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Acute Vibration Induces Peripheral Nerve Sensitization in a Rat Tail Model: Possible Role of Oxidative Stress and Inflammation

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Abstract

Prolonged occupational exposure to hand-held vibrating tools leads to pain and reductions in tactile sensitivity, grip strength and manual dexterity. The goal of the current study was to use a rat-tail vibration model to determine how vibration frequency influences factors related to nerve injury and dysfunction. Rats were exposed to restraint, or restraint plus tail vibration at 62.5 Hz or 250 Hz. Nerve function was assessed using the current perception threshold (CPT) test. Exposure to vibration at 62.5 and 250 Hz, resulted in a reduction in the CPT at 2000 and 250-Hz electrical stimulation (i.e. increased Aβ and Aδ, nerve fiber sensitivity). Vibration exposure at 250 Hz also resulted in an increased sensitivity of C-fibers to electrical stimulation and thermal nociception. These changes in nerve fiber sensitivity were associated with increased expression of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in ventral tail nerves, and increases in circulating concentrations of IL-1 β in rats exposed to 250-Hz vibration. There was an increase in glutathione, but no changes in other measures of oxidative activity in the peripheral nerve. However, measures of oxidative stress were increased in the dorsal root ganglia (DRG). These changes in pro-inflammatory factors and markers of oxidative stress in the peripheral nerve and DRG were associated with inflammation, and reductions in myelin basic protein and post-synaptic density protein (PSD)-95 gene expression, suggesting that vibration-induced changes in sensory function may be the result of changes at the exposed nerve, the DRG and/or the spinal cord. Published by Elsevier Ltd on behalf of IBRO.

Keywords

cytokines; hand-arm vibration syndrome; reactive oxygen species; sensory tests

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DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

INTRODUCTION

Prolonged occupational exposure to hand-held vibrating tools leads to disorders of the peripheral vascular and sensorineural systems. These symptoms, are collectively referred to as hand-arm vibration syndrome (HAVS; (Griffin, 1996; Bovenzi, 2006). Although cold-induced vasospasms and finger blanching are the hallmark characteristics of HAVS, many workers also show abnormalities in sensorineural function, expressed as tingling and numbness in the fingers and hands, reductions in tactile sensitivity and a loss of manual dexterity (Pyykkö et al., 1990; Griffin, 1996; Bovenzi, 2006). These sensorineural symptoms can usually be seen prior to changes in blood flow, and may persist even if a worker has stopped using vibrating hand tools (Cherniack et al., 2004; Stoyneva, 2016; Sorelli et al., 2018).

One of the factors influencing the development of sensorineural disorders is the frequency of the vibration that a worker is exposed to. Studies of the human handarm system, and of the rat tail, have demonstrated that exposure to vibration may produce the most dramatic results when the frequency of the vibration is at or near the resonant frequency of the tissue being exposed (Bovenzi et al., 1996; Bovenzi, 1998; Krajnak et al., 2010; Krajnak et al., 2012a). At the resonant, or natural frequency of the tissue, the shear and bending stress that is associated with vibration exposure is magnified, and there are data demonstrating that this increased tissue stress is associated with an increased risk of developing the peripheral vascular and sensorineural problems seen in workers with HAVS (Dong et al., 2005; Wu et al., 2007; Welcome et al., 2008; Xu et al., 2011; Krajnak et al., 2012b; Krajnak et al., 2015).

The pathology associated with HAVS has been described in a number of experiments (Goldsmith et al., 1994; Takeuchi etal., 1986; Takeuchi etal., 1988). Finger biopsies from patients with HAVS have shown pathological changes in the nerves including increases in Schwann cell activation and fibroblast cell number, fibrosis, loss of the myelin sheath, and reductions in the elastic mem brane and axon size (Goldsmith et al., 1994; Takeuchi et al., 1986; Takeuchi et al., 1988). Animal models of vibration-induced injury have shown that vibration results in functional, morphological, and cellular changes that are consistent with the changes that are seen in biopsy samples collected from workers with HAVS (Krajnak et al., 1994; Govindaraju et al., 2006a, 2006b; Govindaraju et al., 2008; Krajnak et al., 2012a). However, the etiology of HAVS has not been well described and understanding the mechanisms by which occupational exposure to vibration induces problems in peripheral vascular and sensorineural function provides information that can be used by occupational physicians and researchers to identify specific physiological tests and/or biomarkers that can be used to detect vibration-induced changes in function and improve the diagnosis of HAVS.

Studies in humans and animal models have demonstrated that the effects of vibration on the peripheral vascular system are frequency dependent, but the effects of different vibration frequencies on sensorineural function is not as clear (Krajnak et al., 2012b, 2016). In previous experiments, changes in vascular function depended upon the frequency of the vibration exposure, whereas changes in peripheral nerve function occurred at all frequencies (Krajnak et al., 2010, 2012a). It is possible that a frequency-dependent effect of vibration on sensorineural function wasn’t clearly identified because the nerves may be more sensitive to

the exposure, and frequency-dependent changes in nerve function may have occurred at an earlier point during the 10-day vibration exposure used in previous experiments (Krajnak et al., 2012a, 2016). Therefore, the goal of the current experiment was to determine if there were frequency-dependent effects of vibration on sensorineural function after a more acute exposure, and identify which specific physiological or biological markers might be used to diagnose early nerve damage that may occur as a result of exposure to specific vibration frequencies. The resonant frequency of the tail is approximately 250 Hz (Welcome et al., 2008), and as mentioned earlier, it appears that the risk of developing dysfunction or injury is greatest at the resonant frequency. Therefore, this study compared the effects of exposure to vibration at 62.5 Hz (the frequency generated by tools such as drills) with exposure to vibration at 250 Hz (the frequency generated by tools like grinders and sanders). Sensorineural function, and markers of inflammation, injury and oxidative stress were also examined in numerous tissues because previous studies demonstrated that vibration-induced changes in sensory nerve function were associated with inflammation and possibly an increase in oxidative stress, not only in the nerve, but also in the dorsal root ganglia and spinal cord (Krajnak et al., 2012b). Changes in peripheral nerve function that are associated with maintained changes in inflammation, cell signaling and oxidative stress, may lead to the development of chronic pain or reductions in tactile sensitivity (Bovenzi and Zadini, 1989; Morioka and Griffin, 2005; Wu et al., 2006; Leung and Cahill, 2010; Xie et al., 2014). Identifying physiological or biological markers induced after shorter exposures to vibration may allow physicians to detect changes in sensory nerve function before they become permanent.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (6–8 weeks of age; Hilltop Lab Animals, Inc., Scottdale, PA, N = 24) were used for the experiment. The rats were housed in an AAALAC International accredited facility at the National Institute for Occupational Safety and Health, under a 12:12 light:dark cycle (lights on 0600 h), where food and water were available *ad libitum.* We chose to use male rats because the majority of the workers exposed to hand-transmitted vibration through the use of powered handtools are male (Bovenzi, 2005; Bovenzi et al., 2005). Rats were allowed to acclimate to the facility for one week before beginning the experiment. All procedures were approved by the NIOSH Animal Care and Use Committee (ACUC) and were in compliance with policies of the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and use of Laboratory Animals.

Vibration apparatus

The equipment used for vibration exposure as well as control conditions was previously described (Krajnak et al., 2006). Briefly, vibration was generated by V408 electromagnetic shakers and PA100e amplifiers (Ling Dynamic Systems, Royston, UK). To monitor vibration levels an accelerometer (model 353B15) and a signal conditioner (model 482A20) were used (PCB, Piezotronics, Depew, NY). For data acquisition and vibration control, the following instruments were used: PCI-MIO-16XE-10 board, PCI-6713 output board, and

custom Lab-View 5.0 programming software (National Instruments, Austin, Texas). The shakers and restrainers were placed in ventilated, sound attenuating chambers (Med Associates, St. Albans, Vermont).

Vibration and control exposures

Rats were acclimated to restraint by placing them in Broom-style restrainers 3–4 h/day for 3 days prior to the beginning of the exposure (Terrazzino et al., 1995). Rats in the vibrated groups were exposed to 62.5- or 250-Hz vibration (n = 8/group). These frequencies were chosen because previous studies demonstrated that the physical (i.e., biodynamic) response of the rat tail is similar to the responses of the human finger at these frequencies (Welcome et al., 2008). On the days of exposure, rats were placed into their restrainers and put into the exposure chamber. Their tail was gently placed on a vibration platform, secured with 4 elastic straps (each strap 1 cm in width), and exposed to vibration at 62.5 or 250 Hz (acceleration 49 m/s^2) for 4 h. Control rats were also restrained and placed into the exposure chambers with the vibrated rats. However, their tail was secured to a non-vibrating platform. Rats were exposed to restraint or vibration for 3 days. The day following the last exposure, rats were anesthetized with pentobarbital (i.p., 100 mg/kg body weight), and euthanized by exsanguination via cardiac puncture. Ventral tail nerves from vertebrate C10–12 were used to measure gene transcript levels, C13-C18 were dissected into 3 separate segments to be used for enzyme assays and western blot analyses. The right and left dorsal root ganglia (DRG) from each side of lumbar (L) regions 4–6 of the spinal cord were dissected. The left L4 DRG was used for gene transcript measurements. The remaining DRG were used for enzyme and protein analyses. The spinal cord (L4-L6) was also collected and the tissue was dissected in a manner similar to that used for the DRG so that gene transcript, enzyme and protein concentrations could be analyzed. All tissue were dissected, placed into individual cryotubes to be used for each assay, frozen in liquid nitrogen, and stored at –80°C until further analysis.

Current perception threshold (CPT)

Exposure-induced changes in peripheral nerve function were assessed prior to beginning the experiment (pretest, day 1- before exposure) and before and after vibration or restraint exposure on day 2 (day 2 pre- and post-exposure), using the CPT test (American Association of Electrodiagnostic Medicine, 1999). Day 2 was chosen instead of day 3 because the CPT may have transient effects on blood flow in the region being tested (Kiso et al., 2001) and confound measurements of vibration-induced changes in protein levels or gene transcription. CPTs were measured using a Neurometer (Neurotron Inc., Baltimore, MA) as previously described (Krajnak et al., 2007). Briefly, rats were placed in their restrainers and then into a sound-attenuated chamber. Their tail was cleaned with Goldtrobe electrode paste and the residual electrode paste was cleaned off with an isopropyl alcohol swab. Goldtrobe electrode gel was placed onto two electrodes. A stimulating electrode (ATE1925) was secured to the ventral side of the tail at approximately the C13 vertebrate, using Soft tape (Neurotron). The recording electrode (SDE44) was secured 2 cm proximal to the stimulating electrode in the same manner. Transcutaneous electrical stimulation was applied at 3 frequencies (2000, 250, and 5 Hz) to assess the function of the three different nerve fibers: Aβ, Aδ, and C (Krajnak et al., 2007; Krajnak et al., 2016). During the test, the intensity of the stimulus was increased

in 0.5-mA increments for the 2000 Hz, and in 0.1-mA increments for the 250 and 5 Hz. For each frequency, the intensity of the electrical signal was gradually increased until the rat flicked its tail. The intensity that induced the tail flick was recorded as the CPT. CPT measures were collected 3 times at each frequency, and all measurements were made in the same order (2000, 250, and 5 Hz). There was a 1-min inter-test interval between each frequency and each set of measurements. The average CPT at each frequency was used for analyses.

Thermal nociception test

Sensitivity to changes in heat perception was made using an IITC Tail Flick Analgesia Meter model 336TG (IITC Life Science Inc, Woodland Hills, CA). A beam of light (approximately 2 mm in diameter) was focused 4 and 6 mm from the tip of the tail. The intensity of the light gradually increased, thereby increasing the heat generated. When the stimulus became uncomfortable, the rat flicked its tail and a sensor turned off the bean and measured the time (s) from the beginning of the stimulus application until the tail flick. If the rat did not respond after 15 s the test automatically stopped to prevent injury to the tail. The tail-flick latency was measured three times. There was a 1-min interval between the each trial. The average latency to respond (s) was used for analyses.

Mechanical sensitivity

Tactile sensitivity was analyzed using the von Frey monofilaments that delivered approximately logarithmic incremental forces of 10, 15, 26, 60, or 100 mN. Each von Frey monofilament was applied to the tail on the region between C10 and C13 in ascending force and then repeated in descending order. The filament that induced a response (tail flick) when the stimulus was applied in both the ascending and descending order was recorded. Each animal was tested 3 times, with a 1 minute inter-test interval. Animals were tested before and after exposure on day 2 of the experiment. Most animals showed a response at the same tensile strength for all three replications of the test. The other animals responded twice to the filament of a specific tensile strength, and it was that tensile strength that was used for analysis.

Interleukin (IL)-1β and calcitonin gene-related peptide (CGRP) ELISAs

Serum isolated from blood that was collected via cardiac puncture was assayed for IL-1β and CGRP using ELISAs from Caymen Chemicals (Ann Arbor, MI). Assays were performed using the manufacturer's protocols.

Glutathione (GSH) assay

GSH was measured using a GSH colorimetric assay kit and the manufacturer's protocol (Cayman Chemical, Ann Arbor, MI). The tissues were homogenized in lysis buffer (0.2 M 2-(N-morpholino)ethanesulfoic acid, 0.05 M phosphate and 1 mM EDTA), and an aliquot was taken for protein quantification using the BioRad *DC* assay. The remaining aliquots were de-proteinated using metaphosphoric acid (1:1 volume). The supernatant was collected and then incubated with triethanolamine. GSH concentrations were measured in 50-μl aliquots from each sample using methods recommended by the manufacturer (Cayman

Chemical). Average GSH levels were calculated from duplicate samples and used in the analyses. The data analyses were performed on a sample size of $n = 6$ samples/group because of limited tissue sample size.

Lipid peroxidation assay

Ventral tail nerves and dorsal root ganglia were sonicated in 20 mM phosphate-buffered saline (PBS)/5 mM butylated hysdroxytoluene (BHT) on ice for 20 s and centrifuged at 3,000×*g* at 4 °C for 10min. Protein concentrations were determined using the Bio Rad *DC* protein assay (Bio Rad Inc., Hercules, CA) and 200 μg protein was used for malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) colorimetric determination according to manufacturer's protocol (Oxford Biomedical Research, Oxford, MI). Measurement of these two factors have been shown to be a good indicator of lipid peroxidation. Averages from duplicates assayed for each sample were used for analyses. The data analyses were performed on a sample size of $n = 6–8$ samples/group because of limited tissue sample size.

Quantitative reverse transcriptase polymerase chain reaction (qRT–PCR)

A list of transcripts measured by qRT–PCR and the tissues each transcript was measured in are presented in Table 1. Total RNA from the ventral tail nerve, DRG and spinal cord were isolated using RNeasy Lipid Tissue kits (Qiagen, CA) and quantified using the RNA 6000 Nano kit/2100 Bioanalyzer System according to manufacturer's protocol (Agilent Technologies, CA). One μg of total RNA was reverse transcribed using the SuperScript™ first-strand synthesis RT–PCR kit according to manufacturer protocol (Invitrogen, CA). qRT–PCR was performed using either Roche Universal Probe Library probes or SYBR Green as the detection method. Roche Universal Probe Library probe method contained FastStart Universal Probe (ROX) master mix (Roche, IN), 50 ng cDNA, probe, and specific primers designed using the Universal Probe Library Assay Design Center (https://lifescience.roche.com). SYBR Green qRT–PCR experiments were performed using SYBR Green master mix (Qiagen, IN), 1 μg of cDNA and primers. All samples using SYBR Green detection method were subjected to a Melt Curve and samples which did not show one defined melt peak in the 80 °C range were not included in the data set. qRT–PCR reactions were run on an ABI Prism 5700 Sequence Detection System (Applied Biosystems, CA). Data analyses were performed on a sample size of 6–8 samples/group because the transcript being measured was undetectable in some samples.

Western blot analyses

For Western blot analyses, the tail nerve, DRG and spinal cord tissue was homogenized in 50 (nerve) or 100 μl (DRG and spinal cord) lysis buffer (10 mM Tris base, 2 mM EDTA, 150 mM sodium chloride, 1% Triton-X 100, and 1 μl/ml Sigma Protease inhibitor cocktail™), and protein concentrations were measured using Bio-Rad $_{DC}$ assay (Bio-Rad Inc.). The homogenates were diluted with Laemmli buffer (1:1.5), separated on a 10% SDS–PAGE gel, and transferred to nitrocellulose membranes. The membranes were blocked in LI-COR blocking buffer (LI-COR Biosciences, Lincoln NE) and probed for nitrotyrosine (Santa Cruz Biotechnology, Santa Cruz, CA) or Beta-actin (Cell Signaling Technologies, Danvers, MA). The detection was performed with IRDye800-conjugated anti-mouse, or anti-rabbit IgG,

respectively in LI-COR blocking buffer. At the end of incubation period, the membranes were washed 3× with TBST. The membranes were visualized and analyzed on Odyssey IR imaging system (LI-COR Biosciences, Lincoln, NE). Band signal intensities are presented as area intensity relative to control samples after normalization to beta actin as loading control.

Statistical analyses

To determine if physiological and biological data were normally distributed, Shapiro–Wilk's tests were also performed on all data sets, and these analyses found that all data sets were normally distributed. Welch's tests were performed to assess homogeneity of variance, and none of these tests found differences in variance that were significant. CPT and thermal threshold data were analyzed using a 3(condition) × 3 (time points) repeated-measures ANOVA. Significant interactions in the CPT data were analyzed using oneway ANOVAs and Tukey's HSDs for pairwise comparisons. The number of animals responding to a Von Frey filament of a specific tensile strength was analyzed using a Wilcoxon Rank Sum test and chi^2- analyses. All other data were analyzed using one-way ANOVAs and pairwise comparisons were made using a Tukey HSD test. Differences with $p \leq 0.05$ were considered statistically significant. Data were analyzed using Jmp 13.0.0 (SAS Institute, Pittsburgh, PA).

RESULTS

Physiology

Current perception threshold (CPTs).—The function of ventral tail afferent fibers following tail vibration was assessed using the CPT test. Analysis of the CPT at 2000 Hz (which assesses large myelinated Aβ fiber sensitivity), revealed a significant main effect of day of exposure ($F(2,21) = 43.41$, $p < 0.001$). Pairwise comparisons revealed that the 2000-Hz CPT was significantly lower post-exposure than pre-exposure on day 2 of the study (i.e., animals were more sensitive after exposure) in all the animals (Fig. 1A; control: $t(14) = 5.58$, $p < 0.01$; 62.5 Hz: $t(14) = 2.08$, $p < 0.05$; 250 Hz: $t(14) = 23.06$, $p < 0.001$). When post-exposure CPTs on day 2 were analyzed across groups, the CPT in rats exposed to vibration at 250 Hz, was significant lower than the CPT in controls ($t(14) = 2.08$, $p < 0.05$).

Analysis of the 250-Hz CPT, which measures the function of small myelinated Aδ fibers, also revealed a main effect of day ($F(2, 21) = 16.20$, $p < 0.001$). Additional pairwise analyses revealed that the 250-Hz CPT was lower after exposure to restraint control or vibration at 62.5 Hz than before exposure on day 2 (B; $t(14) = 5.91$, $p < 0.001$; $t(14) = 5.75$, $p < 0.05$, respectively). In rats exposed to vibration at 250 Hz, the 250-Hz CPT was lower before exposure on day 2 than it was at the pretest on day 1 ($t(14)$, $=9.19$, $p < 0.01$). Exposure to vibration on day 2 did not result in a further reduction in the 250-HZ CPT.

The 5-Hz CPT measured unmyelinated C-fiber function. Analyses of the data revealed that only the rats exposed to vibration at 250 Hz displayed changes in the 5-Hz CPT (C). On day 2, the pre-exposure CPT was lower than the pretest ($t(14) = 5.31$, $p < 0.001$). In addition, the

post-exposure CPT was lower than the pre-exposure CPT on day 2 of the study ($t(14) = 3.68$, $p < 0.001$).

Thermal nociception and tactile sensitivity.—The results of the tail-flick test are presented in Fig. 2. Animals in all groups showed a similar tail-flick latency in response to heat prior to exposure to restraint or vibration. On day 2 of the experiment, tail-flick latency was faster in rats exposed to vibration at 62.5 and 250 Hz than it was in control rats ($F(2,21) = 5.99$, $p < 0.01$). Tactile sensitivity, tested using the von Frey filaments did not significantly differ after restraint or vibration exposure (Fig. 3A–C).

Cellular and molecular findings

Ventral tail nerves.—Changes in gene transcript levels in the ventral tail nerve are seen in Fig. 4A. Transcript expression of the pro-inflammatory factors, *Il-1*β and *Tnf*-α was higher in ventral tail nerves from rats exposed to 3 days of vibration at 62.5 and 250 Hz than in nerves from control rats ($F(2,15) = 7.32$, $p < 0.01$ and $F(2, 15) = 4.81$, $p < 0.01$, respectively). Interleukin (*Il*)-6 gene expression was significantly higher in rats exposed to vibration at 62.5 Hz than control rats or rats exposed to vibration at 250 Hz ($F(2,14) = 11.94$, $p < 0.001$). Lipid peroxidation (B: expressed as the concentrations of MDA and HAE) was reduced in the nerves of rats exposed to vibration at both frequencies ($F(2,19) = 5.08$, $p < 0.01$) Fig. 4B). There were no effects of vibration on GSH or nitrotyrosine levels (data not shown).

Dorsal root ganglia.—Exposure to vibration did not affect transcription of any of the gene transcript measured (Table 1). However, 2 measures of oxidative stress, nitrotyrosine (Fig. 5A; greater than control $F(2,18) = 4.61$, $p < 0.05$) and GSH (Fig. 5B; *greater than control and 250 Hz, $F(2,19) = 8.95$, $p < 0.01$) were increased in the DRG after exposure to vibration at 62.5 Hz. There were no effects of vibration on lipid peroxidation in the DRG (data not shown).

Lumbar spinal cord.—Exposure to vibration resulted in changes in gene expression in the lumbar spinal cord of rats exposed to vibration. *Mbp* and *Psd-95* mRNAs were significantly reduced in the spinal cord of rats exposed to vibration at 250 Hz (Fig. 6: $F(2,20) = 3.70$, $p < 0.05$; and $F(2,20) = 3.51$, $p = 0.05$). The pro-inflammatory gene, *Tnf*-α was increased in the spinal cord of rats exposed to vibration at both frequencies ($F(2,20) = 4.07$, $p < 0.05$). There were no significant changes in nitrotyrosine, GSH or lipid peroxidation in the spinal cord (data not shown).

Circulating pro-inflammatory factors.—There were no significant changes in circulating CGRP concentrations (data not shown). However, circulating IL-1β concentrations were significantly higher in rats exposed to vibration at 250 Hz than rats exposed to vibration at 62.5 Hz (Fig. 7; $F(2,21) = 5.07$, $p < 0.05$).

DISCUSSION

Previous studies using an animal model similar to the one used in the current study, have shown that the effects of vibration on peripheral vascular function are frequency-dependent

(Krajnak et al., 2010). However it is less clear how the frequency of the vibration exposure affects the development of sensorineural dysfunction since changes in nerve function and biological markers of dysfunction and injury occur at all frequencies after 10 days of vibration exposure (Krajnak et al., 2012a). Because the peripheral nervous system appears to respond more rapidly to vibration, a shorter exposure time was used in the current study to try to determine if the effects of vibration on peripheral nervous system function are frequency-dependent and which physiological markers and/or biomarkers might be most sensitive for detecting early changes in sensorineural function. As in previous studies, with longer exposure durations (Krajnak et al., 2012a), exposure to vibration at both 62.5 and 250 Hz had effects on physiological and biological measures of peripheral nerve dysfunction and injury in the current study. However, there were frequency-dependent changes in the CPT test, circulating IL-1β concentrations, and the transcription of specific genes that support the idea that exposure to vibration at or near the resonant frequency (i.e., 250 Hz) generates dysfunction and injury faster than exposure at other frequencies. In addition, the results of this experiment are consistent with the findings of previous studies demonstrating that the CPT test may be a valuable tool for detecting early changes in sensorineural function in animals and workers exposed to vibration (Krajnak et al., 2007; House, 2016; Krajnak et al., 2016).

The CPT test has been used to assess sensorineural function in workers with HAVS, and in animals exposed to segmental vibration (Krajnak et al., 2007; House et al., 2016; Krajnak et al., 2016). We chose to use the CPT in this study because it by-passes the peripheral sensory end organs and measures sensitivity of the nerve to electrical stimulation (or activation of the nerve). This test measures responses of different nerve fiber types to electrical stimulation, and therefore, can be used to determine the type of nerves being affected by a specific exposure. The sensitivity of bare C-nerve fiber endings was also assessed using the thermal nociception test, and sensitivity of pressure and touch sensitive sensory end organs (e.g. Pacinian or Meissner corpuscles) was assessed using von Frey tactile sensitivity test. Using a number of different tests, the anatomical location(s) of the injury or site of dysfunction could be identified. In addition, these tests provide information about specific peripheral sensory nerve pathways that may be affected by vibration, and knowledge that may lead to the identification of additional tests or biomarkers that can be used to detect vibration-induced injury.

Previous studies in humans and in the rat tail model have shown that the 2000-Hz CPT is most sensitive, and shows the greatest change after exposure to vibration (Krajnak et al., 2007; House et al., 2016; Krajnak et al., 2016). In the current experiment, sensitivity to the 2000-Hz electrical stimulus was increased after 2 days of exposure to restraint or vibration. However, the increased sensitivity (i.e., decrease in the 2000-Hz CPT) was significantly greater in rats exposed to vibration at 250 Hz suggesting that this exposure frequency had greater effects on large myelinated Aβ nerve fibers than 62.5 Hz and control conditions. These findings are consistent with results in both humans and animals showing that the 2000-Hz CPT, is very sensitive to vibration exposure, and that responses to the CPT at this frequency may not only serve as a test for diagnosing vibration-induced injury, but also serve as a marker that can be repeatedly measured to determine if a worker is developing a vibration-induced sensory dysfunction (Krajnak et al., 2012b; House et al., 2016). In

addition, it is not surprising that the 250-Hz vibration exposure had the greatest effect on the 2000-Hz CPT because 250-Hz is the frequency at which Pacinian corpuscles are most sensitive to stimulation, and Aβ-nerve fibers innervate Pacinian corpuscles and carry the vibration signal from the periphery to the central nervous system (Santini, 1969). Thus, the vibration signal was most likely transmitted through the corpuscle to the nerve, altering nerve function.

Vibration exposure at 250 Hz also increased sensitivity to the 5-Hz CPT and resulted in a faster tail-flick latency in thermal sensitivity test. Both of these stimuli activate unmyelinated C-fibers. These fibers carry information about heat or painful stimuli to the central nervous system, and tend to be sensitized when there is inflammation (Hirata et al., 1995; Obata et al., 2004; Tanabe et al., 2009; Leung and Cahill, 2010; Latremoliere et al., 2015). Exposure to vibration at 250 Hz was also associated with an increase in the expression of the pro-inflammatory factors, *Il-1β* and *Tnf-α* in ventral tail nerves. These data are consistent with those from other studies showing that vibration induces inflammation and edema, along with reductions in myeli-nation and the number of nerve fibers (Govindaraju et al., 2006a; Krajnak et al., 2012a; Krajnak et al., 2016). However, acute increases in the expression of pro-inflammatory factors may also induce the expression of growth factors and stimulate repair and regeneration of damaged nerves (Leung and Cahill, 2010). Therefore, additional studies looking at other time points may help clarify the relationship between inflammation, changes in sensory function, and long-term de-innervation and loss of sensation in tissues repeatedly exposed to vibration.

The 250-Hz CPT measures the function of small myelinated Aδ nerve fibers. These nerve fibers carry temperature information, and information regarding light touch, to the central nervous system. Restraint-control rats and rats exposed to vibration at 62.5 Hz had a lower 250-Hz CPT (i.e., increased sensitivity) after exposure to vibration at 62.5 Hz. The increased sensitivity seen in rats exposed to vibration at 62.5 Hz was associated with an increase in the expression of pro-inflammatory factors, and a reduction in lipid peroxidation in the ventral tail nerve. The cell bodies of the nerves that innervate the tail are located in the L4–6 DRG. Exposure to vibration at 62.5 Hz also resulted in an increase in oxidative activity (nitrotyrosine levels) and an increase in levels of the anti-oxidant, GSH in the L4–6 DRG, suggesting that effects of vibration on the ventral tail nerves were transmitted to the DRG. The spinal cord also displayed changes in gene transcription in response to vibration exposure at 62.5 Hz; *Tnf-α* gene expression was increased. Because both control and vibrated rats showed changes in their response to the 250-Hz CPT, but changes in markers of inflammation and oxidative stress were only seen rats exposed to vibration, it doesn't seem likely that the changes in the 250 CPT were due to inflammation or oxidative. Instead, the change in the sensitivity of Aδ fibers to stimulation may have been the result of restraint or having their tail kept in a static posture for 4 h, which reduces blood flow to the tail (Tachi et al., 2004).

The rats exposed to vibration at 250 Hz did not show changes in sensitivity to 250-Hz CPT from pre- to post-exposure on day 2. However, the pre-vibration 250-Hz CPT on day 2 was lower than the pre-test CPT. This suggests that even a single exposure to vibration at 250 Hz may have had a prolonged effect on Aδ fiber sensitivity. Similar to the findings seen in rats

exposed to vibration at 62.5 Hz, vibration exposure at 250 Hz was associated with an increase in the expression of inflammatory factors in the ventral tail nerve, but a reduction in lipid peroxidation. Although there were no significant effects of 250-Hz vibration exposure on gene expression or oxidative stress in the DRG, vibration at this frequency was associated with an increase in expression of *Tnf*-α and a reduction in *Mbp* and the post-synaptic density marker, *Psd-95,* suggesting that vibration exposure at 250 Hz affects both the peripheral and central sensory nervous system more rapidly.

Therefore, based on the results of this experiment, it appears that exposure to vibration at 250 Hz may have a more profound or more rapid effect on sensory function than vibration exposure at 62.5 Hz. There was also an increase in circulating concentrations of IL-1β in animals exposed to vibration at 250 Hz, indicating that vibration exposure at this frequency has systemic effects. These data are consistent with previous studies showing an increase in circulating IL-1 β concentrations after 10 days of exposure to vibration at 250 Hz (Krajnak et al., 2010). This increase in IL-1 β was also associated with changes in sensory function and biomarkers associated with nerve injury (Krajnak et al., 2012a). Exposure to 250 Hz also results in changes in the expression of factors regulating myelination and neural (i.e. synaptic) communication in the spinal cord. These types of changes in the spinal cord have been associated with permanent changes in sensation and the development of neuropathic pain (Leung and Cahill, 2010; Richner et al., 2014; Latremoliere et al., 2015). The results of these experiments are also consistent with findings in workers exposed to hand-transmitted vibration and with other studies performed in animals models; Repeated exposure to vibration initially results in an increase in sensitivity of the nerves to stimulation and a greater sensitivity to noxious stimuli, and this effect is more pronounced at the resonant frequency (Bovenzi, et al., 1997; Krajnak et al., 2012a; House et al., 2016). With long-term exposures, there is permanent nerve damage, a loss of tactile sensitivity, pain and a loss of manual dexterity in the hands (Lundborg et al., 1987; Pyykkö et al., 1990). These changes in peripheral sensory function are usually permanent and persist even after workers are no longer exposed to vibration (Pyykkö et al., 1990; Cherniack et al., 2004). Because this study was designed to look for statistically significant changes that occur as a result of specific exposures, only 8 animals were tested within each group. This sample size is large enough to identify changes between groups due to the exposure, but not large enough to perform correlations between the variables. Therefore, although the results of this study identify potential physiological and biological markers that are indicative of vibration-induced nerve damage, additional studies using more subjects would allow mathematical correlations between changes in CPTs, thermal nociception, circulating IL-1β concentrations and other circulating inflammatory mediators in humans exposed to vibration, or in animals studies that utilize longer exposures. Additional studies could help determine which tests are most reliable for monitoring nerve function in workers exposed to vibration, and to determine if and when different exposure induce these changes. If reliable changes in peripheral sensation or markers of sensory nerve injury can be measured in workers, steps can be taken to protect workers from the exposure before more permanent effects develop.

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Abbreviations:

CPT	current perception threshold
DRG	dorsal root ganglia
HAE	4-hydroxyalkenals
HAVS	hand-arm vibration syndrome
MDA	malondialdehyde
PBS	phosphate-buffered saline
(pSD)-95	post-synaptic density protein

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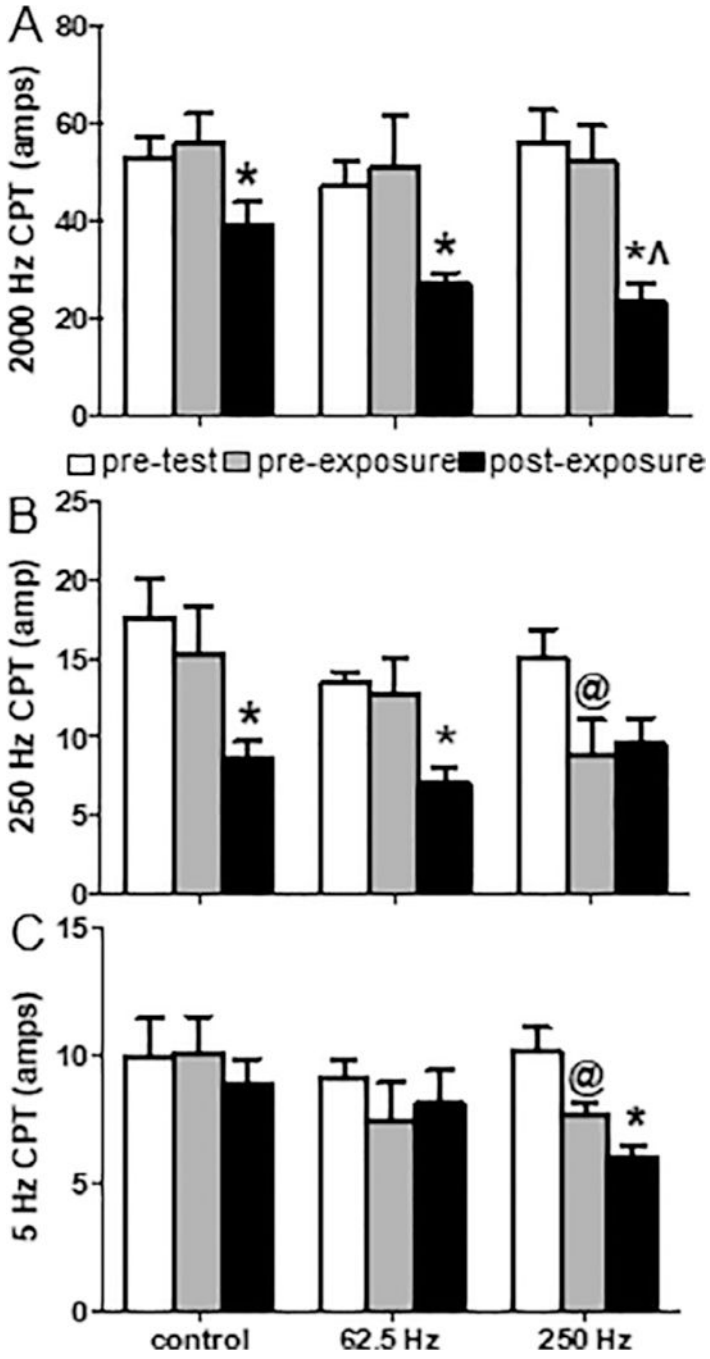

Fig. 1.

Tail-flick responses to applied transcutaneous electrical stimulation. 2000-HZ CPT (A): All groups displayed a reduction in CPT from pre- to post-exposure (*$p < 0.05$). Rats exposed to vibration at 250 Hz also showed a lower post-exposure CPT that rats in the other groups. (^$p < 0.05$). 250-Hz CPT (B): CPTs were lower post- vs pre-exposure in controls and rats exposed to 62.5-Hz vibration (B; *$p < 0.05$). In rats exposed to vibration at 250-Hz vibration, the pre-exposure CPT was lower than the pre-test CPT ($^{@}p < 0.05$). 5 HZ CPT (C): Pre-exposure CPTs were lower than pretest CPTs in rats exposed to 250-Hz vibration

($^{@}p < 0.05$). Post-exposure CPTs were also lower than pre-exposure CPTs in this group ($^{*}p < 0.05$).

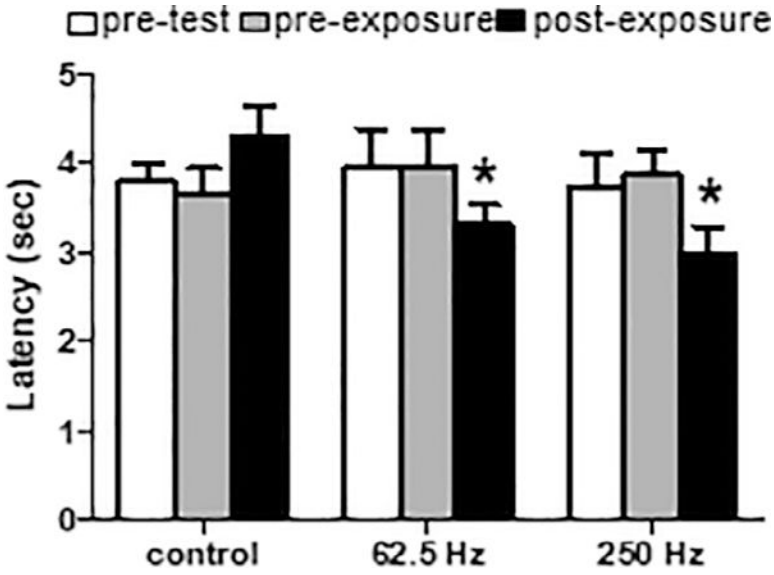

Fig. 2.

Tail-flick latency to applied heat. These data represent the average number of seconds (±SEM) it took for rats to flick their tail away from a noxious heat source. Rats exposed to vibration at 62.5 and 250 Hz showed a significant reduction in the amount of time it took to flick their tail after exposure on day 2 than before exposure (*$p < 0.05$).

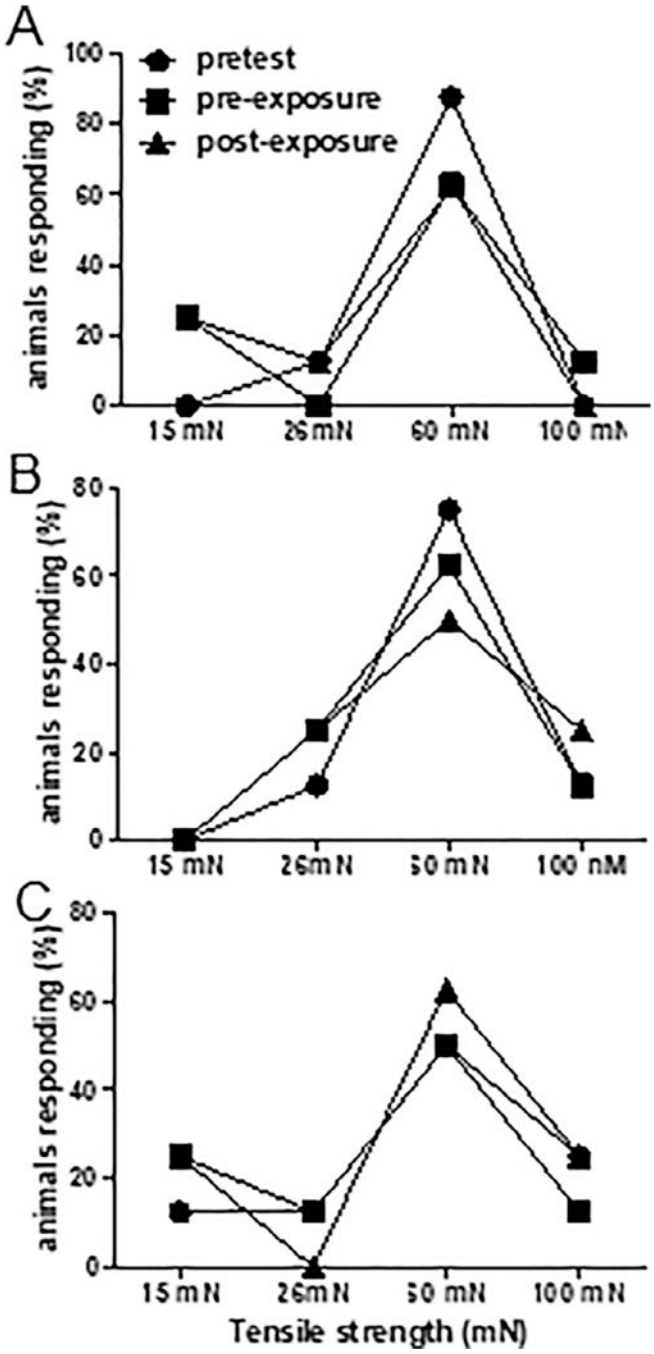

Fig. 3.

Responsiveness to the von Frey stimulation. These data show the percent of animals responding to the von Frey fibers of varying tensile strength. Data are presented for control animals (A); day 2 pre-exposure (B) and day 2 post-exposure (C).

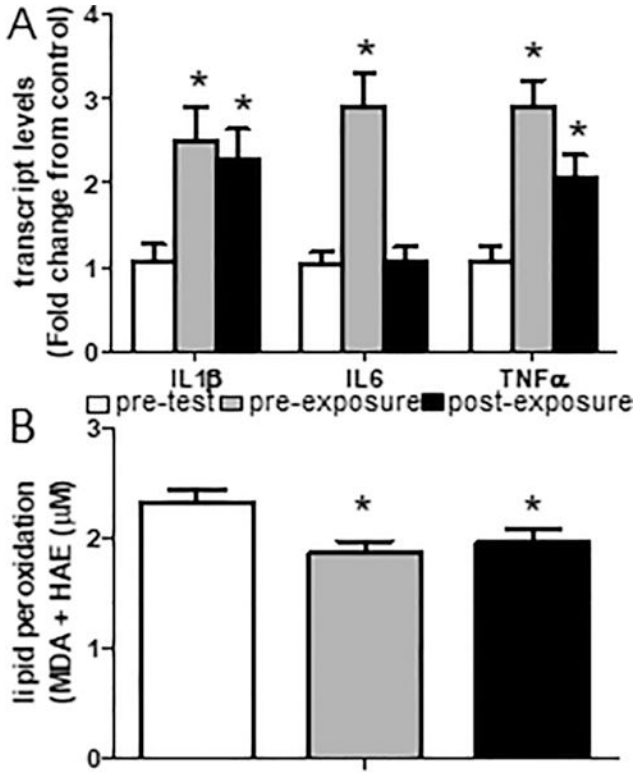

Fig. 4.

Transcript and lipid peroxidation in tail nerves. These data show the average (±sem) transcript levels (A) and lipid peroxidation levels (B: MDA + HAE concentrations). Exposure to vibration at 62.5 and 250 Hz resulted in an increase in *Il1-β* and *Tnf-α* transcript levels. However *Il6* was only increased after exposure at 62.5 Hz (*$p < 0.05$). Exposure to vibration at both frequencies resulted in a reduction in lipid peroxidation (*$p < 0.05$).

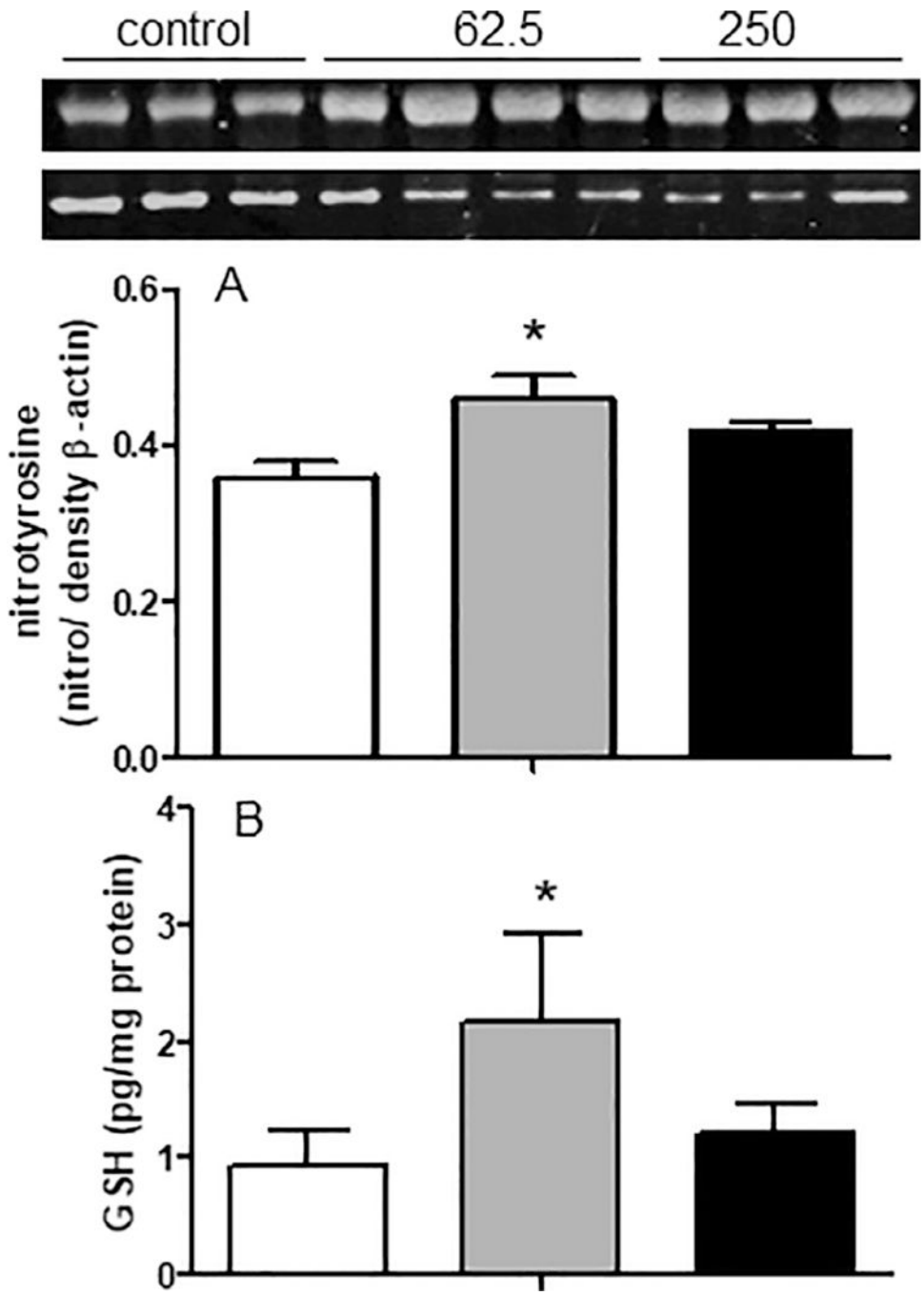

Fig. 5.

Markers of oxidative stress in the DRG. The photo at the top of the figure shows a representative western blot labeled for nitrotyrosine (top band) and b-actin (bottom band) in the DRG. The graphs show average (±sem) band densities for nitrotyrosine (A) and tissue GSH concentrations (B). Exposure to vibration at 62.5 Hz resulted in an increase in nitrotyrosine and GSH concentrations in the DRG (*$p < 0.05$).

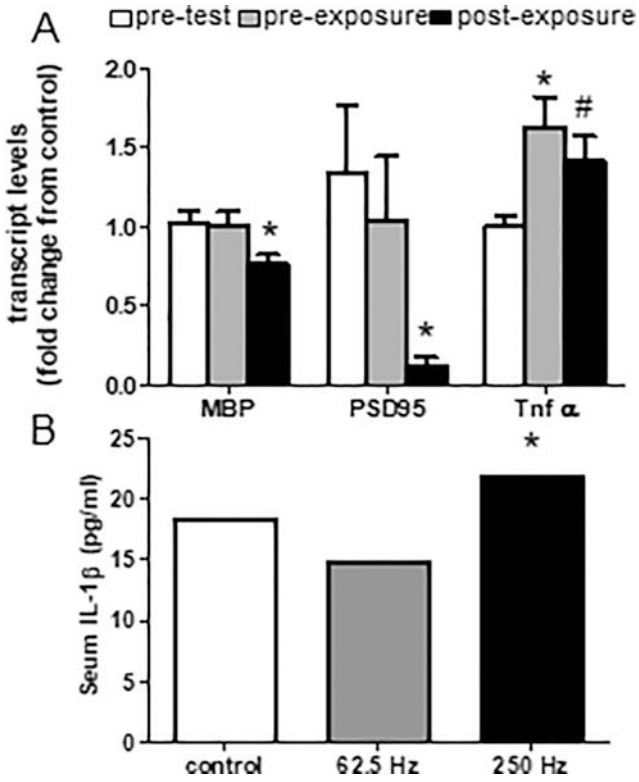

Fig. 6.

Transcript levels in the spinal cord. These data show the average (±sem) transcript levels in the spinal cord. Exposure to vibration at 250 Hz resulted in a reduction in *Mbp* and *Psd-95* gene expression in rats exposed to vibration at 250 Hz (*$p < 0.05$). Exposure to vibration at both 62.5 and 250 Hz resulted in an increase in *Tnf*-α transcript levels (*$p < 0.05$, # less than 0.06).

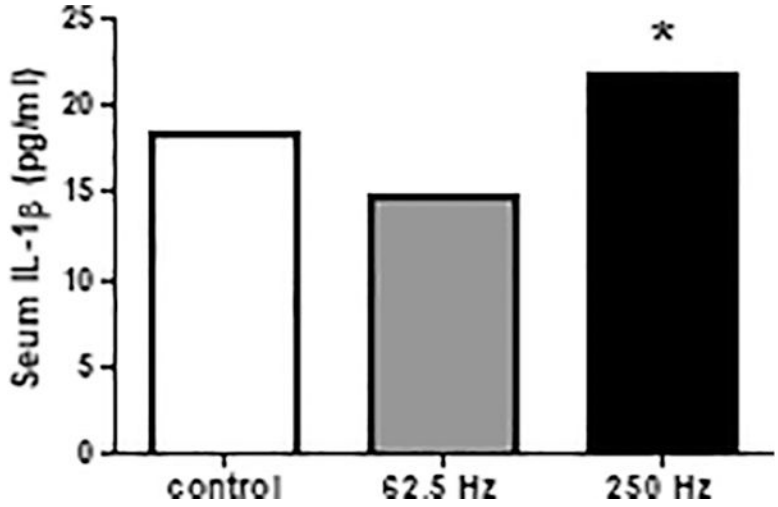

Fig. 7.

Circulating concentrations of IL-1β. Exposure to vibration at 250 Hz resulted in an increase in circulating IL-1β concentrations (*$p < 0.05$; mean ± sem concentrations; control 18.28 ± 1.65; 62.5 Hz 14.77 ± 1.04; 250 Hz 21.76 ± 1.84).

Table 1.

This table lists the transcripts that were measured by qRT–PCR and the tissues each transcript was measured in (highlighted boxes)

Gene	Artery	Nerve	DRG	Spinal cord
18s	■	■	■	■
α2a adrenoreceptor (A2a)				■
α2c adrenoreceptor (A2c)		■		
Acid sensing protein receptor 3 (ASIC3)		■		
c-jun		■	■	■
Calcium calmodulin kinase (camk)				■
Calcitonin gene related peptide (cgrp)		■		■
Calcitonin gene related peptide receptor ($cgrp_r$)			■	■
Cyclo-oxygenase 2 (cox2)	■	■		■
Endothelin-1a (et1a)	■			
Forkhead 3 protein (fox3)		■		
Glutathione peroxidase (gpx)		■	■	■
Glutathione synathase (gsh)		■	■	■
Heme oxygenase-1 (ho-1)		■	■	■
Interleukin 1β (il1β)	■	■	■	■
Interleukin 6 (il6)	■	■		■
Kelch Like ECH Associated Protein 1 (keap)			■	
Metallothionine 1a (mt1a)	■			
Myelin basic protein (mbp)		■		■
n-methyl-d-aspartate receptor ($nmda_r$)			■	
NF-kappa-b (nfkb)		■		
Nitric oxide synthase-1 (nos-1)				
Nitric oxide synthase-2 (nos-2)	■			■
Nitric oxide synthase-3 (nos-3)	■			
Neuronal injury protein family 2 (ninj2)		■		
p75 growth factor receptor (p75)			■	

Gene	Artery	Nerve	DRG	Spinal cord
p-selectin	■			
Post-synaptic density protein-95 (psd95)		■	■	■
Runx-1	■			
Supraoxide dismutase-1 (sod1)		■	■	■
Supraoxide dismutase-2 (sod2)			■	■
Tumor necrosis factor α (tnfα)		■	■	■
Transient receptor potential cation channel subfamily V member 1		■	■	