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EXERCISE PREVENTS DEVELOPMENT OF AUTONOMIC DYSREGULATION AND HYPERALGESIA IN A MOUSE MODEL OF CHRONIC MUSCLE PAIN

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Abstract

Chronic musculoskeletal pain (CMP) conditions, like fibromyalgia, are associated with widespread pain and alterations in autonomic function. Regular physical activity prevents development of CMP and can reduce autonomic dysfunction. We tested if there were alterations in autonomic function in sedentary mice with CMP, and if exercise reduced the autonomic dysfunction and pain induced by CMP. CMP was induced by two intramuscular injections of pH 5 in combination with a single fatiguing exercise task. A running wheel was placed into cages so that the mouse had free access for either 5 days or 8 weeks (exercise groups) and these animals were compared to sedentary mice without running wheels. Autonomic function and nociceptive withdrawal thresholds of the paw and muscle were assessed before and after induction of CMP in exercised and sedentary mice. In sedentary mice, we show decreased baroreflex sensitivity, increased blood pressure variability, decreased heart rate variability and decreased withdrawal thresholds of the paw and muscle 24h after induction of CMP. There were no sex differences after induction of the CMP in any outcome measure. We further show that both 5 days and 8 weeks of physical activity prevent the development of autonomic dysfunction and decreases in withdrawal threshold induced by CMP. Thus, this study uniquely shows development of autonomic dysfunction in animals with chronic muscle hyperalgesia that can be prevented with as little as 5 days of physical activity, and suggest that physical activity may prevent the development of pain and autonomic dysfunction in people with CMP.

Keywords

autonomic; exercise; pain; muscle; cardiovascular; analgesia; hyperalgesia

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1. Introduction

Chronic musculoskeletal pain (CMP) conditions, like fibromyalgia, affect between 11–24 % of the population [15;38] and result in significant disability [2]. CMP has been associated with autonomic dysfunction in humans, albeit with substantial heterogeneity of dysfunction among subjects [42;61]. Decreases in heart rate variability and baroreflex sensitivity, and abnormal autonomic responses to physiological challenges have been most consistently observed in people with chronic pain [16;19;26;32;54;69;72;73]. Autonomic dysfunction predicts premature death post-myocardial infarction and in heart failure [43], and is associated with cardiovascular and renal complications in diabetes [37]. It is unclear if induction of muscle pain induces autonomic dysfunction, or if these changes parallel the hyperalgesia. Therefore, a better understanding of autonomic regulation in chronic pain is needed.

Repeated muscle insults produce long-lasting and widespread hyperalgesia in mice and rats [22;84;98]. In particular, repeated intramuscular acid injections, or acid injection in combination with muscle fatigue, result in hyperalgesia of the muscle, skin and viscera without overt tissue injury [22;30;84;88;98;99]. The hyperalgesia in these models, once developed, is independent of peripheral input and requires continued activation of central pathways to maintain the hyperalgesia [20;84;93]. Furthermore, these models are sensitive to treatments commonly used in CMP including opioids, dual and serotonin reuptake inhibitors, pregabalin/gabapentin and exercise [22;64;85;98]. Thus, these animal models of chronic muscle pain mimic the signs, symptoms and responsiveness to treatment observed clinically in fibromyalgia, further validating the models.

Regular exercise may reduce cardiovascular risk in part by decreasing sympathetic tone and increasing parasympathetic tone and baroreflex sensitivity [46;52;67]. In addition, regular exercise can prevent and reduce pain and disability in humans with CMP [8;10], and hyperalgesia in animal models of pain [6;78;85;91]. In fact, regular physical activity reduces the risk of both heart disease [4] and development of chronic pain [44;45]. Whilst some studies have shown that exercise can correct autonomic dysfunction in fibromyalgia, others do not show beneficial effects [27;42]. It is possible that different types of exercise, different intensities of exercise, or different durations of exercise underlie these conflicting data. Our previous study shows that 5 days of physical activity prevented development of exercise-induced secondary hyperalgesia but not primary hyperalgesia, and 8 weeks of exercise prevented both primary and secondary hyperalgesia [85]. While it is generally recommended that several weeks of exercise are required to induce training effects [33], this has not been tested in animals with chronic muscle pain. The primary aims of this study were to 1) extensively characterize the autonomic dysfunction induced by CMP, 2) determine if regular physical activity prevents the autonomic dysfunction induced by CMP, and 3) determine if prevention of autonomic dysfunction occurs with a short-duration of physical activity (5 days) or requires a more long-term exercise task (8 weeks). In parallel, we examined the ability of short- and long-duration physical activity to prevent primary and secondary hyperalgesia induced by CMP.

2. Methods

2.1. Mice

All experiments were approved by the University of Iowa Animal Care and Use Committee and were conducted in accordance with National Institutes of Health guidelines. Mice used were on a C57BL/6J background and were bred at the University of Iowa Animal Care Facility or purchased from Jackson Laboratories. Both male (n=40) and female (n=32) mice were used in these studies. Animals were 9–10 weeks old when starting the study.

2.2. Physical activity in running wheels

Running wheels (Columbus Instruments or University of Iowa Animal Care Facility) were placed in home cages and all mice were housed individually. Thus mice had free access to running wheels. Running wheel activity was recorded and converted to average distance per day. Sedentary groups consisted of housing mice individually in cages for the same duration as the running wheel group.

2.3. Induction of fatigue-induced chronic musculoskeletal pain (CMP) model

After exercise training or sedentary living (5 days or 8 weeks), the CMP model was induced by combining 2 intramuscular injections of pH 5.0 sterile saline solution (20µl) with a 2h fatigue task as previously published [99]. To inject acidic saline, mice were briefly anaesthetized with isoflurane (3%), and the left gastrocnemius muscle was injected. Two injections were given 5 days apart. Just prior to the second injection, mice were run in exercise wheels at a self-selected speed. If mice stopped running, the exercise wheel was gently tapped to encourage continued running.

2.4. Measurement of Nociceptive Behaviors

For behavioral assays, mice were separated into 4 groups: sedentary 5 days in cages (n=12; 8 Males, 4 Females), 5 days running wheel (n=12; 8 Males, 4 Females), sedentary 8 weeks in cages (n=6; 3 Males, 3 Females), 8 weeks running wheel (n=8; 4 Males, 4 Females). Nociceptive behavioral tests were performed before the first injection of acidic saline, 5 days after the first injection of acidic saline, and 24 hours after the second injection of acidic saline. Behavioral testing was separated across multiple days, mice were randomly assigned to groups, and sedentary and running wheel groups were evenly distributed. In these experiments the behavioral tester was not blinded to group.

Mice were tested for mechanical withdrawal thresholds of the paw and muscle, both ipsilateral and contralateral to the injection site, as we previously published [85]. Mice were acclimated to behavioral procedures 2x per day for two days prior to beginning the experiment. To acclimate for mechanical withdrawal thresholds of the paw, mice were placed in small clear cubicles on the wire mesh table for 20 minutes. To acclimate for mechanical withdrawal thresholds of the muscle, mice were placed in a glove and their hind limb was gently stroked.

Mechanical withdrawal thresholds of the paw were tested using Von-Frey filaments (0.008–2 g) using the Dixon up down method. Testing was initiated with a 0.4-gm filament in the

middle of the series and withdrawal was determined if a mouse elevated or licked the paw after the filament was pressed against the paw. A decrease in withdrawal threshold (force) was interpreted as cutaneous hyperalgesia.

Muscle withdrawal thresholds were measured with a pair of force-sensitive forceps, built in house, and applied to the belly of the gastrocnemius muscle [87]. Mice were held in a gardener's glove while the hind quarters were pulled into extension. The muscle was squeezed with the forceps until the mouse withdrew the hindlimb. Three trials were conducted and an average was taken. A decrease in withdrawal threshold was interpreted as muscle hyperalgesia.

2.5. Radiotelemetry

For telemetry protocols, separate groups of mice were randomly divided into 4 groups: non-runners 5 days in cages (n=8–10; 3–5 Males, 5 Females), 5 days running wheel (n=8–12; 2–6 Males, 6 Females), non-runners 8 weeks in cages (n=5; 2 Males, 3 Females), 8 weeks running wheel (n=7; 4 Males, 3 Females). All mice underwent the CMP protocol after the wheel-running or sedentary intervention. The intervention of 5 days or 8 weeks was performed and running wheels removed from cages for the duration of the experiment. Telemetry recordings were obtained at 3 time points in every mouse: 1) baseline, control after recovery from implant surgery before the start of the wheel-running or sedentary intervention, 2) 5 days or 8 weeks after initiating the wheel-running or sedentary intervention and before induction of the CMP model, and 3) 24 hours after induction of the CMP model. After the first baseline testing period, the runners were put in cages with running wheels attached and allowed to run (8 weeks or 5 days), while non-runners (sedentary) were put in individual cages without running wheels for the same duration of time. The experimenter was blinded to group.

A telemetry probe (TA11PA-C10, DSI) was inserted into the thoracic aorta *via* the left common carotid artery in mice anaesthetized with ketamine (91 mcg/g, i.p.) and xylazine (9.1 mcg/g, i.p.) at 9–10 weeks of age as described previously [74]. Animals were allowed to recover from the surgery for 7 days before beginning the protocols.

2.6. Assessment of Cardiovascular and Autonomic Indices

For assessment of cardiovascular and autonomic indices, mice were transferred to the telemetry room, placed into a novel cage, and acclimated for an hour before data were collected. Blood pressure and heart rate were recorded continuously at 2000 Hz for 1–2 hours between 10:00 and 13:00 to enable collection of beat-to-beat data for assessment of baroreflex sensitivity, blood pressure variability, heart rate variability, and vasomotor sympathetic tone [74].

Baroreflex sensitivity for control of heart rate was calculated from spontaneous fluctuations in systolic blood pressure and heart rate measured over a 15 minute period when mice were relatively active (based on the locomotor activity signal) using the well-established sequence technique [47;74]. Sequences of four-or-more consecutive blood pressure pulses where systolic pressure and pulse interval (inversely related to heart rate) were positively

correlated ($r^2 > 0.85$) were detected using a customized software (Hemolab). Baroreflex sensitivity was calculated as the average slope of the systolic pressure-pulse interval relationships (ms/mmHg). In addition, blood pressure variability was calculated as the standard deviation (SD) of the systolic blood pressure values measured over the 15 minute period [74].

To estimate resting autonomic tone, heart rate variability was calculated from beat-to-beat measurements of pulse intervals collected over a 5 minute period when mice were relatively inactive using Hemolab and Batch Processor software. The standard deviation (SD) of the recorded pulse intervals and the root mean square of successive differences in pulse intervals (RMSSD) provided measures of overall heart rate variability and rapid, parasympathetic-mediated heart rate variability, respectively [11;74]. Resting sympathetic vasomotor tone was estimated by measuring the decrease in mean arterial pressure induced by injection of the ganglionic blocker chlorisondamine (12 mcg/g, IP, Tocris) when the mice were relatively inactive as described previously [74].

2.7. Data Analysis

The results are expressed as means $\pm$ standard error of the mean (SEM). Statistical evaluation was performed using paired and unpaired t-tests to compare measurements obtained before and after an intervention in the same mice and measurements between two groups of mice, respectively. Repeated measures, 2 factor ANOVA with Fishers PLSD *post-hoc* test was used to determine effects of CMP and wheel-running activity on nociceptive behaviors, and cardiovascular and autonomic indices (StatView SAS Institute, Cary, NC). A secondary analysis examined for sex differences by combining the control sedentary mice from the 5-day and the 8-week running groups (n=8 female, n=5–7 male). This allowed us to perform a repeated measures ANOVA (baseline, post CMP) with an analysis for sex. Significant differences were defined at $P < 0.05$.

3. RESULTS

3.1. Effects of Inducing CMP Model on Cardiovascular, Autonomic, and Nociceptive Measures

As prior clinical studies have shown alterations in heart rate variability and other indices of autonomic nervous system activity in people with chronic pain, we examined if cardiovascular and autonomic measures are altered in our CMP model. We combined the sedentary comparison groups from the 5 day and the 8 week exercise groups to give us a total of 15 mice (n=8 female; n=5–7 male) for analysis of autonomic function. In comparison to baseline, induction of the CMP model significantly decreased baroreflex sensitivity by 30 % from 2.4 ± 0.12 to 1.7 ± 0.06 ms/mmHg, and increased blood pressure variability by 100 % from 3.2 ± 0.23 to 6.5 ± 0.44 mmHg (Fig 1). In addition, sedentary mice with CMP showed decreased heart rate variability measured as both the SD and the RMSSD of pulse intervals (Fig 1). The decrease in RMSSD, a specific measure of parasympathetic-mediated heart rate variability, was particularly pronounced averaging 49 % (10.2 ± 1.3 to 5.2 ± 0.92 ms, Fig 1). Interestingly, there were no changes in mean arterial pressure (117 ± 2.2 vs. 119 ± 1.8 mmHg), mean heart rate (664 ± 8 vs. 665 ± 14 bpm), or resting sympathetic

vasomotor tone measured as the depressor response to ganglionic blockade (-59 ± 2.9 vs. -63 ± 1.6 mmHg) after induction of the CMP model in sedentary mice (Fig 1). Cardiovascular and autonomic measurements were measured twice under control conditions, first under baseline conditions after recovery from the telemetry implant surgery and again after an additional 5 days of sedentary cage-living. There were no significant differences between the two sets of measurements. All changes elicited by induction of the CMP model noted above were also significant when measurements were compared with the second set of control measurements (Table 1). Table 1 provides the mean and SEM for each measure in the sedentary non-runner groups before and after induction of chronic muscle pain.

As we have previously shown [83;99], the combination of exercise-induced fatigue with pH 5.0 intramuscular injections resulted in decreased both paw and muscle withdrawal thresholds, bilaterally, 24h after the second intramuscular acid injection in the group of mice that did not have access to running wheels ($n=18/\text{group}$). Decreases were significant ($P=0.001-0.00001$) when comparing the withdrawal thresholds prior to the first or second injection with the withdrawal thresholds after the second injection (Fig 2).

As a secondary exploratory analysis, we pooled the data from the control sedentary groups from the 5-day and the 8-week running wheel groups and examined for sex differences. For autonomic dysfunction measures ($n=8$ females; $n=5-7$ males), there were significant differences for sex for MAP ($p=0.01$), for sympathetic tone ($p=0.01$), and for baroreceptor sensitivity ($p=0.01$) but not for HR, heart rate variability (SD, RMSSD), or blood pressure variability (Table 2). There was no interaction between time of measurement and sex suggesting that differences in MAP, sympathetic tone and baroreceptor sensitivity occur at both time periods and are unaffected by induction of CMP. For nociceptive measures there were no sex differences in muscle or paw withdrawal thresholds before or after induction of CMP (Table 2).

3.2. Effects of Regular Exercise on Cardiovascular, Autonomic and Nociceptive Measures

Regular physical activity reduces the risk for development of chronic muscle pain in human subjects, prevents the development of chronic muscle pain in mice [44;45;85] and has protective effects on the cardiovascular and autonomic nervous systems [46;52;97]. Therefore we tested if regular physical activity, by allowing free access to running wheels, prevented cardiovascular and autonomic dysfunction in our CMP model. To produce cardiovascular and autonomic adaptations with exercise training, guidelines typically recommend several weeks of regular exercise to induce training effects [33;62]. In contrast, we have shown that a relatively short duration of wheel-running activity of 5 days can prevent hyperalgesia in rodent models of CMP [85], and changes in vasculature can occur within days [92]. Therefore, we examined effects of both 8 weeks and 5 days of voluntary wheel-running activity on, arterial blood pressure, heart rate, autonomic indices, and nociceptive behaviors in the present study. Sex differences were not analyzed due to the small number of mice per sex.

3.2.1. Overall physical activity—Mice in the 5 day running group ran an average of 3.4 ± 0.44 km/day over the 5 day period. Mice in the 8 week running group ran an average of

6.5±0.19 km/day over the 8 week period. The first several days of running in the 8 week group were lower (4.2 km/day, 3.6 km/day, and 4.7 km/day on days 1–3) and this increased to a peak of 7.8 km/day by the 3rd week. Body weights of the mice with running wheels were similar to sedentary mice (Table 1).

3.2.2. 8 weeks of exercise prevents autonomic dysfunction and hyperalgesia in CMP model—Mice subjected to 8 weeks of wheel-running activity exhibited significantly lower mean heart rate and sympathetic vasomotor tone than age-matched sedentary mice prior to induction of the fatigue-induced pain model (Fig 3, Table 1). Mean arterial pressure tended to be lower and baroreflex sensitivity and heart rate variability tended to be higher in runners vs. non-runners, but the differences were not significant (Fig 3, Table 1).

While induction of the CMP model in sedentary mice (non-runners) did not change mean arterial pressure, heart rate, or sympathetic vasomotor tone, it markedly decreased baroreflex sensitivity ($P=0.001$) and heart rate variability (SD and RMSSD of pulse intervals, $P=0.016$); and increased blood pressure variability ($P=0.017$) (Fig 3, Table 1). All of these CMP-induced changes observed in sedentary mice were abolished in the mice previously subjected to 8 weeks of wheel-running activity (Fig 3, Table 1). After induction of the CMP model, the wheel-running mice exhibited significantly lower mean arterial blood pressure (104±2 vs. 117±5 mmHg), blood pressure variability (2.5±0.6 vs. 6.9±1.1 mmHg), mean heart rate (510±6 vs. 645±8 bpm) and sympathetic vasomotor tone (−26±3 vs. −59±4 ? mmHg); and significantly higher baroreflex sensitivity (2.6±0.2 vs. 1.6±0.1 ms/mmHg), and heart rate variability (21.9±2.7 vs. 5.7±2.1 ms for SD of PI; and 15.1±2.7 vs. 2.7±1.2 ms for RMSSD) compared with sedentary mice.

Eight weeks of wheel-running activity prevented the decrease in withdrawal threshold of the paw after induction of the CMP model. The exercise-trained mice exhibited significantly greater withdrawal thresholds when compared with sedentary controls ($P=0.04$ ipsilateral; $P=0.009$ contralateral) (Fig 4). The decreased withdrawal threshold of the muscle was also significantly higher ipsilaterally in the group that performed 8 weeks of wheel-running activity when compared with sedentary mice ($P=0.01$, Fig 4).

3.2.3. 5 days of exercise prevents autonomic dysfunction and hyperalgesia in CMP model—Mice with access to running wheels for 5 days exhibited significantly lower mean arterial pressure, mean heart rate, and sympathetic vasomotor tone; and higher heart rate variability than age-matched sedentary mice prior to induction of the CMP model (Fig 5, Table 1). Induction of the CMP model did not change mean arterial pressure, heart rate, or sympathetic vasomotor tone in sedentary mice (Fig 5, Table 1). In contrast, induction of the CMP model decreased baroreflex sensitivity ($P=0.01$) and heart rate variability (SD and RMSSD, $P=0.002$), and increased blood pressure variability ($P=0.001$) (Fig 5, Table 1). The CMP-induced changes observed in sedentary mice were abolished in the mice previously subjected to 5 days of wheel-running activity (Fig 5, Table 1). After induction of the CMP model, the runners exhibited significantly lower mean arterial blood pressure, blood pressure variability, mean heart rate, and sympathetic vasomotor tone; and significantly higher baroreflex sensitivity and heart rate variability compared with sedentary mice (Fig 5, Table

1). Interestingly, the effects of 5 days of wheel-running on cardiovascular and autonomic measures (Fig 5) were similar to the effects of 8 weeks of wheel-running (Fig 3, Table 1).

As expected, 5 days of wheel-running activity prevented the decrease in withdrawal threshold of the paw in the CMP model with mice showing a significantly greater withdrawal threshold of the paw (ipsilateral $P=0.03$; contralateral $P=0.04$) when compared to sedentary mice (Fig 6). In contrast, 5 days of wheel-running activity had no effect on the decreased withdrawal threshold of the muscle with mice showing similar decreases in muscle withdrawal thresholds when compared to sedentary mice after induction of the CMP model (Fig 6).

4. Discussion

4.1. Autonomic dysfunction in CMP

The current study provided a comprehensive assessment of autonomic function and uniquely shows CMP decreases baroreflex sensitivity, increases blood pressure variability, and decreases heart rate variability without altering HR, MAP, or sympathetic vasomotor tone. The marked decrease in RMSSD indicates decreased heart rate variability reflects loss of parasympathetic modulation. Abnormalities in autonomic regulation occur in people with CMP, with substantial heterogeneity between studies that is attributed to co-morbidities, subgroups with different underlying mechanisms, methodological issues, medication effects, age, or sex [42;61]. Despite the heterogeneity, decreased heart rate variability and baroreflex sensitivity are found consistently in people with CMP [14;16;19;26;32;54;69;72;73]. The current data are consistent with our prior study showing changes in heart rate variability and baroreceptor sensitivity after induction of CMP by repeated acid injections in rats [65], and in people with CMP. We extend our prior study by performing a more comprehensive analysis and show changes in heart rate variability reflect altered parasympathetic, but not sympathetic, activity.

The current study shows no sex differences in autonomic dysfunction after induction of CMP. We further show that there are no sex differences in nociceptive measures after induction of CMP which is consistent with our prior studies using repeated intramuscular acid injections [86], but not with our prior study which induced muscle pain by combining a localized isometric contraction with pH 5.0 injections [30]. These data suggest that different muscle stimuli result in uniquely different manifestations across the sexes. Prior work summarizes the complexities of sex differences in pain and analgesia concluding that the biological mechanisms are complex and could include sex hormones, endogenous opioid function, and genetics [5].

The baroreceptor reflex minimizes blood pressure variability by altering heart rate and vascular resistance in response to blood pressure fluctuations [40]. As a result, low baroreflex sensitivity manifests as decreased heart rate variability and increased blood pressure variability [55] that can cause end-organ damage and increase cardiovascular risk [50;55]. As predicted, the decrease in baroreflex sensitivity in our mouse model of CMP was accompanied by a 2-fold increase in blood pressure variability. Furthermore, activation of arterial baroreceptors and vagal afferents reflexively inhibit nociception in animals and

humans [9;71], and spontaneous baroreflex sensitivity is inversely related to severity of pain in humans [14;24;72;73]. To our knowledge, the possibility that blood pressure variability may contribute to CMP has not been investigated previously.

People with fibromyalgia exhibit increases in the ratio of low frequency (LF) to high frequency (HF) heart rate variability (LF/HF)[16;26;54]. While LF/HF is indicative of cardiac sympathovagal balance, it does not correlate with sympathetic nerve activity to either heart or skeletal muscle [28;39;51]. Prior evidence suggests that decreased parasympathetic-mediated HF heart rate variability is responsible for the increase in LF/HF ratio in people with fibromyalgia [16;26;32;54;72;73]. We further show no difference in sympathetic vasomotor tone in our model of CMP, and prior studies suggest, sympathetic nerve activity contributes to pain [3;53;75]. However, direct measurement of sympathetic nerve activity to skeletal muscle shows conflicting results in people with fibromyalgia - one study shows increased activity [26], and another no difference [25]. Taken together, these results suggest a strong positive correlation between decreased baroreflex sensitivity and heart rate variability and increased pain.

4.2. Effects of exercise on autonomic dysregulation

The current study shows regular physical activity prevents development of autonomic dysfunction induced by CMP, and this effect can be prevented with as little as 5 days of running wheel activity. These studies are the first to show that autonomic dysfunction induced by CMP can be reversed by regular physical activity, and that 5 days of exercise produces an equivalent effect on normalizing autonomic dysfunction as 8 weeks of exercise. Regular exercise produces numerous adaptations that influence autonomic regulation in humans and animals. Exercise training for 4 weeks to 6 months decreases resting heart rate in part by increasing parasympathetic tone [1], and is associated with increased heart rate variability, decreased sympathetic tone and blood pressure, and altered baroreflex sensitivity [1;13;23;48;52;57;66;76]. Responses vary between studies depending on subject age, presence of disease, and assessment methods. Most notably, baroreflex sensitivity for control of heart rate is decreased [13;23;63;89] or unchanged [48;76;79] after exercise training in healthy humans and animals, whereas it is consistently increased by training in pathological states associated with impaired baroreflex sensitivity, such as hypertension and heart failure [46;48;52;57;66;82]. Similarly, we show that neither 5 days nor 8 weeks of wheel running altered baroreflex sensitivity in control mice, but both training regimens restored baroreflex sensitivity and heart rate variability in mice with CMP.

The ability of 5 days of wheel-running activity to alter cardiovascular responses was surprising since guidelines and numerous studies recommend several weeks of exercise to induce training effects [33]. Consistent with the current study, treadmill exercise (5 days/wk) improves baroreflex sensitivity in spontaneously hypertensive rats within 1 week and wheel running begins to decrease heart rate in mice after 5 days [1;57]. However, exercise-induced decreases in blood pressure occur much later between 4 and 8 weeks of training [1;57]. It also is possible that the rapid changes in cardiovascular responses observed after 5 days of wheel running reflect attenuation of stress-induced autonomic responses that occur when moving sedentary mice to a novel cage to obtain measurements.

Another possibility is that ‘postexercise hypotension’ contributes to our results as a single bout of aerobic exercise produces long-lasting decreases in sympathetic activity and blood pressure and increases in baroreflex sensitivity after exercise is terminated [12;17;31;90].

4.3. Effects of exercise on hyperalgesia

The current study shows that prior running wheel activity can prevent the onset of muscle and paw hyperalgesia induced by muscle insult, and is similar to our prior studies using free access to running wheels [85], and are consistent with clinical studies showing that physical activity reduces the risk for development of chronic musculoskeletal pain [44;45]. We further show that 5 days of running wheel activity prevented the hyperalgesia of the paw but not the muscle in our CMP model induced by combining fatigue with intramuscular acid, and is similar to our prior study in CMP models [85]. On the other hand, 8 weeks of exercise prevents both muscle and paw hyperalgesia in several models of CMP [85]. It is likely that once developed the hyperalgesia in the CMP models becomes independent of nociceptive input and has a strong central component [22]. Indeed, prior studies show that our CMP models increase central neuronal activity, including enhanced phosphorylation of the NMDA receptor, in brainstem neurons in the rostral ventromedial medulla (RVM) [83;87], and that regular physical activity prevents this increased activity [85]. Although long-term effects of exercise were not examined in the current study, we previously show the effects of exercise are temporary with hyperalgesia returning 1–2 week after stopping exercise [85]. Analgesia produced by regular exercise is reversed by blockade of opioid receptors with naloxone systemically in animal models of muscle pain and nerve injury [6;77;91], there is release of endogenous opioids in central inhibitory pathways including the RVM [91], and blockade of central but not peripheral opioid receptors reverses the analgesia [91]. Thus, these studies suggest that exercise produces analgesia by activation of central inhibitory pathways that reduce increased excitability of neurons in the RVM.

4.4. Potential overlapping mechanisms between autonomic dysfunction and pain

Extensive anatomical and functional interactions exist between central cardiovascular and pain regulatory systems [7;18;29;56;68;94]. The mechanisms underlying the changes in autonomic function induced by CMP, and the normalization of those by regular exercise remain to be determined. Sustained periods of physical exercise induce neuroplasticity in nervous system circuits regulating efferent parasympathetic and sympathetic activity, sometimes rapidly [12;41;59;60;81]. Many of the neurotransmitter pathways affecting autonomic regulation also inhibit pain, including activation of central α -2-adrenergic receptors, release of endogenous opiates, and modulation of NMDA receptors [6;80;83].

Anatomical and functional characteristics of the RVM strongly suggest that it could play a role in the integration of nociceptive and autonomic responses. The RVM receives inputs from the periaqueductal grey (PAG) which is known to coordinate autonomic and nociceptive stimuli [34;49] and projects to the spinal cord where it targets autonomic and nociceptive neurons [35;36]. In CMP models, the PAG and RVM are both involved in facilitation of nociceptive responses as evidenced by increases in glutamate release, increases in phosphorylation of NMDA receptors (NR1 subunit), and reversal of hyperalgesia after blockade of NMDA receptors in the RVM [20–22;70;85;93]. For

autonomic function, microinjection of glutamate or NMDA agonists into the RVLM or RVM increases blood pressure, and autonomic responses induced by stimulation of RVLM are blocked by anesthetic in the RVM [18;94;95]. Furthermore, exercise produces analgesia through central release of endogenous opioids in the PAG and RVM [58;91], and blockade of opioids in the RVM alters autonomic responses associated with opioid withdrawal [96]. Together these data suggest an interaction between motor and cardiovascular systems occurs within brainstem nuclei particularly the RVM and PAG.

4.5.

In *summary*, induction of CMP alters the cardiovascular system in sedentary mice, but not those that are physically active. These data suggest that physical activity may prevent development of pain and autonomic dysfunction observed in people with CMP. It further suggests an overlap between nociceptive and autonomic pathways underlies these co-morbid conditions.

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References

1. Adlam D, De Bono JP, Danson EJ, Zhang MH, Casadei B, Paterson DJ, Channon KM. Telemetric analysis of haemodynamic regulation during voluntary exercise training in mouse models. *Exp Physiol*. 2011; 96:1118–1128. [PubMed: 21824998]
2. Arnold LM, Crofford LJ, Mease PJ, Burgess SM, Palmer SC, Abetz L, Martin SA. Patient perspectives on the impact of fibromyalgia. *Patient Educ Couns*. 2008; 73:114–120. [PubMed: 18640807]
3. Backman E, Bengtsson A, Bengtsson M, Lennmarken C, Henriksson KG. Skeletal muscle function in primary fibromyalgia Effect of regional sympathetic blockade with guanethidine. *Acta Neurol Scand*. 1988; 77:187–191. [PubMed: 3376744]
4. Barlow PA, Otahal P, Schultz MG, Shing CM, Sharman JE. Low exercise blood pressure and risk of cardiovascular events and all-cause mortality: systematic review and meta-analysis. *Atherosclerosis*. 2014; 237:13–22. [PubMed: 25190307]
5. Bartley EJ, Fillingim RB. Sex differences in pain: a brief review of clinical and experimental findings. *Br J Anaesth*. 2013; 111:52–58. [PubMed: 23794645]
6. 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. [PubMed: 16181935]
7. Benarroch EE. Pain-autonomic interactions. *Neurol Sci*. 2006; 27(Suppl 2):130–S133.
8. Bidonde J, Busch AJ, Webber SC, Schachter CL, Danyliw A, Overend TJ, Richards RS, Rader T. Aquatic exercise training for fibromyalgia. *Cochrane Database Syst Rev*. 2014; 10:011336.
9. Bruhl S, Chung OY. Interactions between the cardiovascular and pain regulatory systems: an updated review of mechanisms and possible alterations in chronic pain. *Neurosci Biobehav Rev*. 2004; 28:395–414. [PubMed: 15341037]
10. Busch AJ, Webber SC, Brachanec M, Bidonde J, Bello-Haas VD, Danyliw AD, Overend TJ, Richards RS, Sawant A, Schachter CL. Exercise therapy for fibromyalgia. *Curr Pain Headache Rep*. 2011; 15:358–367. [PubMed: 21725900]
11. Chapleau MW, Sabharwal R. Methods of assessing vagus nerve activity and reflexes. *Heart Fail Rev*. 2011; 16:109–127. [PubMed: 20577901]
12. Chen CY, Bonham AC. Postexercise hypotension: central mechanisms. *Exerc Sport Sci Rev*. 2010; 38:122–127. [PubMed: 20577060]

13. Chen CY, Dicarlo SE, Scislo TJ. Daily spontaneous running attenuated the central gain of the arterial baroreflex. *Am J Physiol.* 1995; 268:H662–H669. [PubMed: 7864192]
14. Chung OY, Bruehl S, Diedrich L, Diedrich A, Chont M, Robertson D. Baroreflex sensitivity associated hypoalgesia in healthy states is altered by chronic pain. *Pain.* 2008; 138:87–97. [PubMed: 18164819]
15. Clauw DJ, Crofford LJ. Chronic widespread pain and fibromyalgia: what we know, and what we need to know. *Best Practice & Research in Clinical Rheumatology.* 2003; 17:685–701.
16. Cohen H, Neumann L, Alhosshe A, Kotler M, Abu-Shakra M, Buskila D. Abnormal sympathovagal balance in men with fibromyalgia. *J Rheumatol.* 2001; 28:581–589. [PubMed: 11296963]
17. Convertino VA, Adams WC. Enhanced vagal baroreflex response during 24 h after acute exercise. *Am J Physiol.* 1991; 260:R570–R575. [PubMed: 2001006]
18. Cox BF, Brody MJ. Evidence for two functionally distinct vasomotor subregions of rostral ventral medulla. *Clin Exp Hypertens A.* 1988; 10(Suppl 1):11–18. [PubMed: 3242985]
19. da Cunha Ribeiro RP, Roschel H, Artioli GG, Dassouki T, Perandini LA, Calich AL, de Sa Pinto AL, Lima FR, Bonfa E, Gualano B. Cardiac autonomic impairment and chronotropic incompetence in fibromyalgia. *Arthritis Res Ther.* 2011; 13:190.
20. da Silva LFS, DeSantana JM, Sluka KA. Activation of NMDA receptors in the brainstem, rostral ventromedial medulla, and nucleus reticularis gigantocellularis mediates mechanical hyperalgesia produced by repeated intramuscular injections of acidic saline in rats. *Pain.* 2010; 111:378–387.
21. da Silva LFS, Walder RY, Davidson BL, Wilson SP, Sluka KA. Changes in expression of NMDA-NR1 receptor subunits in the rostral ventromedial medulla modulates pain behaviors. *Pain.* 2010; 151:155–161. [PubMed: 20688433]
22. DeSantana JM, Sluka KA. Central mechanisms in the maintenance of chronic widespread noninflammatory muscle pain. *Curr Pain Headache Rep.* 2008; 12:338–343. [PubMed: 18765138]
23. Dicarlo SE, Bishop VS. Exercise training attenuates baroreflex regulation of nerve activity in rabbits. *Am J Physiol.* 1988; 255:H974–H979. [PubMed: 3177687]
24. Duschek S, Muck I, Reyes Del Paso GA. Relationship between baroreceptor cardiac reflex sensitivity and pain experience in normotensive individuals. *Int J Psychophysiol.* 2007; 65:193–200. [PubMed: 17553582]
25. Elam M, Johansson G, Wallin BG. Do patients with primary fibromyalgia have an altered muscle sympathetic nerve activity? *Pain.* 1992; 48:371–375. [PubMed: 1594259]
26. Furlan R, Colombo S, Perego F, Atzeni F, Diana A, Barbic F, Porta A, Pace F, Malliani A, Sarzi-Puttini P. Abnormalities of cardiovascular neural control and reduced orthostatic tolerance in patients with primary fibromyalgia. *J Rheumatol.* 2005; 32:1787–1793. [PubMed: 16142879]
27. Gavi MB, Vassalo DV, Amaral FT, Macedo DC, Gava PL, Dantas EM, Valim V. Strengthening exercises improve symptoms and quality of life but do not change autonomic modulation in fibromyalgia: a randomized clinical trial. *PLoS One.* 2014; 9:90767.
28. Goldstein DS, Benth O, Park MY, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp Physiol.* 2011; 96:1255–1261. [PubMed: 21890520]
29. Green AL, Wang S, Owen SL, Xie K, Bittar RG, Stein JF, Paterson DJ, Aziz TZ. Stimulating the human midbrain to reveal the link between pain and blood pressure. *Pain.* 2006; 124:349–359. [PubMed: 16781077]
30. Gregory NS, Gibson-Corley K, Frey-Law L, Sluka KA. Fatigue-enhanced hyperalgesia in response to muscle insult: Induction and development occur in a sex-dependent manner. *Pain.* 2013; 154:2668–2676. [PubMed: 23906552]
31. Halliwill JR, Buck TM, Lacewell AN, Romero SA. Postexercise hypotension and sustained postexercise vasodilatation: what happens after we exercise? *Exp Physiol.* 2013; 98:7–18. [PubMed: 22872658]
32. Hallman DM, Lyskov E. Autonomic regulation, physical activity and perceived stress in subjects with musculoskeletal pain: 24-hour ambulatory monitoring. *Int J Psychophysiol.* 2012; 86:276–282. [PubMed: 23075754]

33. Heran BS, Chen JM, Ebrahim S, Moxham T, Oldridge N, Rees K, Thompson DR, Taylor RS. Exercise-based cardiac rehabilitation for coronary heart disease. *Cochrane Database Syst Rev*. 2011:001800.
34. Hermann DM, Luppi PH, Peyron C, Hinckel P, Jouvett M. Afferent projections to the rat nuclei raphe magnus, raphe pallidus and reticularis gigantocellularis pars alpha demonstrated by iontophoretic application of cholera toxin (subunit b). *J Chem Neuroanat*. 1997; 13:1–21. [PubMed: 9271192]
35. Holstege G, Kuypers HGJM. The anatomy of brainstem pathways to the spinal cord in cat A labeled amino acid tracing study. *Prog Brain Res*. 1982; 57:45–75.
36. Holstege JC, Kuypers HG. Brainstem projections to spinal motoneurons: an update. *Neurosci*. 1987; 23:809–821.
37. Iyngkaran P, Anavekar N, Majoni W, Thomas MC. The role and management of sympathetic overactivity in cardiovascular and renal complications of diabetes. *Diabetes Metab*. 2013; 39:290–298. [PubMed: 23871308]
38. Jones GT, Atzeni F, Beasley M, Fluss E, Sarzi-Puttini P, Macfarlane GJ. The prevalence of fibromyalgia in the general population: a comparison of the american college of rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis Rheumatol*. 2015; 67:568–575. [PubMed: 25323744]
39. Kingwell BA, Thompson JM, Kaye DM, McPherson GA, Jennings GL, Esler MD. Heart rate spectral analysis, cardiac norepinephrine spillover, and muscle sympathetic nerve activity during human sympathetic nervous activation and failure. *Circulation*. 1994; 90:234–240. [PubMed: 8026003]
40. Kirchheim HR. Systemic arterial baroreceptor reflexes. *Physiol Rev*. 1976; 56:100–177. [PubMed: 174143]
41. Kleiber AC, Zheng H, Schultz HD, Peuler JD, Patel KP. Exercise training normalizes enhanced glutamate-mediated sympathetic activation from the PVN in heart failure. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294:R1863–R1872. [PubMed: 18385465]
42. Kulshreshtha P, Deepak KK. Autonomic nervous system profile in fibromyalgia patients and its modulation by exercise: a mini review. *Clin Physiol Funct Imaging*. 2013; 33:83–91. [PubMed: 23383685]
43. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity-heart-rate variability in prediction of total cardiac mortality after myocardial infarction ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet*. 1998; 351:478–484. [PubMed: 9482439]
44. Landmark T, Romundstad P, Borchgrevink PC, Kaasa S, Dale O. Associations between recreational exercise and chronic pain in the general population: evidence from the HUNT 3 study. *Pain*. 2011; 152:2241–2247. [PubMed: 21601986]
45. Landmark T, Romundstad PR, Borchgrevink PC, Kaasa S, Dale O. Longitudinal associations between exercise and pain in the general population--the HUNT pain study. *PLoS One*. 2013; 8:65279.
46. Laterza MC, de Matos LD, Trombetta IC, Braga AM, Roveda F, Alves MJ, Krieger EM, Negrao CE, Rondon MU. Exercise training restores baroreflex sensitivity in never-treated hypertensive patients. *Hypertension*. 2007; 49:1298–1306. [PubMed: 17438307]
47. Laude D, Baudrie V, Elghozi JL. Applicability of recent methods used to estimate spontaneous baroreflex sensitivity to resting mice. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294:R142–R150. [PubMed: 17989145]
48. Liu JL, Kulakofsky J, Zucker IH. Exercise training enhances baroreflex control of heart rate by a vagal mechanism in rabbits with heart failure. *J Appl Physiol* (1985). 2002; 92:2403–2408. [PubMed: 12015354]
49. Loyd DR, Murphy AZ. The role of the periaqueductal gray in the modulation of pain in males and females: are the anatomy and physiology really that different? *Neural Plast*. 2009; 2009:462879. [PubMed: 19197373]
50. Mancia G, Frattola A, Parati G, Santucci C, Ulian L. Blood pressure variability and organ damage. *J Cardiovasc Pharmacol*. 1994; 24(Suppl A):6–11.

51. Martelli D, Silvani A, McAllen RM, May CN, Ramchandra R. The low frequency power of heart rate variability is neither a measure of cardiac sympathetic tone nor of baroreflex sensitivity. *Am J Physiol Heart Circ Physiol*. 2014; 307:H1005–H1012. [PubMed: 25063795]
52. Martinez DG, Nicolau JC, Lage RL, Toschi-Dias E, de Matos LD, Alves MJ, Trombetta IC, Dias da Silva VJ, Middlekauff HR, Negrao CE, Rondon MU. Effects of long-term exercise training on autonomic control in myocardial infarction patients. *Hypertension*. 2011; 58:1049–1056. [PubMed: 22025377]
53. Martinez-Lavin M. Fibromyalgia as a sympathetically maintained pain syndrome. *Curr Pain Headache Rep*. 2004; 8:385–389. [PubMed: 15361323]
54. Martinez-Lavin M, Hermosillo AG, Rosas M, Soto ME. Circadian studies of autonomic nervous balance in patients with fibromyalgia: a heart rate variability analysis. *Arthritis Rheum*. 1998; 41:1966–1971. [PubMed: 9811051]
55. Martinka P, Fielitz J, Patzak A, Regitz-Zagrosek V, Persson PB, Stauss HM. Mechanisms of blood pressure variability-induced cardiac hypertrophy and dysfunction in mice with impaired baroreflex. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288:R767–R776. [PubMed: 15563577]
56. Mason P. Deconstructing endogenous pain modulations. *J Neurophysiol*. 2005; 94:1659–1663. [PubMed: 16105951]
57. Masson GS, Costa TS, Yshii L, Fernandes DC, Soares PP, Laurindo FR, Scavone C, Michelini LC. Time-dependent effects of training on cardiovascular control in spontaneously hypertensive rats: role for brain oxidative stress and inflammation and baroreflex sensitivity. *PLoS One*. 2014; 9:94927.
58. Mathes WF, Kanarek RB. Chronic running wheel activity attenuates the antinociceptive actions of morphine and morphine-6-glucuronide administration into the periaqueductal gray in rats. *Pharmacol Biochem Behav*. 2006; 83:578–584. [PubMed: 16712909]
59. Michelini LC, Stern JE. Exercise-induced neuronal plasticity in central autonomic networks: role in cardiovascular control. *Exp Physiol*. 2009; 94:947–960. [PubMed: 19617267]
60. Mueller PJ. Physical (in)activity-dependent alterations at the rostral ventrolateral medulla: influence on sympathetic nervous system regulation. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298:R1468–R1474. [PubMed: 20357021]
61. Naschitz JE, Rozenbaum M, Fields MC, Enis S, Manor H, Dreyfuss D, Peck S, Peck ER, Babich JP, Mintz EP, Sabo E, Slobodin G, Rosner I. Cardiovascular reactivity in fibromyalgia: evidence for pathogenic heterogeneity. *J Rheumatol*. 2005; 32:335–339. [PubMed: 15693096]
62. National Guideline Clearinghouse N. Cardiac rehabilitation. A national clinical guideline. Agency for Healthcare Research and Quality (AHRQ);
63. Negrao CE, Moreira ED, Brum PC, Denadai ML, Krieger EM. Vagal and sympathetic control of heart rate during exercise by sedentary and exercise-trained rats. *Braz J Med Biol Res*. 1992; 25:1045–1052. [PubMed: 1342828]
64. Nielsen AN, Mathiesen C, Blackburn-Munro G. Pharmacological characterisation of acid-induced muscle allodynia in rats. *Eur J Pharmacol*. 2004; 487:93–103. [PubMed: 15033380]
65. Oliveira LR, de Melo VU, Macedo FN, Barreto AS, Badaue-Passos D Jr, Viana dos Santos MR, Dias DP, Sluka KA, DeSantana JM, Santana-Filho VJ. Induction of chronic non-inflammatory widespread pain increases cardiac sympathetic modulation in rats. *Auton Neurosci*. 2012; 167:45–49. [PubMed: 22266357]
66. Pagani M, Somers V, Furlan R, Dell’Orto S, Conway J, Baselli G, Cerutti S, Sleight P, Malliani A. Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension*. 1988; 12:600–610. [PubMed: 3203964]
67. Patel KP, Zheng H. Central neural control of sympathetic nerve activity in heart failure following exercise training. *Am J Physiol Heart Circ Physiol*. 2012; 302:H527–H537. [PubMed: 22101524]
68. Pereira EA, Lu G, Wang S, Schweder PM, Hyam JA, Stein JF, Paterson DJ, Aziz TZ, Green AL. Ventral periaqueductal grey stimulation alters heart rate variability in humans with chronic pain. *Exp Neurol*. 2010; 223:574–581. [PubMed: 20178783]

69. Qiao ZG, Vaeroy H, Morkrid L. Electrodermal and microcirculatory activity in patients with fibromyalgia during baseline, acoustic stimulation and cold pressor tests. *J Rheumatol.* 1991; 18:1383–1389. [PubMed: 1757941]
70. Radhakrishnan R, Sluka KA. Increased glutamate and decreased glycine release in the rostral ventromedial medulla during induction of a pre-clinical model of chronic widespread muscle pain. *Neurosci Lett.* 2009; 457:141–145. [PubMed: 19429181]
71. Randich A, Maixner W. Interactions between cardiovascular and pain regulatory systems. *Neurosci Biobehav Rev.* 1984; 8:343–367. [PubMed: 6095151]
72. Reyes Del Paso GA, Garrido S, Pulgar A, Duschek S. Autonomic cardiovascular control and responses to experimental pain stimulation in fibromyalgia syndrome. *J Psychosom Res.* 2011; 70:125–134. [PubMed: 21262414]
73. Reyes Del Paso GA, Garrido S, Pulgar A, Martin-Vazquez M, Duschek S. Aberrances in autonomic cardiovascular regulation in fibromyalgia syndrome and their relevance for clinical pain reports. *Psychosom Med.* 2010; 72:462–470. [PubMed: 20467004]
74. Sabharwal R, Zhang Z, Lu Y, Abboud FM, Russo AF, Chapleau MW. Receptor activity-modifying protein 1 increases baroreflex sensitivity and attenuates Angiotensin-induced hypertension. *Hypertension.* 2010; 55:627–635. [PubMed: 20100989]
75. Schlereth T, Birklein F. The sympathetic nervous system and pain. *Neuromolecular Med.* 2008; 10:141–147. [PubMed: 17990126]
76. Seals DR, Chase PB. Influence of physical training on heart rate variability and baroreflex circulatory control. *J Appl Physiol* (1985). 1989; 66:1886–1895. [PubMed: 2732182]
77. Shankarappa SA, Piedras-Renteria ES, Stubbs EB Jr. Forced-exercise delays neuropathic pain in experimental diabetes: effects on voltage-activated calcium channels. *J Neurochem.* 2011; 118:224–236. [PubMed: 21554321]
78. Sharma NK, Ryals JM, Gajewski BJ, Wright DE. Aerobic exercise alters analgesia and neurotrophin-3 synthesis in an animal model of chronic widespread pain. *Phys Ther.* 2010; 90:714–725. [PubMed: 20338916]
79. Sheldahl LM, Ebert TJ, Cox B, Tristani FE. Effect of aerobic training on baroreflex regulation of cardiac and sympathetic function. *J Appl Physiol* (1985). 1994; 76:158–165. [PubMed: 8175501]
80. Shyu BC, Andersson SA, Thoren P. Endorphin mediated increase in pain threshold induced by long-lasting exercise in rats. *Life Sci.* 1982; 30:833–840. [PubMed: 7070198]
81. Shyu BC, Thoren P. Circulatory events following spontaneous muscle exercise in normotensive and hypertensive rats. *Acta Physiol Scand.* 1986; 128:515–524. [PubMed: 3028042]
82. Silva GJ, Brum PC, Negrao CE, Krieger EM. Acute and chronic effects of exercise on baroreflexes in spontaneously hypertensive rats. *Hypertension.* 1997; 30:714–719. [PubMed: 9323011]
83. Sluka KA, Danielson J, Rasmussen L, Dasilva LF. Exercise-Induced Pain Requires NMDA Receptor Activation in the Medullary Raphe Nuclei. *Med Sci Sports Exerc.* 2012; 44:420–427. [PubMed: 21795998]
84. Sluka KA, Kalra A, Moore SA. Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. *Muscle & Nerve.* 2001; 24:37–46. [PubMed: 11150964]
85. 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.* 2013; 114:725–733. [PubMed: 23271699]
86. Sluka, KA.; Price, MP.; Wemmie, JA.; Welsh, MJ. ASIC3, but not ASIC1, channels are involved in the development of chronic muscle pain. In: Dostrovsky, JO.; Carr, DB.; Koltzenburg, M., editors. *Proceedings of the 10th World Congress on Pain.* Seattle: IASP Press; 2003. p. 71-79.
87. Sluka KA, Radhakrishnan R, Benson CJ, Eshcol JO, Price MP, Babinski K, Audette KM, Yeomans DC, Wilson SP. ASIC3 in muscle mediates mechanical, but not heat, hyperalgesia associated with muscle inflammation. *Pain.* 2007; 129:102–112. [PubMed: 17134831]
88. Sluka KA, Rasmussen LA. Fatiguing exercise enhances hyperalgesia to muscle inflammation. *Pain.* 2010; 148:188–197. [PubMed: 19632780]
89. Smith ML, Graitzer HM, Hudson DL, Raven PB. Baroreflex function in endurance- and static exercise-trained men. *J Appl Physiol* (1985). 1988; 64:585–591. [PubMed: 3372416]

90. Somers VK, Conway J, LeWinter M, Sleight P. The role of baroreflex sensitivity in post-exercise hypotension. *J Hypertens Suppl.* 1985; 3:S129–S130. [PubMed: 2856688]
91. Stagg NJ, Mata HP, Ibrahim MM, Henriksen EJ, Porreca F, Vanderah TW, Philip MT Jr. Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain model: role of endogenous opioids. *Anesthesiol.* 2011; 114:940–948.
92. Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT, Green DJ. Impact of inactivity and exercise on the vasculature in humans. *Eur J Appl Physiol.* 2010; 108:845–875. [PubMed: 19943061]
93. Tillu DV, Gebhart GF, Sluka KA. Descending facilitatory pathways from the RVM initiate and maintain bilateral hyperalgesia after muscle insult. *Pain.* 2008; 136:331–339. [PubMed: 17764841]
94. Varner KJ, Grosskreutz CL, Cox BF, Brody MJ. Differential regulation of sympathetic nerve activity by lateral and medial subregions of the rostral ventral medulla. *Prog Brain Res.* 1989; 81:99–103. [PubMed: 2616791]
95. Varner KJ, Vasquez EC, Brody MJ. Lesions in rostral ventromedial or rostral ventrolateral medulla block neurogenic hypertension. *Hypertension.* 1994; 24:91–96. [PubMed: 8021014]
96. Vera-Portocarrero LP, Ossipov MH, Lai J, King T, Porreca F. Descending facilitatory pathways from the rostroventromedial medulla mediate naloxone-precipitated withdrawal in morphine-dependent rats. *J Pain.* 2011; 12:667–676. [PubMed: 21354865]
97. Voulgari C, Pagoni S, Vinik A, Poirier P. Exercise improves cardiac autonomic function in obesity and diabetes. *Metabolism.* 2013; 62:609–621. [PubMed: 23084034]
98. Yokoyama T, Audette KM, Sluka KA. Pregabalin reduces muscle and cutaneous hyperalgesia in two models of chronic muscle pain in rats. *J Pain.* 2007; 8:422–429. [PubMed: 17293165]
99. Yokoyama T, Lisi TL, Moore SA, Sluka KA. Muscle Fatigue Increases the Probability of Developing Hyperalgesia in Mice. *J Pain.* 2007; 8:692–699. [PubMed: 17627895]

Figure 1. Effects of inducing the chronic musculoskeletal pain (CMP) model on cardiovascular and autonomic indices in sedentary mice

Measurements were obtained under baseline control conditions (black bars) and 10 days later, after induction of the CMP model (gray bars) in the same mice (n = 13–15). Sedentary animals from the 5-day group and from the 8-week group were combined. Induction of CMP decreased baroreflex sensitivity and heart rate (HR) variability, and increased systolic blood pressure (BP) variability, without altering mean BP, mean HR or sympathetic vasomotor tone. *P < 0.05, CMP vs. control period, paired t-test. Data are means ± SEM. SD of PI, standard deviation of pulse intervals, a general measure of HR variability. RMSSD, root mean square of successive differences in pulse intervals, a measure of parasympathetic-mediated HR variability.

Figure 2. Effects of fatigue combined with acid injections on paw and muscle withdrawal threshold

Bar graphs representing the paw withdrawal threshold and the muscle withdrawal threshold before injection 1, before injection 2 and 24h after the second acid injection for all animals (n=18) for the ipsilateral (black bars) and the contralateral (gray bars). A significant decrease in withdrawal threshold of the paw and muscle occurred bilaterally in both groups, *, $p < 0.001$. Data are the means $\pm$ SEM.

Figure 3. Effects of 8 weeks of wheel running on cardiovascular and autonomic indices during the control period and after induction of the CMP model

Measurements were obtained after either 8 weeks of 'sedentary' living (Non-Runners, black bars, n = 5) or 8 weeks of wheel-running activity (Runners, gray bars, n = 7). Mice subjected to 8 weeks of wheel running exhibited lower mean heart rate (HR) and lower sympathetic vasomotor tone during the control period than sedentary mice. Baroreflex sensitivity, blood pressure (BP) variability, and HR variability did not differ between the groups. Mean BP, mean HR, and sympathetic vasomotor tone measured after induction of CMP were lower in mice previously subjected to wheel running than in sedentary mice. Furthermore, the prior

period of wheel running prevented CMP-induced decreases in baroreflex sensitivity and HR variability, and the CMP-induced increase in BP variability. * $P < 0.05$, Runners vs. Non-Runners; † $P < 0.05$, CMP vs. control period.

Figure 4. Effects of 8 weeks of exercise training on paw and muscle withdrawal thresholds in the fatigue-induced pain model

Line graphs showing the mechanical withdrawal threshold of the paw before the first injection, before the second injection on day 5 (D5) and 24h after the second acid injection (D6) in the group with access to running wheels for 8 weeks (solid circles) when compared to the sedentary group (open circles). The withdrawal thresholds were greater in the running wheel group compared to the non-running group 24h after the second acid injection (*, $p < 0.05$). Data are the means $\pm$ SEM.

Figure 5. Effects of 5 days of wheel running on cardiovascular and autonomic indices during the control period and after induction of the CMP model

Measurements were obtained after either 5 days of 'sedentary' living (Non-Runners, black bars, $n = 8-10$) or 5 days of wheel-running activity (Runners, gray bars, $n = 8-12$). Mice subjected to 5 days of wheel running exhibited lower mean blood pressure (BP) and heart rate (HR) and lower sympathetic vasomotor tone during the control period than sedentary mice. Baroreflex sensitivity and BP variability did not differ between the groups, whereas HR variability (SD of PI) was increased in the runners. Mean BP, mean HR, and sympathetic vasomotor tone measured after induction of CMP were lower in mice

previously subjected to wheel running than in sedentary mice. Furthermore, the prior period of wheel running prevented CMP-induced decreases in baroreflex sensitivity and HR variability, and the CMP-induced increase in BP variability. * $P < 0.05$, Runners vs. Non-Runners; † $P < 0.05$, CMP vs. control.

Figure 6. Effects of 5 days of exercise training on paw and muscle withdrawal thresholds in the fatigue-induced pain model

Line graphs showing the mechanical withdrawal threshold of the paw before the first injection, before the second injection on day 5 (D5) and 24h after the second acid injection (D6) in the group with access to running wheels for 5 days (solid circles) when compared to the sedentary group (open circles). The withdrawal thresholds were greater in the running wheel group compared to the non-running group 24h after the second acid injection (*, $p < 0.05$). Data are the means $\pm$ SEM.

Table 1

Cardiovascular and autonomic indices in sedentary (Non-Runners) and exercised (runners) mice before (Control) and after induction of chronic musculoskeletal pain (CMP). Corresponding values from 8 weeks and 5 day protocols are shown in the table.

	Non-Runners		Runners	
	Control	CMP	Control	CMP
Body Weight (g)				
8 weeks	20±4	20±3	22±4	21±2
5 days	21±3	20±3	20±3	20±1
Mean BP (mmHg)				
8 weeks	112±3	117±5	105±2	104±2*
5 days	121±2	119±1	108±1*	105±2*
BP Variability (mmHg)				
8 weeks	3.2±0.6	6.9±1.1 [†]	2.2±0.3	2.5±0.6*
5 days	3.5±0.7	6.3±0.9 [†]	3.2±0.4	3.0±0.7*
Baroreflex sensitivity (ms/mmHg)				
8 weeks	2.5±0.2	1.6±0.1 [†]	2.9±0.2	2.6±0.2*
5 days	2.3±0.2	1.7±0.1 [†]	2.6±0.1	2.4±0.1*
Sympathetic Tone (AmmHg)				
8 weeks	-53±4	-59±4	-35±4*	-26±3 [†] *
5 days	-63±1	-66±2	-37±3*	-34±3*
Mean HR (bpm)				
8 weeks	655±15	645±8	527±8*	510±6*
5 days	680±13	664±8	535±14*	505±7*
HR Variability-SD of PI (ms)				
8 weeks	18.4±4.0	5.7±2.1 [†]	24.1±3.9	21.9±2.7*
5 days	13.8±1.2	9.0±1.0 [†]	18.8±1.6	16.4±1.1*
HR Variability-RMSSD (ms)				
8 weeks	11.1±2.5	2.7±1.2 [†]	15.8±3.3	15.1±2.7*
5 days	9.8±1.2	4.7±0.7 [†]	12.7±1.5	11.6±1.6

BP: blood pressure; HR: heart rate; SD: overall HR variability; RMSSD: parasympathetic-mediated HR variability. Data are means ± SEM.

* Runners vs. Non-Runners, P<0.05

[†] CMP vs. Control, P<0.05

Table 2
Sex Differences for Autonomic and Nociceptive Measures at Baseline and After CMP.

Measure	Baseline		CMP	
	Male	Female	Male	Female
HR (bpm) (n=8 female, n=7 male)	660±12	653±20	649±8	666±9
MAP (mmHg) (n=8 female, n=7 male) *, p=0.01	122±2.8	112±2.6	121±1.5	116±2.6
SympTone (mmHg) (n=8 female, n=7 male) *, p=0.01	-65±2.4	-53±2.6	-66±2.4	-61±2.4
BRS (ms/mmHg) (n=8 female, n=7 male) *, p=0.02	2.1±0.4	2.6±.17	1.6±.10	1.7±.07
SDNN (ms) (n=8 female, n=5 male)	13.1±1.8	17.9±2.7	6.0±1.7	8.8±1.1
RMSSD (ms) (n=8 female, n=5 male)	8.3±1.1	11.5±1.7	2.7±3.9	4.7±.69
Blood Pressure Variability (n=8 female, n=5 male)	2.7±.46	3.6±.20	6.9±.59	6.2±.62
Muscle withdrawal threshold (mN) (n=7 female, n=11 male)	1724±26	1566±77	1011±52	1027±88
	1758±24	1540±68	1188±69	1288±115
Paw withdrawal threshold (g) (n=7 female, n=11 male)	.35±.06	.14±.03	.037±.007	.031±.011
	.45±.10	.33±.08	.14±.08	.039±.013

* significant effect for sex (repeated measures ANOVA), p<0.05