distal sites of the nerve and 0.5 cm of the site of ligatures were harvested. Levels of IL-10, IL-6, and IL-1β in the collected tissues were quantified using enzyme-linked immunosorbent assay (ELISA). ION samples of naïve high-EIH (n = 6) and low-EIH (n = 6) rats served as control.

The tissue samples were weighed and placed separately in 300.25-μL medium containing 300 L of CelLytic MT mammalian tissue lysis/extraction reagent (Sigma Chemical) and 0.25 μl of protease inhibitor cocktail (Sigma Chemical). Tissue homogenization was performed using the Polytron PT1200E (Kinematica), and tissue samples were centrifuged (×13,000 g for 10 minutes). Employing standard procedures, IL-1β, IL-6, and IL-10 content in the samples was quantified using an ELISA microplate absorbance reader (Bio-Rad Laboratories) according to the manufacturer's instructions (R&D Systems). All specimens were tested in duplicate, where 50 ug of total protein was loaded into each well to assess inter-assay variability. The cytokine content was expressed in pictograms of cytokine per milliliter per milligram.

Drugs and Dosages

Naltrexone hydrochloride, a nonspecific opioid receptor antagonist (N3136, Sigma-Aldrich), was administered intraperitoneally to low- and high-EIH rats (low-EIH n = 13, high-EIH n = 15) at a dose of 1 mL/kg per rat.[27] EIH and MA at the ION territory were tested prior to and 15 minutes following the naltrexone administration. AM251, a CB1 antagonist, and AM630 (Sigma Aldrich), a CB2 antagonist, were injected subcutaneously into low- and high-EIH rats (AM251: low n = 14, high n = 16; AM630: low n = 15, high n = 13) at a dose of 1 mL/kg per rat.[28,29] EIH and MA at the ION territory were tested before and 10 minutes following the antagonists' administration.

Data Analysis

The distribution of outcome measures was tested for normality using Shapiro-Wilk test. The results indicated that the response to mechanical stimulation data before and after exercise, with and without antagonists, were significantly skewed. Therefore, nonparametric approaches, such as Wilcoxon rank sum test, were used to compare the medians of the two groups (high and low EIH). Steel-Dwass multiple comparison method was used to control the overall type I error in pairwise comparisons. Nonparametric Wilcoxon test was used to analyze repeated outcomes within the group.

For normally distributed outcomes, Student *t* test was used to compare the means of the high- and low-EIH groups; multivariate analysis of variance (MANOVA) was used for the analysis of repeated-measures data. Analysis of variance (ANOVA) was used to compare the means across multiple groups with Tukey multiple comparison method to control the overall type I error in pairwise comparisons.

Classification of the rats into high- and low-EIH responders was based on the percent reduction in the number of responses to the 30 mechanical stimuli applied to each rat's paw. A reduction $\geq$ 70% was considered a high response (high EIH), and a reduction of $\leq$ 30% was considered a low response (low EIH). The data for the affected and contralateral nerve territories were analyzed separately. The significance

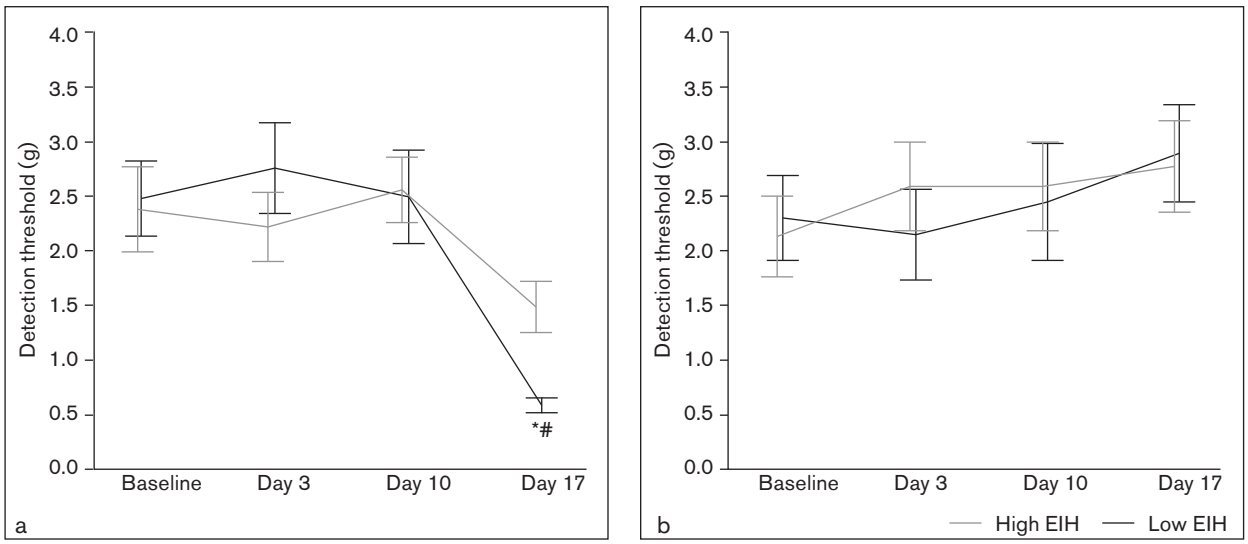

Fig 1 Mechanical allodynia (MA) before and after infraorbital nerve injury. **(a)** MA at the injured nerve territory. No significant differences were found between low- and high-EIH rats at baseline, 3, or 10 days postinjury. Low-EIH rats had a significantly lower threshold (ie, more pain) at day 17 postsurgery compared to high-EIH rats (#). Low-EIH rats' detection threshold was significantly lower compared to baseline on day 17 postsurgery compared to baseline (*). while the reduction in high-EIH rats did not approach significance. **(b)** MA at the contralateral nerve territory. No significant differences were found between low- and high-EIH rats at any time point; neither high- nor low-EIH rats developed significant allodynia compared to baseline.

level was set at α = .05, and a confidence interval (CI) of 95% was set. A sample size analysis relying on previous EIH and pain behavior methods similar to those employed in this study suggested that 10 rats in each study group would provide a power of 80%. Descriptive statistics are presented as mean and standard error of the mean (SEM). Statistical analyses were performed using JMP Pro 13.0.0 (SAS Institute).

Results

A total of 229 rats were initially screened (high EIH = 96 [42%], medium EIH = 78 [34%], low EIH = 55 [24%]). A total of 58 high-EIH and 55 low-EIH rats were included in the study. The rats were selected randomly for the EIH groups.

Experiment 1

Pain behavior following nerve injury. *Mechanical Allodynia.* On the ION-CCI side, no significant differences were found between low- and high-EIH rats at baseline (high: 2.38 ± 0.39; low: 2.48 ± 0.34), day 3 postsurgery (high: 2.22 ± 0.31; low: 2.76 ± 0.42), or day 10 postsurgery (high: 2.56 ± 0.30, low: 2.50 ± 0.42). Low-EIH rats had a significantly lower threshold (ie, more pain) at day 17 postsurgery compared to high-EIH rats (high: 1.49 ± 0.23, low: 0.58 ± 0.01; *P* = .003; Fig 1a). Low-EIH rats' detection threshold was significantly lower on day 17 postsurgery compared to baseline (*P* < .001). While the detection threshold decreased in high-EIH rats, it did not approach significance (*P* = .101; Fig 1a).

On the contralateral ION side, no significant differences were found between low- and high-EIH rats at baseline (high: 2.13 ± 0.37; low: 2.30 ± 0.39), day 3 postsurgery (high: 2.60 ± 0.41; low: 2.14 ± 0.41), day 10 postsurgery (high: 2.58 ± 0.42; low: 2.45 ± 0.54), or day 17 postsurgery (high: 2.78 ± 0.42; low: 2.90 ± 0.45; Fig 1b). Neither high- nor low-EIH rats developed a significant threshold reduction at days 3, 10, or 17 postsurgery compared to baseline (Fig 1b).

Cytokine levels. The affected and contralateral nerves were harvested at the end of the experiment on day 17 postsurgery. Data are presented as mean ± SEM (pg/mg).

IL-1β levels. On the ION-CCI side, IL-1β levels were significantly higher in low-EIH rats compared to high-EIH rats (high: 35.83 ± 18.00; low: 87.50 ± 11.29; *P* = .018). Low-EIH rats that underwent nerve injury had significantly higher IL-1β levels compared to naïve low-EIH rats (naïve low: 2.50 ± 2.50, *P* = .003). The difference between high-EIH ION-CCI rats and high-EIH naïve rats (naïve high: 1.67 ± 1.67) was not statistically significant (Fig 2a).

On the contralateral ION, there was no significant difference between high- and low-EIH rats (high: 31.90 ± 14.77; low: 48.33 ± 21.77). The difference

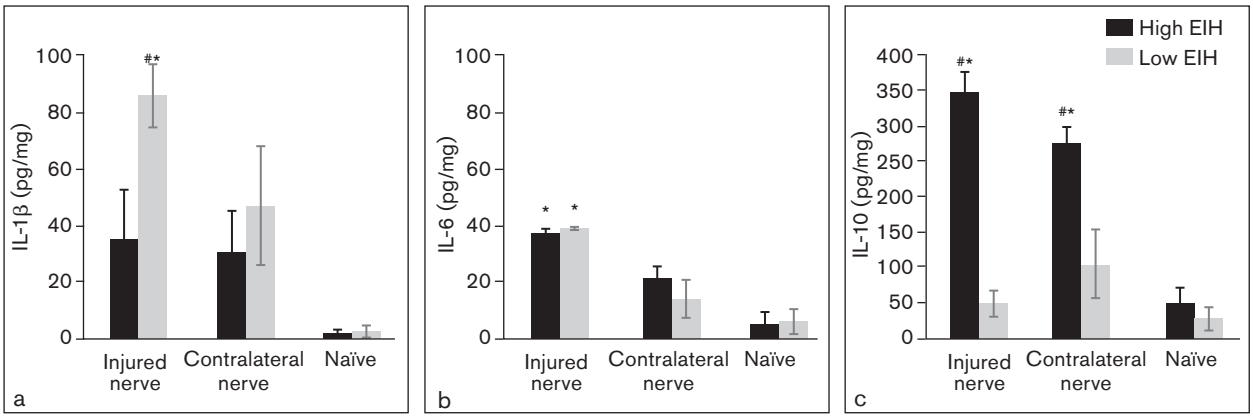

Fig 2 Cytokine levels in the injured and contralateral infraorbital nerves 17 days following injury. **(a)** IL-1β. Low-EIH rats' levels were significantly higher compared to high-EIH rats (#) and compared to naïve low-EIH rats (*). The difference between high-EIH postsurgery rats and high-EIH naïve rats was not statistically significant. On the contralateral side, there were no significant differences in IL-1β levels between high- and low-EIH rats, or between high- and low-EIH rats postsurgery and naïve high- and low-EIH rats. **(b)** IL-6. On the injured side, there were no significant differences in IL-6 levels between high-EIH and low-EIH rats. High-EIH postsurgery rats had significantly higher IL-6 levels compared to high-EIH naïve rats, and low-EIH postsurgery rats had significantly higher IL-6 levels compared to naïve low-EIH rats (*). On the contralateral side, there were no significant differences in IL-6 levels between high-EIH and low-EIH rats, or between high- and low-EIH rats postsurgery and naïve high- and low-EIH rats. **(c)** IL-10. On the injured side, IL-10 levels were significantly higher in high-EIH rats compared to low-EIH rats postsurgery (#) and naïve high-EIH rats (*). No significant differences were found between low-EIH rats postsurgery and naïve low- or high-EIH rats. On the contralateral side, IL-10 levels were significantly higher in high-EIH compared to low-EIH rats postsurgery (#) and compared to naïve high-EIH rats (*). No significant differences were found between low-EIH rats postsurgery and naïve low-EIH rats.

between high-EIH ION-CCI rats and naïve high-EIH rats (1.67 ± 6.01), as well as the difference between low-EIH ION-CCI rats and low-EIH naïve rats (2.50 ± 2.50), was not statistically significant.

IL-6 levels. On the ION-CCI side, there was no significant difference in IL-6 levels between high-EIH and low-EIH rats (high: 38.00 ± 1.63; low: 39.67 ± 0.82). High-EIH rats that underwent nerve injury had significantly higher IL-6 levels compared to high-EIH naïve rats (naïve high: 5.80 ± 5.00; $P = .004$), and low-EIH rats that underwent nerve injury had significantly higher IL-6 levels compared to naïve low-EIH rats (naïve low: 6.67 ± 4.94, $P = .003$; Fig 2b).

On the contralateral ION side, there was no significant difference between IL-6 levels in high-EIH and low-EIH rats (high: 31.90 ± 14.77; low: 48.33 ± 21.71). There were also no differences between high- and low-EIH rats that underwent nerve injury and naïve high and low rats.

IL-10 levels. On the ION-CCI side, IL-10 levels were significantly higher in high-EIH compared to low-EIH rats (high: 345.83 ± 73.58, low: 38.89 ± 17.39; $P = .008$) and compared to naïve high-EIH rats (50 ± 55.82; $P = .005$). No significant difference was found between low-EIH rats that underwent nerve injury and naïve low- or high-EIH rats (28.00 ± 16.09).

On the contralateral side, IL-10 levels were significantly higher in high-EIH rats (275.83 ± 53.70) compared to low-EIH rats (105.00 + 49.77, $P = .044$) and compared to naïve high-EIH rats (50 ± 55.82, $P = .005$). No significant difference was found between low-EIH rats that underwent nerve injury and naïve low-EIH rats (28.00 ± 16.09; Fig 2c).

Experiment 2

Antagonist effect on EIH. *CB1 antagonist.* CB1 antagonist (AM251) administration blocked the exercise hypoalgesic effect in high-EIH rats (EIH: 75.36 ± 3.57; EIH following antagonist administration: 24.18 ± 5.07; $P < .001$), but had no significant effect in low-EIH rats (EIH: 20.80 ± 2.96; EIH following antagonist administration: 18.40 ± 2.75; Fig 3a).

CB2 antagonist. CB2 antagonist (AM630) administration did not have a significant effect in high-EIH rats (EIH: 76.00 ± 3.51; EIH following CB1 administration: 76.81 ± 3.63) or low-EIH rats (EIH: 15.60 ± 3.30; EIH following CB1 antagonist administration: 20.10 ± 2.77; Fig 3b).

Opioid antagonist. Opioid antagonist (naltrexone) administration blocked the exercise hypoalgesic effect in high-EIH (EIH: 77.18 ± 3.51; EIH following naltrexone administration: 19.18 ± 3.59; $P < .001$) and low-EIH rats (EIH: 25.00 ± 2.64; EIH following naltrexone administration: 11.60 ± 2.67; $P = .009$; Fig 3c).

Antagonist effect on mechanical allodynia. *CB1 antagonist.* Exercise significantly increased the high-EIH rats' mechanical detection threshold, indicating reduced sensitivity (before exercise: 0.96. ± 0.12; following exercise: 1.78 ± 0.26; $P = .004$), but

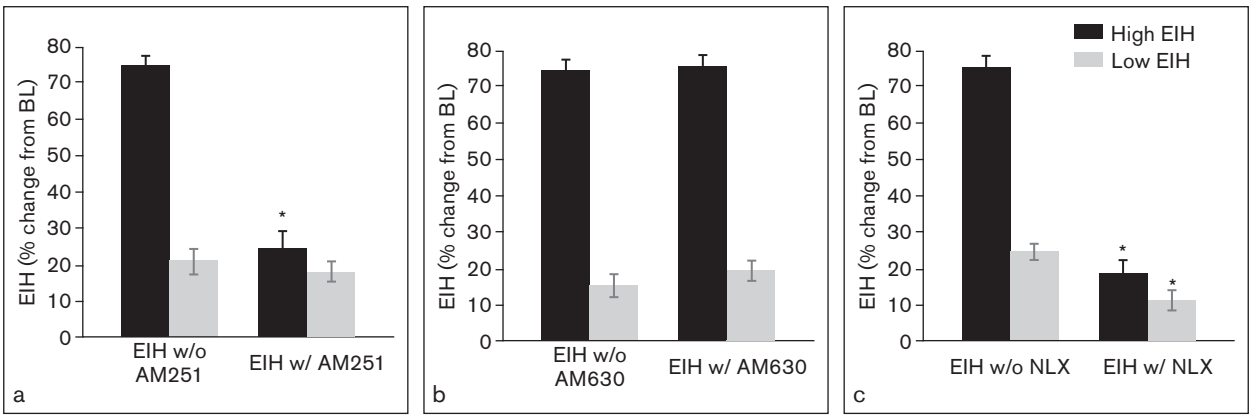

Fig 3 Effect of cytokine antagonist administration on EIH. (a) CB1 antagonist AM251 blocked the exercise hypoalgesic effect in high-EIH rats (*), but had no significant effect in low-EIH rats. (b) CB2 antagonist AM630 did not have a significant effect in high-EIH or low-EIH rats. (c) Opioid antagonist naltrexone (NLX) blocked the exercise hypoalgesic effect in high- and low-EIH rats. BL = baseline.

had a nonsignificant effect in low-EIH rats (before exercise: 0.79 ± 0.17; following exercise: 0.98 ± 0.14). Administration of AM251 blocked the hypoalgesia induced by exercise in high-EIH rats and significantly increased the tested area's sensitivity (before exercise: 0.97 ± 0.12; following exercise: 0.56 ± 0.05; P = .019). However, it had no significant effect in low-EIH rats (before exercise: 1.03 ± 0.21; following exercise: 0.83 ± 0.22). The antagonist administration did not affect the threshold before exercise in high- or low-EIH rats (Fig 4a).

CB2 antagonist. Exercise increased the high-EIH rats' mechanical detection threshold significantly (before exercise: 0.94. ± 0.15; following exercise: 2.04 ± 0.29; P = .003) but did not have a significant effect in low-EIH rats (before exercise: 0.81 ± 0.15; following exercise: 1.17 ± 0.34). CB2 antagonist administration did not have a significant effect on the hypoalgesia induced by exercise in high-EIH rats (before exercise: 1.04. ± 0.11; following exercise: 1.83 ± 0.24) or in low-EIH rats (before exercise: 0.97 ± 0.17; following exercise: 1.14 ± 0.19). The antagonist administration did not have a significant effect on the threshold before exercise in high- or low-EIH rats (Fig 4b).

Opioid antagonist. Exercise increased the high-EIH rats mechanical detection threshold significantly (before exercise: 1.23. ± 0.19; following exercise: 2.55 ± 0.21; P < .001), but did not have a significant effect in low-EIH rats (before exercise: 1.19 ± 0.18; following exercise: 1.51 ± 0.37). Naltrexone administration in high-EIH rats significantly reduced the detection threshold before exercise compared to naïve rats (without naltrexone: 1.23 ± 0.19; with naltrexone: 0.60 ± 0.13; P = .011) but did not block the hypoalgesia (ie, threshold elevation) induced by exercise (before exercise: 0.60 ± 0.13; following exercise: 2.49 ± 0.25; P < .001). Administration in low-EIH rats did not significantly change the response before exercise compared to naïve rats (without naltrexone: 1.19 ± 0.18; with naltrexone: 0.71. ± 0.24); however, exercise induced a significant hypoalgesia (before exercise: 0.71. ± 0.68; following exercise: 1.68 ± 0.2; P = .007; Fig 4c).

Discussion

This study investigated the role of EIH profile in rats in the development of neuropathic pain following ION injury and explored its association with pro- and anti-inflammatory cytokine levels in the injured nerve. Additionally, the effect of systemic opioids and cannabinoid antagonists on EIH and the response to mechanical stimulation in the ION territory were also investigated.

The key findings of this study are the substantial differences between rats with high- and low-EIH profiles. Low-EIH rats developed significantly more pain following ION injury compared to high-EIH rats. Differences were also noted in the pro- and anti-inflammatory cytokine levels at the injured nerve and in the responses to opioid and cannabinoid antagonists.

In clinical practice and in animal studies, exercise has been shown to have a palliative effect on various painful conditions, including neuropathic pains.[30–37] Regular physical activity may also play a role in preventing the development of chronic pain and the activation of central neurons.[38] Exercise is presently included in the treatment protocols for chronic painful conditions such as arthritis, fibromyalgia, chronic fatigue, and low back pain.[39–44] However, less is known about the effect of exercise on neuropathic orofacial

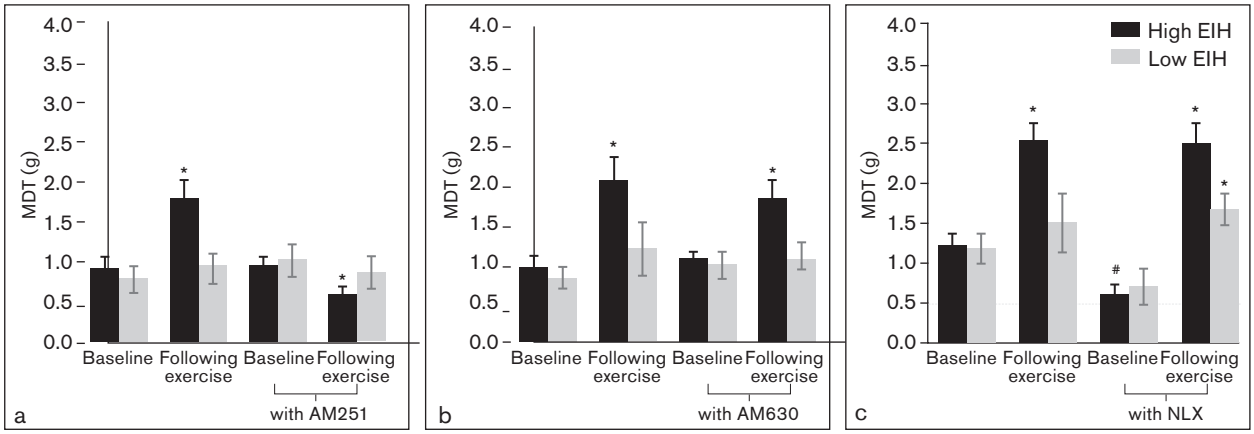

Fig 4 Effect of cytokine antagonist administration on mechanical allodynia (MA). The mechanical detection threshold (MDT) to Semmes-Weinstein monofilaments was tested before and after exercise, with and without antagonist administration. A lower score denotes increased sensitivity. **(a)** Exercise significantly increased the MDT in high-EIH rats but had a nonsignificant effect in low-EIH rats. CB1 antagonist AM251 did not have a significant effect on the responses before exercise; however, in high-EIH rats, the antagonist blocked the hypoalgesia induced by exercise, and the detection threshold following exercise decreased significantly (*). **(b)** Exercise increased the MDT in high-EIH rats significantly, but did not have a significant effect in low-EIH rats. CB2 antagonist AM630 did not have a significant effect on the hypoalgesia induced by exercise or on the threshold before exercise in high-EIH or low-EIH rats. **(c)** Exercise increased the MDT in high-EIH rats significantly, but did not have a significant effect in low-EIH rats. Opioid antagonist naltrexone (NLX) significantly reduced the MDT in high-EIH rats before exercise compared to naïve rats, but had no significant effect in low-EIH rats. NLX did not block the threshold elevation following exercise in the high- or low-EIH rats.

pain, as previous studies have focused on masticatory myalgia.[45] Orofacial or trigeminal nerve neuropathic pain share mechanisms and features with spinal-nerve neuropathic painful conditions; however, the trigeminal nerve seems to display some unique pain syndromes unknown in other anatomical sites. These may include trigeminal neuralgia, BMS, and primary headaches, such as migraines and trigeminal autonomic cephalalgias. It also seems to react differently to trauma with less electrophysiologic activity at the level of the neuroma,[2] a lower incidence of neuropathic pain,[46] and no sympathetic-sensory relationships at the level of the dorsal root ganglion, as seen in the sciatic nerve.[1,47] Additionally, there is a significantly lower incidence of other painful syndromes in the trigeminal system, such as complex regional pain syndrome and painful diabetic neuropathy.

A limited number of studies assessed the association between pain modulation and neuropathic orofacial pain[48,49]; however, it has been shown that patients with less efficient pain modulation developed more chronic pain following root canal treatment,[13] and patients suffering from BMS have a less efficient pain modulatory system.[14] In contrast, a study employing the blink reflex test to measure pain modulation and capsaicin as a painful stimulus could not demonstrate altered pain modulation in atypical odontalgia patients.[50]

Although exercise is commonly used to evaluate the inhibitory pain modulation system,[51] the majority of the studies on orofacial pains and all of the studies on neuropathic orofacial pain employed the conditioned pain modulation (CPM) paradigm, whereby a painful conditioning stimulus inhibits the test stimulus (pain inhibits pain). CPM and EIH share common mechanisms, such as activation of the opioid, cannabinoid, serotonergic, noradrenergic, and dopaminergic systems[17,19,52–56]; however, they are not identical phenomena.[57] An important difference is the effect duration—while the CPM effect is limited to the time at which the conditioning painful stimulus is provided, EIH endures beyond the exercise period.[58]

An association between pain modulation profile and postsurgical pain was first demonstrated in a prospective study in patients undergoing thoracotomy, where patients with less efficient pain modulation suffered more from significant persistent postsurgical neuropathic pain.[59]

In a previous study employing the same method for assessing EIH in rats, the present authors demonstrated that the severity of the pain developed following sciatic nerve injury is related to the rat's EIH profile.[24] Rats with less efficient hypoalgesia following exercise (low EIH) not only developed more severe neuropathic pain following the nerve injury, but also developed pain in the contralateral intact paw. It is not clear whether the low- and high-EIH

rats are endowed with this trait or whether behavioral change, such as an increase in activity (such as daily exercise), can change EIH profile.[17,60]

The current study is the first to use EIH profile in the prediction of developing chronic pain following nerve injury in the trigeminal nerve. As expected, and in line with the study on sciatic nerve injury, low-EIH rats developed significantly more pain compared to high-EIH rats. However, ION injury induced pain only in the affected nerve territory, with no detectable pain in the contralateral nerve territory. This may be another difference between the trigeminal system and spinal nerves.

In various clinical studies, most of the participants demonstrated hypoalgesia following exercise at some point of the study. However, while some individuals consistently demonstrated hypoalgesia, some changed their response from hypoalgesic to hyperalgesic, or vice versa, when tested on different days.[61–65] To avoid potential inconsistency in the response to exercise, the current study included only rats that demonstrated a clear high (reduction of $\geq$ 70%) or low (reduction of $\leq$ 30%) EIH profile in three tests performed on 3 consecutive days.

It is not clear whether exercise alleviates all kinds of pain and works for all patients suffering from chronic pain, as variations have been noted among patients with different pain conditions, such as musculoskeletal pain, whiplash, and osteoarthritis.[66–68] Exercise can also induce or augment existing pain, especially in some of the patients suffering from chronic pain conditions.[56,69,70] EIH profile, or each patient's ability to modulate pain, and differences in the underlying mechanisms may play a role in the differences among patient responses to exercise and in the transition from acute postinjury pain to chronic neuropathic pain.

In addition to its palliative effect, exercise has been shown to modulate inflammation, immune response, and cytokine expression.[71–75] It has been suggested that the imbalance between pro- and anti-inflammatory cytokines might play a role in the development of chronic pain states.[76–80]

The cytokines assessed in this study are known to be involved in the initiation and maintenance of neuropathic pain.[78,81] While IL-1β and IL-6 are known to induce pain, IL-10 may have a role in alleviating pain.[82–84]

The primary hypothesis of this study, that IL-1β levels would be higher in rats with a less efficient pain modulation system (low EIH), while IL-10 would be higher in rats with a more efficient pain modulation system (high EIH), was confirmed. IL-6 levels were higher in the injured nerves, but with no differences between high- and low-EIH rats. IL-10 levels were higher in high-EIH rats in the affected and contralateral nerves, while IL-1β and IL-6 levels were elevated significantly only in the injured nerve. The cytokine levels were assessed only at one time (17 days following injury) and only at the peripheral nerve. Cytokine levels at different time points and at the DRG or higher levels of the nervous system could show a different distribution.

It appears that, at least in part, high-EIH rats' excessive IL-10 levels in the affected and contralateral nerves play a role in the lower pain levels following ION injury, while the high IL-1β in low EIH rats may increase the risk of developing pain.

The high-EIH rats' contralateral nerve increase in IL-10 levels may also play a part in protecting from the development of widespread pain following injury.

The exact mechanisms of how exercise alleviates pain are not fully understood, though the endogenous opioid and cannabinoid systems and activation of the pain modulatory system may play a major role in this phenomenon.[17–19] A majority of studies have demonstrated that opioid antagonists can block EIH[85]; however, some studies have shown limited or no effect on EIH.[19,56] The role of the endocannabinoid system in EIH has been supported by studies that demonstrated an increase in circulating endocannabinoids following exercise[19] and prevention of EIH in rodents with endocannabinoid antagonists.[28] Although interaction between the endogenous opioid and endocannabinoid systems has been reported,[86] it has not been assessed in relation to EIH and not in orofacial pain.

In the present study, both the CB1 antagonist and the nonspecific opioid antagonist (naltrexone) prevented EIH. However, while naltrexone blocked EIH in both high- and low-EIH rats, the CB1 antagonist's effect was limited to high-EIH rats.

As expected, exercise induced a hypoalgesic effect to mechanical stimuli applied to the face (ION territory); however, the reduction in the response was significant only in high-EIH rats. CB1 antagonist administration did not change the pre-exercise response to mechanical stimuli, while naltrexone administration resulted in increased sensitivity in high-EIH rats. The effect of CB1 antagonists and naltrexone on the hypoalgesia induced by exercise had opposite trends. Following CB1 antagonist administration, the ION territory became more sensitive to mechanical stimuli in high-EIH rats, while following naltrexone administration, exercise induced a significant hyposensitivity to the mechanical stimuli in both high- and low-EIH rats.

These findings suggest that although both CB1 antagonists and naltrexone reverse EIH, the endocannabinoid pathway is more prominent in rats that have a more efficient EIH and a more efficient pain modulatory system.

The mechanisms underlying EIH and the individual differences in response to exercise are complex and not yet completely understood. However, based on the present study's findings, rats with less efficient inhibito-

Table 1 Effect of Antagonists on EIH

	CB1 antagonist (AM251)	CB2 antagonist (AM630)	Opioid antagonist (naltrexone)
High EIH			
EIH	Decreased EIH effect	None	Decreased EIH effect
MA before exercise	None	None	Increased sensitivity
MA following exercise	Exercise increase sensitivity	None	EIH
Low EIH			
EIH	None	None	Decreased EIH effect
MA before exercise	None	None	None
MA following exercise	None	None	EIH

EIH = exercise-induced hypoalgesia; CB1/CB2 = cannabinoid 1/2 ; MA = mechanical allodynia.

ry pain modulation (low EIH) have a higher risk of developing pain following injury to the trigeminal nerve. The endocannabinoid system is more active in rats with a more efficient inhibitory pain modulation system (high EIH), while the endogenous opioid system effect is not necessarily associated with EIH efficiency. Table 1 summarizes the antagonists' effects on EIH and on the response to mechanical stimulation before and after exercise. The inhibitory pain modulation efficiency is also associated with cytokine profiles characteristic of rats with efficient or nonefficient pain modulation systems, suggesting differences in the inflammatory response following nerve injury.

It is not clear whether daily exercise can help a less efficient pain inhibitory system become a more efficient one or whether it will reduce the risk of developing chronic pain following injury. Athletes have been shown to have reduced sensitivity to cold and mechanical stimuli[87,88]; however, overall, there are no significant differences in pain modulation between elite athletes and healthy controls.[89] Future rodent research that includes daily exercise, EIH assessment, biomarkers such as cytokine and endocannabinoid levels, and responses to an antagonist may provide an answer to this question.

Key Findings/Highlights

- Rats with less efficient inhibitory pain modulation (low EIH) have a higher risk of developing pain following injury to the trigeminal nerve.
- The endocannabinoid system is more active in rats with a more efficient inhibitory pain modulation system (high EIH).
- Inhibitory pain modulation efficiency is also associated with cytokine profiles characteristic of rats with efficient or nonefficient pain modulation systems.

Acknowledgments

The authors report no conflict of interest. All authors contributed to the conceptualization, planning, analysis, writing, and submission of the manuscript.

References

1. Benoliel R, Eliav E, Tal M. No sympathetic nerve sprouting in rat trigeminal ganglion following painful and non-painful infraorbital nerve neuropathy. Neurosci Lett 2001;297:151–154.
2. Tal M, Devor M. Ectopic discharge in injured nerves: Comparison of trigeminal and somatic afferents. Brain Res 1992;579:148–151.
3. Korczeniewska, OA, Katzmann Rider G, Gajra S, et al. Differential gene expression changes in the dorsal root versus trigeminal ganglia following peripheral nerve injury in rats. Eur J Pain 2020;24:967–982.
4. Graven-Nielsen T, Kendall SA, Henriksson KG. Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. Pain 2000;85:483–491.
5. Staud R, Vierck CJ, Cannon RL, Mauderli AP, Price DD. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. Pain 2001;91:165–175.
6. Price DD, Staud R, Robinson ME, Mauderli AP, Cannon R, Vierck CJ. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. Pain 2002;99:49–59.
7. Staud R, Robinson ME, Vierck CJ Jr, Price DD. Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. Pain 2003;101:167–174.
8. Kleinböhl D, Hölzl R, Möltner A, Rommel C, Weber C, Osswald PM. Psychophysical measures of sensitization to tonic heat discriminate chronic pain patients. Pain 1999;81:35–43.
9. Ashina S, Bendtsen L, Ashina M, Magerl W, Jensen R. Generalized hyperalgesia in patients with chronic tension-type headache. Cephalalgia 2006;26:940–948.
10. Weissman-Fogel I, Sprecher E, Granovsky Y, Yarnitsky D. Repeated noxious stimulation of the skin enhances cutaneous pain perception of migraine patients in-between attacks: Clinical evidence for continuous sub-threshold increase in membrane excitability of central trigeminovascular neurons. Pain 2003;104:693–700.
11. Kleinböhl D, Görtelmeyer R, Bender HJ, Hölzl R. Amantadine sulfate reduces experimental sensitization and pain in chronic back pain patients. Anesth Analg 2006;102:840–847.
12. Piché M, Bouin M, Arsenault M, Poitras P, Rainville P. Decreased pain inhibition in irritable bowel syndrome depends on altered descending modulation and higher-order brain processes. Neuroscience 2011;195:166–175.

13. Nasri-Heir C, Khan J, Benoliel R. Altered pain modulation in patients with persistent postendodontic pain. Pain 2015;156:2032–2041.
14. Nasri-Heir C, Shigdar D, Alnaas D, Korczeniewska OA, Eliav R, Heir GM. Primary burning mouth syndrome: Literature review and preliminary findings suggesting possible association with pain modulation. Quintessence Int 2017;49:49–60.
15. Nishihara C, Watanabe K, Ozasa K, et al. Altered pain modulation to noxious heat thermal stimuli in burning mouth syndrome. Oral Dis 2020;26:1777–1782.
16. Black J, Chesher GB, Starmer GA, Egger G. The painlessness of the long distance runner. Med J Aust 1979;1:522–523.
17. Stagg NJ, Mata HP, Ibrahim MM, et al. Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain model: Role of endogenous opioids. Anesthesiology 2011;114:940–948.
18. Bement MK, Sluka KA. Low-intensity exercise reverses chronic muscle pain in the rat in a naloxone-dependent manner. Arch Phys Med Rehabil 2005;86:1736–1740.
19. Koltyn KF, Brellenthin AG, Cook DB, Sehgal N, Hillard C. Mechanisms of exercise-induced hypoalgesia. J Pain 2014;15: 1294–1304.
20. Dietrich A, McDaniel WF. Endocannabinoids and exercise. Br J Sports Med 2004;38:536–541.
21. Bobinski F, Ferreira TAA, Córdova MM, et al. Role of brainstem serotonin in analgesia produced by low-intensity exercise on neuropathic pain after sciatic nerve injury in mice. Pain 2015;156:2595–2606.
22. Martins DF, Mazzardo-Martins L, Soldi F, Stramosk J, Piovezan AP, Santos ARS. High-intensity swimming exercise reduces neuropathic pain in an animal model of complex regional pain syndrome type I: Evidence for a role of the adenosinergic system. Neuroscience 2013;234:69–76.
23. Lovick TA. Integrated activity of cardiovascular and pain regulatory systems: Role in adaptive behavioural responses. Prog Neurobiol 1993;40:631–644.
24. Khan J, Benavent V, Korczeniewska OA, Benoliel R, Eliav E. Exercise-induced hypoalgesia profile in rats predicts neuropathic pain intensity induced by sciatic nerve constriction injury. J Pain 2014;15:1179–1189.
25. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110.
26. Imamura Y, Kawamoto H, Nakanishi O. Characterization of heat-hyperalgesia in an experimental trigeminal neuropathy in rats. Exp Brain Res 1997;116:97–103.
27. Morgan RW, Nicholson KL. Characterization of the antinociceptive effects of the individual isomers of methadone after acute and chronic administrations. Behav Pharmacol 2011;22: 548–557.
28. Galdino G, Romero TR, Silva JF, et al. The endocannabinoid system mediates aerobic exercise-induced antinociception in rats. Neuropharmacology 2014;77:313–324.
29. Pharmacology of the cannabinoid system. In: Beaulieu P, Lussier D, Porreca F, Dickenson A (eds). Pharmacology of Pain. Seattle: IASP, 2010:111–138.
30. Kuphal KE, Fibuch EE, Taylor BK. Extended swimming exercise reduces inflammatory and peripheral neuropathic pain in rodents. J Pain 2007;8:989–997.
31. Chen YW, Li YT, Chen YC, Li ZY, Hung CH. Exercise training attenuates neuropathic pain and cytokine expression after chronic constriction injury of rat sciatic nerve. Anesth Analg 2012;114:1330–1337.
32. Shen J, Foxw LE, Cheng J. Swim therapy reduces mechanical allodynia and thermal hyperalgesia induced by chronic constriction nerve injury in rats. Pain Med 2013;14:516–525.
33. Bobinski F, Ferreira TAA, Córdova MM, et al. Role of brainstem serotonin in analgesia produced by low-intensity exercise on neuropathic pain after sciatic nerve injury in mice. Pain 2015;156:2595–2606.
34. Detloff MR, Quiros-Molina D, Javia AS, et al. Delayed exercise is ineffective at reversing aberrant nociceptive afferent plasticity or neuropathic pain after spinal cord injury in rats. Neurorehabil Neural Repair 2016;30:685–700.
35. Anheyer D, Klose P, Koch AK, Haller H, Dobos G, Cramer H. Comparative efficacy of different exercise interventions in chronic non-specific low back pain: Protocol of a systematic review and network meta-analysis. BMJ Open 2020;10:e036050.
36. de Zoete RM, Armfield NR, McAuley JH, Chen K, Sterling M. Comparative effectiveness of physical exercise interventions for chronic non-specific neck pain: A systematic review with network meta-analysis of 40 randomised controlled trials. Br J Sports Med 2020;bjsports-2020-102664.
37. Bakdach WMM, Hadad R. Effectiveness of supplemental vibrational force in reducing pain associated with orthodontic treatment: A systematic review. Quintessence Int 2020;51:742–752.
38. Sluka KA, O'Donnell JM, Danielson J, Rasmussen LA. Regular physical activity prevents development of chronic pain and activation of central neurons. J Appl Physiol (1985) 2013;114:725–733.
39. Brosseau L, Wells GA, Tugwell P, et al. Ottawa Panel evidence-based clinical practice guidelines for aerobic fitness exercises in the management of fibromyalgia: Part 1. Phys Ther 2008;88:857–871.
40. Jansen MJ, Viechtbauer W, Lenssen AF, Hendriks EJM, de Bie RA. Strength training alone, exercise therapy alone, and exercise therapy with passive manual mobilisation each reduce pain and disability in people with knee osteoarthritis: A systematic review. J Physiother 2011;57:11–20.
41. Stewart MJ, Maher CG, Refshauge KM, Herbert RD, Bogduk N, Nicholas M. Randomized controlled trial of exercise for chronic whiplash-associated disorders. Pain 2007;128:59–68.
42. van Middelkoop M, Rubinstein SM, Verhagen AP, Ostelo RW, Koes BW, van Tulder MW. Exercise therapy for chronic nonspecific low-back pain. Best Pract Res Clin Rheumatol 2010;24:193–204.
43. Fransen M, McConnell S, Hernandez-Molina G, Reichenbach S. Exercise for osteoarthritis of the hip. Cochrane Database Syst Rev 2014;(4):CD007912.
44. Busch AJ, Webber SC, Richards RS, et al. Resistance exercise training for fibromyalgia. Cochrane Database Syst Rev 2013;2013:CD010884.
45. Nasri-Heir C, Patil AG, Korczeniewska OA, et al. The effect of nonstrenuous aerobic exercise in patients with chronic masticatory myalgia. J Oral Facial Pain Headache 2019;33:143–152.
46. Benoliel R, Birenboim R, Regev E, Eliav E. Neurosensory changes in the infraorbital nerve following zygomatic fractures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99: 657–665.
47. Bongenhielm U, Boissonade FM, Westermark A, Robinson PP, Fried K. Sympathetic nerve sprouting fails to occur in the trigeminal ganglion after peripheral nerve injury in the rat. Pain 1999;82: 283–288.
48. Moana-Filho EJ, Herrero Babiloni A, Theis-Mahon NR. Endogenous pain modulation in chronic orofacial pain: A systematic review and meta-analysis. Pain 2018;159:1441–1455.
49. Khan J, Zusman T, Wang Q, Eliav E. Acute and chronic pain in orofacial trauma patients. J Endod 2019;45:S28–S38.
50. Baad-Hansen L, List T, Kaube H, Jensen TS, Svensson P. Blink reflexes in patients with atypical odontalgia and matched healthy controls. Exp Brain Res 2006;172:498–506.
51. Lannersten L, Kosek E. Dysfunction of endogenous pain inhibition during exercise with painful muscles in patients with shoulder myalgia and fibromyalgia. Pain 2010;151:77–86.
52. Bement MK, Sluka KA. Low-intensity exercise reverses chronic muscle pain in the rat in a naloxone-dependent manner. Arch Phys Med Rehabil 2005;86:1736–1740.
53. Crombie KM, Brellenthin AG, Hillard CJ, Koltyn KF. Endocannabinoid and opioid system interactions in exercise-induced hypoalgesia. Pain Med 2018;19:118–123.
54. Dietrich A, McDaniel WF. Endocannabinoids and exercise. Br J Sports Med 2004;38:536–541.
55. Droste C, Meyer-Blankenburg H, Greenlee MW, Roskamm H. Effect of physical exercise on pain thresholds and plasma beta-endorphins in patients with silent and symptomatic myocardial ischaemia. Eur Heart J 1988;9(suppl N):s25–s33.

56. Lima LV, Abner TSS, Sluka KA. Does exercise increase or decrease pain? Central mechanisms underlying these two phenomena. J Physiol 2017;595:4141–4150.
57. Vaegter HB, Handberg G, Graven-Nielsen T. Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. Pain 2014;155:158–167.
58. Vaegter HB, Handberg G, Graven-Nielsen T. Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. Pain 2014;155:158–167.
59. Yarnitsky D, Crispel Y, Eisenberg E, et al. Prediction of chronic post-operative pain: Pre-operative DNIC testing identifies patients at risk. Pain 2008;138:22–28.
60. Lazaridou A, Paschali M, Schreiber K, et al. The association between daily physical exercise and pain among women with fibromyalgia: The moderating role of pain catastrophizing. Pain Rep 2020;5:e832.
61. Gomolka S, Vaegter HB, Nijs J, et al. Assessing endogenous pain inhibition: Test-retest reliability of exercise-induced hypoalgesia in local and remote body parts after aerobic cycling. Pain Med 2019;20:2272–2282.
62. Hviid JT, Thorlund JB, Vaegter HB. Walking increases pain tolerance in humans: An experimental cross-over study. Scand J Pain 2019;19:813–822.
63. Vaegter HB, Bjerregaard LK, Redin MM, Rasmussen SH, Graven-Nielsen T. Hypoalgesia after bicycling at lactate threshold is reliable between sessions. Eur J Appl Physiol 2019;119:91–102.
64. Vaegter HB, Dørge DB, Schmidt KS, Jensen AH, Graven-Nielsen T. Test-retest reliabilty of exercise-induced hypoalgesia after aerobic exercise. Pain Med 2018;19:2212–2222.
65. Vaegter HB, Lyng KD, Yttereng FW, Christensen MH, Sørensen MB, Graven-Nielsen T. Exercise-induced hypoalgesia after isometric wall squat exercise: A test-retest reliabilty study. Pain Med 2019;20:129–137.
66. Van Oosterwijck J, Nijs J, Meeus M, Van Loo M, Paul L. Lack of endogenous pain inhibition during exercise in people with chronic whiplash associated disorders: An experimental study. J Pain 2012;13:242–254.
67. Fingleton C, Smart KM, Doody CM. Exercise-induced hypoalgesia in people with knee osteoarthritis with normal and abnormal conditioned pain modulation. Clin J Pain 2017;33:395–404.
68. Vaegter HB, Handberg G, Graven-Nielsen T. Hypoalgesia after exercise and the cold pressor test is reduced in chronic musculoskeletal pain patients with high pain sensitivity. Clin J Pain 2016;32:58–69.
69. Dailey DL, Keffala VJ, Sluka KA. Do cognitive and physical fatigue tasks enhance pain, cognitive fatigue, and physical fatigue in people with fibromyalgia? Arthritis Care Res (Hoboken) 2015;67:288–296.
70. Staud R, Robinson ME, Price DD. Isometric exercise has opposite effects on central pain mechanisms in fibromyalgia patients compared to normal controls. Pain 2005;118:176–184.
71. Bote ME, García JJ, Hinchado MD, Ortega E. An exploratory study of the effect of regular aquatic exercise on the function of neutrophils from women with fibromyalgia: Role of IL-8 and noradrenaline. Brain Behav Immun 2014;39:107–112.
72. Pilat C, Frech T, Wagner A, et al. Exploring effects of a natural combination medicine on exercise-induced inflammatory immune response: A double-blind RCT. Scand J Med Sci Sports 2015;25:534–542.
73. Zheng Q, Cui G, Chen J, et al. Regular exercise enhances the immune response against microbial antigens through up-regulation of toll-like receptor signaling pathways. Cell Physiol Biochem 2015;37:735–746.
74. Amaral LSB, Souza CS, Volpini RA, et al. Previous exercise training reduces markers of renal oxidative stress and inflammation in streptozotocin-induced diabetic female rats. J Diabetes Res 2018;2018:6170352.
75. Rezende RM, Gouveia Pelúzio MdCG, de Jesus Silva F, et al. Does aerobic exercise associated with tryptophan supplementation attenuates hyperalgesia and inflammation in female rats with experimental fibromyalgia? PLoS One 2019;14:e0211824.
76. Clark AK, Old EA, Malcangio M. Neuropathic pain and cytokines: Current perspectives. J Pain Res 2013;6:803–814.
77. Noma N, Khan J, Chen IF, et al. Interleukin-17 levels in rat models of nerve damage and neuropathic pain. Neurosci Lett 2011;493:86–91.
78. Khan J, Ramadan K, Korczeniewska O, Anwer MM, Benoliel R, Eliav E. Interleukin-10 levels in rat models of nerve damage and neuropathic pain. Neurosci Lett 2015;592:99–106.
79. Khan J, Wang Q, Ren Y, et al. Exercise induced hypoalgesia profile in rats is associated with IL-10 and IL-1 beta levels and pain severity following nerve injury. Cytokine 2021;143:155540.
80. Khan J, Hassun H, Zusman T, Korczeniewska O, Eliav E. Interleukin-8 levels in rat models of nerve damage and neuropathic pain. Neurosci Lett 2017;657:106–112.
81. Eliav E, Benoliel R, Herzberg U, Kalladka M, Tal M. The role of IL-6 and IL-1beta in painful perineural inflammatory neuritis. Brain Behav Immun 2009;23:474–484.
82. da Silva MD, Bobinski F, Sato KL, Kolker SJ, Sluka KA, Santos AR. IL-10 cytokine released from M2 macrophages is crucial for analgesic and anti-inflammatory effects of acupuncture in a model of inflammatory muscle pain. Mol Neurobiol 2015;51:19–31.
83. Shao Q, Li Y, Wang Q, Zhao J. IL-10 and IL-1beta mediate neuropathic-pain like behavior in the ventrolateral orbital cortex. Neurochem Res 2015;40:733–739.
84. Soderquist RG, Sloane EM, Loram LC, et al. Release of plasmid DNA-encoding IL-10 from PLGA microparticles facilitates long-term reversal of neuropathic pain following a single intrathecal administration. Pharm Res 2010;27:841–854.
85. Koltyn KF. Analgesia following exercise: A review. Sports Med 2000;29:85–98.
86. Welch SP. Interaction of the cannabinoid and opioid systems in the modulation of nociception. Int Rev Psychiatry 2009;21:143–151.
87. Tesarz J, Gerhardt A, Schommer K, Treede RD, Eich W. Alterations in endogenous pain modulation in endurance athletes: An experimental study using quantitative sensory testing and the cold-pressor task. Pain 2013;154:1022–1029.
88. Janal MN, Glusman M, Kuhl JP, Clark CW. Are runners stoical? An examination of pain sensitivity in habitual runners and normally active controls. Pain 1994;58:109–116.
89. McDougall J, Jutzeler CR, Scott A, Crocker PRE, Kramer JLK. Conditioned pain modulation in elite athletes: A systematic review and meta-analysis. Scand J Pain 2020;20:429–438.