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Contact area affects frequency-dependent responses to vibration in the peripheral vascular and sensorineural systems

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Abstract

Repetitive exposure to hand-transmitted vibration is associated with development of peripheral vascular and sensorineural dysfunctions. These disorders and symptoms associated with it are referred to as hand-arm vibration syndrome (HAVS). Although the symptoms of the disorder have been well characterized, the etiology and contribution of various exposure factors to development of the dysfunctions are not well understood. Previous studies performed using a rat-tail model of vibration demonstrated that vascular and peripheral nervous system adverse effects of vibration are frequency-dependent, with vibration frequencies at or near the resonant frequency producing the most severe injury. However, in these investigations, the amplitude of the exposed tissue was greater than amplitude typically noted in human fingers. To determine how contact with vibrating source and amplitude of the biodynamic response of the tissue affects the risk of injury occurring, this study compared the influence of frequency using different levels of restraint to assess how maintaining contact of the tail with vibrating source affects the transmission of vibration. Data demonstrated that for the most part, increasing the contact of the tail with the platform by restraining it with additional straps resulted in an enhancement in transmission of vibration signal and elevation in factors associated with vascular and peripheral nerve injury. In addition, there were also frequency-dependent effects, with exposure at 250 Hz generating greater effects than vibration at 62.5 Hz. These observations are consistent with studies in humans demonstrating that greater contact and exposure to frequencies near the resonant frequency pose the highest risk for generating peripheral vascular and sensorineural dysfunction.

Introduction

Repetitive exposure to hand-transmitted vibration in the workplace has been associated with development of a number of different symptoms including cold-induced vasospasms and reductions in tactile sensitivity (Griffin 1996, 2004; Griffin and Bovenzi 2002). Additional effects might include muscle weakness, chronic pain, and increased risk of developing arthritis in the upper limbs and neck (Bovenzi, Fiorito, and Volpe 1987; Bovenzi, Petronio, and DiMarino 1980; Bovenzi, Prodi, and Mauro 2015; Roquelaure, Y., Ha, C., Rouillon, C.,

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Fouquet, N., Leclerc, A., Descatha, A., Touranchet, A., Goldberg, M., Imbernon, E., 2009). These symptoms are collectively referred to as hand-arm vibration syndrome (HAVS) (Griffin 1996). Vibration-related characteristics that mediate the risk of developing HAVS include (1) dominant frequency emitted by the tool (Welcome et al. 2008), (2) magnitude of the vibration, (3) duration of tool use, (4) contact between tool user and vibrating tool, and (5) grip force applied while the tool is in use (Griffin 1996). Although there are data to demonstrate that all these factors pose some risk, understanding the level of risk associated with each of these factors may be used by manufacturers to improve tools or other personal protective equipment, or it may be utilized to update exposure-response curves presented in the International Standards Organization (ISO)-5349 (ISO 2001) or American National Standards Institute (ANSI 2006) standards on hand-transmitted vibration exposure.

The National Institute for Occupational Safety and Health (NIOSH) developed and characterized a rat-tail model of vibration-induced injury, and utilized this model to demonstrate that development of vascular and sensorineural dys-function are frequency-dependent, where the highest risk of injury occurs with exposure to vibration within the frequency range that induces the greatest tissue stress and strain (Krajnak et al. 2006a, 2010; Welcome et al. 2008). Exposure to 10 consecutive days of vibration resulted in a maintained vasoconstriction of the ventral tail artery, and rise in the expression of transcripts known to be involved in vascular remodeling (Krajnak et al. 2010). These changes were more pronounced at 250 Hz (which is in the range of the resonant frequency of both the rat tail and human fingers) than at 63 or 125 Hz (Welcome et al. 2008). Alterations in gene transcription indicative of peripheral nerve injury and remodeling also tended to be greater when the frequency animals were exposed to was at or near the resonant frequency (Krajnak et al. 2012a). Based upon these data and findings from epidemiological (Bovenzi et al. 2011; Bovenzi, Prodi, and Mauro 2015), lab-based (Dong et al. 2007, 2001, 2004; Dong, Welcome, and Wu 2005a, 2005b; Dong et al. 2005c; Griffin 2012; Griffin, Bovenzi, and Nelson 2003; Ye and Griffin 2013; Griffin 1996) and mathematical modeling studies (Dong et al. 2007; Wu et al. 2006, 2008a, 2007), it was suggested that occupational exposure to vibration at or near the resonant frequency may actually induce local injury more quickly than exposure to vibration at other frequencies.

One of the major differences between the physical (i.e. biodynamic) response of the rat-tail and human finger is that although the frequency-dependent response of the tail is similar to the response of the human finger (i.e. the resonant frequency range is similar), the magnitude of the tissue response of the tail is greater than that of the human finger when exposed to vibration within the resonant frequency range (Welcome et al. 2008). The greater magnitude in the tail compared to human finger may be attributed to 2 factors; one is that the stiffness of human fingers is greater than the stiffness of the rat-tail (Wu et al. 2008b; Xu et al. 2011), and two, when a worker is using a tool, his/her hand is gripping the tool to maintain contact and control of the tool. Although the tail cannot grip a tool, the contact surface and stiffness is enhanced by increasing the number of straps that are employed to secure the tail to the platform. Thus, in this study, it was postulated that increasing contact between vibration source and tissue by using additional straps enhances transmission of vibration energy from the vibrating source to the tail, and results in elevation in indices of remodeling and injury.

Methods

Animals

Male Sprague-Dawley rats ([H1a:(SD) CVF rats; 6 weeks of age at arrival weighing 250–275 g (Hilltop Lab Animals, Inc, Scottsdale, PA) were used in this study. Rats were maintained in a colony room with a 12-hr light:dark cycle (lights off 0700 h) with Teklad 2918 rodent diet and tap water available *ad libitum*, at the National Institute for Occupational Safety and Health (NIOSH) facility, which is accredited by AAALAC International. Rats were allowed to acclimate to the lab for 1 week prior to beginning the experiments. All procedures were approved by the NIOSH Animal Care and Use Committee and were in compliance with 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 exposures

The equipment and protocol for exposing animals to vibration has been previously described (Wu et al. 2008b). Briefly, rats were placed in Broome style restrainers for 1–2 hr a day for 5 days to acclimate them to the restrainer. After a week of acclimation to restraint, rats were randomly assigned to a cage-control group, restraint-control group or one of three vibration groups, where vibration exposure frequencies were 62.5 or 250 Hz. During exposures, animals had their tails secured to a vibrating platform. Restraint-control rats were placed in the chambers along with vibrated rats, but their tails strapped to platforms that were mounted onto isolation blocks with either four or seven straps (Krajnak et al. 2006a; Welcome et al. 2008). Each exposure was performed between 0900 and 1300 hr, and was 4 hr in duration. The acceleration magnitude employed for all vibration frequencies was 49 m/s^2 root mean squared (r.m.s). After each treatment rats were returned to their home cages and housed in the colony room. Animals were exposed to vibration or restraint for 10 consecutive days for 4 hr/day. This exposure duration was selected based upon previous studies demonstrating that after 10 days of treatment there were marked changes in biomarkers indicative of peripheral vascular and sensorineural dysfunctions (Krajnak et al. 2012b, 2010, 2009). The daily duration of the exposure was selected based upon the ANSI 2.70 and ISO 5349–1 frequency weighting curve which suggests that when utilizing a tool with a dominant frequency of 250 Hz, this is the maximal length of time a worker should be exposed to vibration. These frequencies were also chosen because previous investigators found that there were frequency-related differences in the effects of these two exposures on sensorineural and peripheral vascular function (Curry et al. 2002; Krajnak et al. 2012a). The accelerations used in this study are similar to the unweighted accelerations a worker would be exposed to if utilizing a riveter (62.5 Hz), or a sander or grinder (125–250 Hz). Previously Krajnak et al. (2010) examined frequency-dependent effects of vibration in unrestrained portions of the tail. To determine if similar results could be obtained if a larger proportion of the tail was restrained, a four strap (as in previous studies) versus seven strap restraint system was compared; the four strap restraint kept approximately 25% and the seven strap restraint kept approximately 44% of the tail in contact with the platform during the treatment. Care was taken to ensure that the straps did not put additional pressure on the tail with the straps. With the seven strap system, the portion of the tail restrained was sufficient to reduce bending stress that occurs at the intervertebral joints. Because there were

not enough exposure systems to run all animals at the same time, animals were run in blocks, with each block consisting two control and two vibration exposed rats (within each frequency).

Tissue samples

One day following the final exposure, rats were anesthetized using pentobarbital (100 mg/kg) and exsanguinated by cardiac puncture. Ventral tail arteries were dissected from the C15–18 regions of the tail. These segments were selected because the biodynamic response (amplitude of the tail/amplitude of the vibrating platform) to vibration is frequency-dependent in these regions of the tail; with the response at 62.5 Hz being around 1 (unity) and the response at 250 Hz being between two and three (i.e. resonance (Welcome et al. 2008)). The ventral tail artery, dissected from the C15–16 region of the tail was immediately placed into a cryovial, frozen in liquid nitrogen, and stored at −80°C until RNA was isolated. The C17–18 segments of each tail were placed in 15 ml conical tubes and immersion fixed overnight using 4% paraformaldehyde + 0.1M phosphate buffer, pH 7.3. The next morning, the ventral-tail artery was dissected from the fixed segment and placed in 2 ml cryovials containing 1.5 ml 10 mM phosphate buffered saline pH 7.4. Vials were stored at 4°C until processed for morphological analyses. Nerve samples were also collected from the C15–16 and C17–18 regions, along with dorsal root ganglia (DRG) adjacent to the L4–6 region of the spinal cord. These tissues were placed into cryovials, immediately frozen in liquid nitrogen and stored at −80°C until used for RT-PCR or measurement anti-oxidant enzymes.

Measurement of anti-oxidant enzymes

Glutathione-s-transferase (GST), superoxide dismutase-1 (SOD1) and SOD2 were measured using ELISA kits from (Oxis Inc., USA). Tissues were prepared and enzymes assayed following the manufacturer’s instructions.

Morphology

Fixed artery samples were dehydrated at room temperature with agitation using increasing concentrations of ethanol. Dehydrated samples were embedded employing a JB4 Embedding Kit (Electron Microscopy Sciences, Hatfield, PA) following manufactures instructions. Briefly, samples were incubated in JB4 Infiltration solution at 4°C overnight with agitation. The next morning fresh solution was added and after 4-hr incubation, arteries were removed and placed in 2 × 15 × 5 mm molding trays (Electron Microscopy Sciences). Embedding solution (1.2 ml) was added to each mold. Samples were allowed to polymerize on the bench at room temperature overnight.

Artery cross-sections, 4-μm thick, were cut using a Sorvall Microtome and 0.5-inch glass knives. Sections were wet-mounted onto microscope slides and dried at 60°C for 5 min. Arteries were stained utilizing Lee’s Methylene Blue, dehydrated in 95% ethanol, dried, and cover-slipped with Permount (Fisher Scientific, Pittsburgh, PA). Images of arteries were captured on an AX70 Olympus microscope using a 20× air objective and a 2000R Retiga color camera. Images were imported into Scion Image (ImageJ version 1.51, vendor). For each section (n = 5 sections/animal), the external perimeter of each artery was outlined and area within the outline calculated. The lumen was then outlined, and area within lumen

measured. To determine the area of the each artery that was comprised of vascular smooth muscle (VSM), the area within the lumen was subtracted from the total area. Averages were calculated for each animal and analyzed as described below.

Quantitative RT-PCR

qRT-PCR was performed to (1) confirm vibration-induced changes in transcript levels previously associated with vibration-induced vascular and sensorineural dysfunctions, and (2) determine how vibration induced alterations in expression of pro-inflammatory cytokines, apoptotic factors or vaso-modulating factors involved in vascular remodeling were affected under differing conditions. Ribosomal 18s was used as a loading control. Transcripts examined and primer sequences are listed in Table 1. RNA was isolated from tissues, and first-strand cDNA was synthesized from 1 μg total RNA using Reverse Transcription System (Invitrogen, Carlsbad, CA) as previously described (Krajnak et al. 2006b). Control RNA from heart or brain tissue was run at 10× dilutions for each transcript to establish a standard curve of relative transcript levels, and relative RNA levels calculated using the efficiency calculated from this curve. Samples that did not show a single defined melt peak in the 80°C range were not included in the dataset.

Analyses

Changes in % area of VSM and lumen, along with gene expression in artery, nerve and DRG, and antioxidant concentrations in the nerve, were analyzed using a 2 (62.5 v 250 Hz) × 2 (4 v 7 straps) × 2 (control v vibrated) ANOVA with the block the animal was run in as a random variable. Additional analyses were performed using oneway and two-way ANOVA and Student's t-tests. Differences with $p < 0.05$ were considered significant unless otherwise noted.

Results

Histology

The effects of varying frequency and strap number are shown in Figure 1. The photomicrographs illustrate an artery from controls (A) and an artery from a rat exposed to 250 Hz vibration when seven straps are used for restraint (B). The graphs below show changes in lumen size (C) and thickness of vascular smooth muscle (VSM; D). When four straps were employed for restraint, there were no significant effects on area of the lumen and amount of vascular smooth muscle in rats exposed to 250 Hz vibration (Figure 1C and1D). In contrast, when seven straps were used for restraint, there was a significant reduction in area of lumen and increase in percentage area of VSM in arteries of animals exposed at 250 Hz (Figure 1D).

qRT-PCR

Figure 2 demonstrates the influence of vibration and strap number on gene transcription in the ventral tail artery. Exposure to vibration at 250 Hz resulted in a rise in transcript number for metallothionen (*mt*)-*1a*, intracellular adhesion molecule-1 (*i-cam1*), and myeloid leukemia-1 protein (*runx*) when the tails were restrained with four or seven straps (Figure 2A, 2C and 2D). Restraint with four straps decreased expression of cox_2 when rats were

vibrated at 62.5 Hz (Figure 2B). Restraint with seven straps diminished expression of *cox*$_2$ in arteries of controls, but an increase in arteries of rats exposed to 250 Hz vibration.

In nerves (Figure 3) exposure to vibration at 250 Hz increased expression of GTP cyclohydrolase 1 (*gch-1*) in ventral tail nerves with both four and seven strap restraint (Figure 3A). However, the elevation in expression of *gch-1* was significantly greater with treatment at 250 Hz than 62.5 Hz. Exposure to 250 Hz vibration also resulted in an increase in *hif-1α* expression in nerves in tail restrained with either four or seven straps, and an elevation in *hif-1α* expression with exposure to 62.5 Hz vibration (seven strap restraint only; Figure 3B). Treatment with 62.5 Hz vibration (four strap restraint) produced a rise in myelin associated glycoprotein (mag; Figure 3C).

Concentrations of the anti-oxidant activity levels GST, SOD1 and SOD2 (Figure 4A–4C) were measured by ELISA and data presented in Figure 4. Vibration or restraint did not markedly affect GST or SOD1 activity levels. However, SOD2 activity levels were significantly increased in nerves tails of rats exposed to vibration at 250 Hz and restrained with seven straps (Figure 4C).

The effects of vibration and strap number on gene expression in the DRG are presented in Figure 5. There was an increase in transcription of *cox*$_2$ at 62.5 Hz (with both four and seven straps) and a decrease in *cox*$_2$ in DRG with exposure to vibration at 250 Hz (with both four and seven straps; Figure 5A). Acid-sensing proton-gated ion channel three (*asic* or *drasic*) was not markedly altered in DRG from rats whose tails were restrained with four straps (Figure 5B). However, restraint with seven straps enhanced the expression of *drasic* in controls. Tyrosine receptor kinase (*ntrk*) was reduced in the DRG after exposure to 62.5 or 250 Hz (when either four or seven straps were used for restraint; Figure 5C), but rose in DRG of rats exposed to 250 Hz vibration (seven strap restraint). Transient vanilloid protein receptor 1 (*trpv1*) was increased in DRG rats exposed to vibration at 62.5 Hz (four straps), but lowered with exposure to vibration at 250 Hz (seven straps; Figure 5D).

Discussion

The goal of the current study was to use our rat tail model to determine how frequency and amplitude of the tissue response and coupling between vibrating source and exposed tissue interact to affect vascular morphology and expression of genes that are markers of vibration-induced vascular and sensorineural dysfunction. Previous studies demonstrated that in rats and humans, the risk of developing a vibration-induced injury or disorder is frequency-dependent, with exposure to vibration near the resonant frequency of the tissue generating the more severe injury (Dong et al. 2007, 2012; Krajnak et al. 2010; Welcome et al. 2008). The effects of amplitude and interaction between amplitude and frequency were examined in acute studies in humans These investigators found that increasing the grip force or coupling between the vibrating source and the hand did not exert significant effects on the biodynamic response of the fingers during an acute exposure (Dong et al. 2004). In the current study, an animal model of vibration-induced injury examined the influence of amplitude and coupling to the vibrating source on markers of vibration-induced injury. Based upon the results of this

investigation increasing the coupling between vibration source and tissue enhanced markers of dysfunction and injury, even though the amplitude of the tissue response was reduced.

In our previous study (Krajnak et al. 2010), 10 consecutive days of exposure to vibration at 250 Hz (four strap restraint) significantly reduced the diameter of lumen and elevated the thickness of the ventral tail artery smooth muscle wall. In this study, exposure to vibration at 62.5 Hz under either restraint condition did not markedly alter measures of vascular morphology compared with controls. These findings are consistent with those of Krajnak et al. (2010), showing that exposure to vibration at frequencies below the resonant frequency range (between 100 and 500 Hz) pose a lower risk of generating vibration-induced dysfunctions.

Krajnak et al. (2010) showed that repeated exposure to vibration at 250 Hz results in a vasoconstriction that might be measured as a reduction in the size of the lumen. However, in the current study, there was either thrombosis, or growth of the VSM into the lumen, and because of this, the lumen was not round. Because the lumen was not round in some sections, calculations of the diameter of the lumen and the thickness of the vascular smooth muscle would have been difficult to make. Therefore, the area inside the external boundary of the artery was determined, and the area inside the perimeter of the lumen subtracted to obtain % vascular smooth muscle. Measurements of VSM and lumen were more variable, and thus with four strap restraint, there were no significant differences. However, when seven straps were used for restraint, exposure to vibration at 250 Hz resulted in significant reductions in luminal area and increases in thickness of vascular smooth muscle wall. The maintenance of contact induced by the seven strap restraint produced greater constriction of the artery and diminished variability measurements.

As an additional measure of the effects of vibration exposure, qPCR was performed on tissues from ventral tail artery, peripheral tail nerve and DRG. The gene transcripts analyzed in each tissue were selected based upon previous studies (Krajnak et al. 2012b, 2010) demonstrating these specific transcripts were affected by vibration exposure. Exposure to vibration at 62.5 Hz resulted in a reduction in arterial *cox*$_2$ transcript levels but only when a four strap restraint was employed. Restraint with seven straps lowered in *cox*$_2$ levels in arteries of controls, but a rise in arterial *cox*$_2$ expression occurred following 250 Hz treatment. The vibration-induced elevation in vascular *cox*$_2$ levels is consistent with findings of previous studies (Krajnak et al. 2010) examining the influence of vibration at this frequency and suggests that vibration exposure at 250 Hz (seven straps) may have induced inflammation in the peripheral vasculature leading to increased *cox*$_2$ levels. *Cox*$_2$ might produce vasodilation by increasing inducible nitric oxide synthase, and enhance the response to inflammation by inducing the transcription of prostaglandins (Toriyabe et al. 2004). Increases in inflammatory factors and responses to inflammatory factors may result in remodeling and repair of injured tissue, or if inflammation is maintained chronically, lead to long-term damage and chronic dysfunctions. Treatment with vibration at both frequencies and under both restraint conditions increased transcription of *mt1a, i-cam* and *runx*. However, the increases were more pronounced in rats in the seven strap restraint condition. These findings are consistent with previous observations showing that vibration exposure at or near the resonant frequency results in a rise in transcription factors that are associated

with vascular dysfunction/remodeling (Krajnak et al. 2012b, 2012a). Figure 3 illustrates how vibration and restraint affected the transcription of genes involved in signaling pain and peripheral nerve functions. Exposure to vibration at 250 Hz resulted in enhanced transcription of *gch1* (A) and *hif1α* (B) under both restraint conditions. *Gch1* is a transcription factor, and transcription of this gene is usually increased when there is nerve damage and pain (Campbell et al. 2009; Doehring et al. 2009). Previous investigators reported that specific point-mutations in this gene are associated with diminished sensitivity to pain (Campbell et al. 2009; Doehring et al. 2009; Krajnak et al. 2016, 2007; Nasser et al. 2013) using the rat tail model demonstrated a correlation between elevation in *gch1*transcription and hyperalgesia. Nerves are particularly sensitive to reduction in oxygen, vibration or maintained pressure which result in vasoconstriction within blood vessels of nerves and less oxygen delivered to the tissue (Cho et al. 2015). Interestingly, exposure to vibration at 62.5 Hz produced a decrease in expression of *hif1α*, a marker of hypoxia, under both restraint conditions. This finding was unexpected. However, in these investigations gene transcription was measured at a single time point. Based upon previous studies, it is likely that the effects of exposure to vibration at 62.5 or 250 Hz induce similar changes (Krajnak et al. 2010), but that the time-course over which these changes occur is different. Therefore, it is possible that there was also a hypoxic event following vibration treatment at 62.5 Hz, but it was not detectable at the time point the measurement was made. Hypoxic events, if maintained produce nerve damage. However, acute hypoxic events, and activation of *hif1α* might induce neurogenesis (Cho et al. 2015). Exposure to vibration at 62.5 Hz enhanced expression of *mag* when tails were restrained with 4 straps. The transcription of this gene is usually increased in response to myelin damage and is involved in myelin repair processes. These findings support previous observations that exposure to vibration results in alterations in the expression of genes and proteins involved in pain signaling and in repair and regeneration of the myelin sheath that surrounds peripheral nerves to guide regeneration after injury (Krajnak et al. 2012a, 2013).

Vibration at 250 Hz also elevated the anti-oxidant enzyme, SOD2, but only when seven straps were being used for restraint. SOD2 is an anti-oxidant that decreases the effects of injury-induced rise in reactive oxygen species (ROS), inflammation and pain (Afonso et al. 2007; Toriyabe et al. 2004; Xie et al. 2014). The increase in SOD2 may have been, in part, a response to elevation in *gch-1* transcription (Campbell et al. 2009; Doehring et al. 2009; Nasser et al. 2013). *Gch-1* is a precursor for tetrahydrobiopterin (BH4) which is an enzyme required for production of nitric oxide (NO) (Latremoliere et al. 2015). A rise in GCH-1, BH4 and NO in the DRG and spinal cord has been associated with development of hyperalgesia (Doehring et al. 2009; Latremoliere et al. 2015; Nasser et al. 2013). In addition, single point mutations in the *gch-1* gene were found to affect sensitivity to painful stimuli and development of chronic pain (Nasser et al. 2013).

Not only did vibration exposure affect peripheral nerves, but also resulted in changes in gene expression within DRG. Although many responses to tactile stimulation are dealt with directly at the level of the synapse, other alterations in response to more chronic stimulation might affect gene transcription at the level of peripheral nerve cell bodies which are located within the DRG. In the current study, exposure to vibration at 62.5 Hz enhanced cox_2 signaling when both four and seven straps were used for restraint. As with the arteries, an

increase in *cox2* signaling indicates that there were enhanced inflammatory reactions. In contrast, treatment with 250 Hz vibration resulted in reduction in *cox2* expression in the DRG under both restraint conditions. Previously Krajnak et al. (2012b) showed that exposure to vibration at 250 Hz resulted in inflammation of sensory nerves and pro-inflammatory responses within the DRG; thus, it is likely that varying effects of exposure to 62.5 and 250 Hz may be due to the fact that there are differences in the timing of the effects of varying frequencies on gene expression; and vibration at either frequency might generate an inflammatory response, but the timing of that response varies depending upon the time point samples were collected.

Exposure to vibration also altered the expression of ion channels (*drasic*, and *trpv-1*) in sensory nerves and DRG. Increases in expression of these receptors, if maintained, are associated with development of neuropathic pain (Dai 2016; Honda et al. 2017; Palhares et al. 2017). Several studies demonstrated that repeated exposure to vibrating hand tools is associated with development of pain in the short-term (Ekenvall, Gemne, and Tegner 1989; Lundborg et al. 1987). However, when workers keep using vibrating hand-tools, there is a degeneration of the sensory nerves and loss of sensation in the fingers and hands (Lundborg et al. 1987; Pyykkö et al. 1990). Once injuries progress to this point, there is loss of sensory function and a decline in manual dexterity. The diminished dexterity exerts significant effects on quality of life with workers being unable to perform tasks that require fine motor coordination, such as buttoning a shirt (House, Krajnak, and Jiang 2016). Consequently, several investigations focused on finding engineering (Hewitt et al. 2015; Krajnak et al. 2015; McDowell et al. 2016; Welcome et al. 2016) or managerial (Cherniack et al. 2004; Krajnak et al. 2014) controls that diminish exposure. In cases where it is difficult or not possible to lower the exposure, the assessment of biological or physiological markers that might be reliably used to identify workers at risk of developing a permanent vibration-induced sensor-ineural injury are being investigated such that appropriate interventions may be employed to prevent development of permanent injuries.

There were also increases in the expression of the *nTrk* receptor, which when activated by nerve growth hormone and brain-derived neurotrophic factor, might serve as a signal for repair and nerve regeneration (Curtis et al. 1998; Obata et al. 2004; Richner et al. 2014). Previous investigators examined the effects of repetitive vibration on nerve injury and gene expression in peripheral nervous system and noted findings consistent with current data that increased expression of this receptor is indicative of nerve repair and regeneration (Krajnak et al. 2012a, 2013; ; Xu et al. 2011; Govindaraju et al. 2006a; Matloub et al. 2005; Yan et al. 2005). Based upon previous observations (Govindaraju et al. 2006b; Krajnak et al. 2016; Matloub et al. 2005), peripheral sensorineural function might return to pre-injury levels if exposure to vibration is terminated for a period of time in an otherwise healthy worker. However, in workers where symptoms developed or progressed to the point where there is a reduction in sensory function and loss of manual dexterity, recovery of normal function usually does not occur (Cherniack et al. 2004; House, Krajnak, and Jiang 2016). Therefore, in jobs where workers are exposed to hand-transmitted vibration, having a marker or test that might be used to identify changes in sensorineural function may be critical for early detection of an injury.

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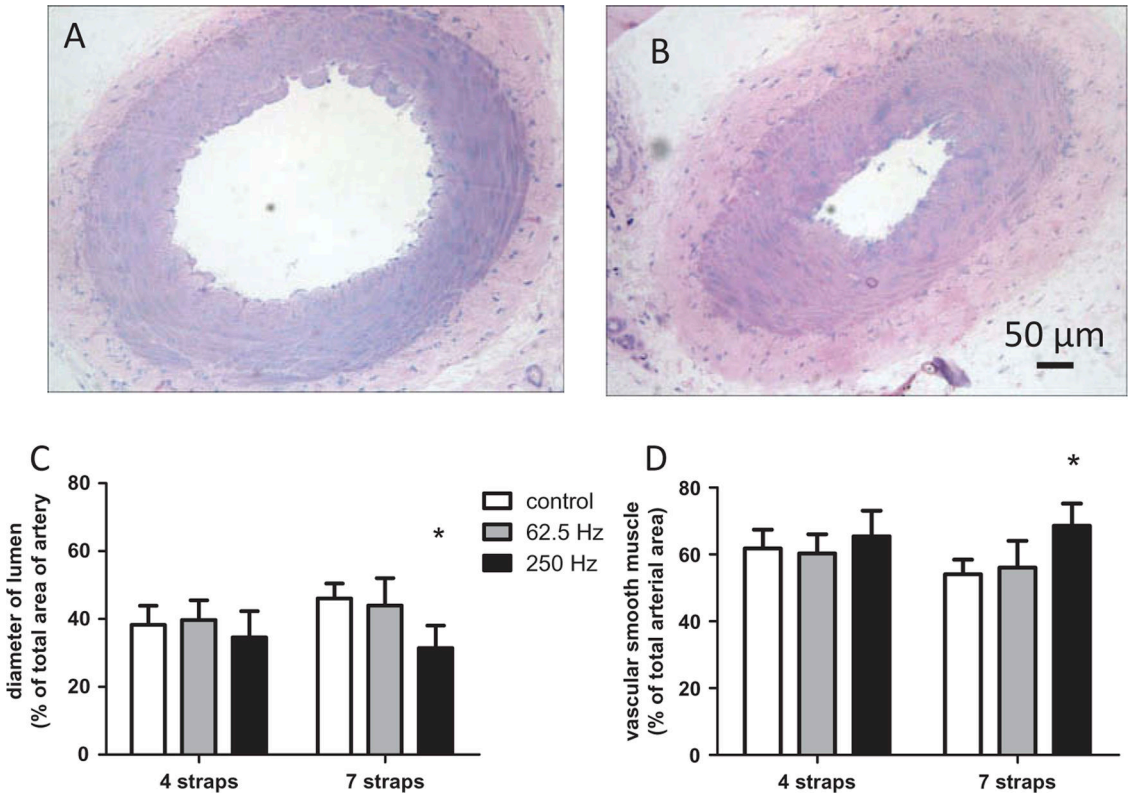

Figure 1.

Photomicrographs of arteries exposed to vibration at 250 Hz with 7 strap restraint (A is control and B is exposed animal). The graphs below show changes in area of the lumen (C) and VSM (D). Exposure to vibration at either frequency did not alter the area of lumen or vascular smooth muscle with 4 strap restraint. However, with 7 strap restraint, both area of the lumen was reduced and VSM was significantly increased at 250 Hz (*$p < 0.05$). Bar in Fig B = 50 µM.

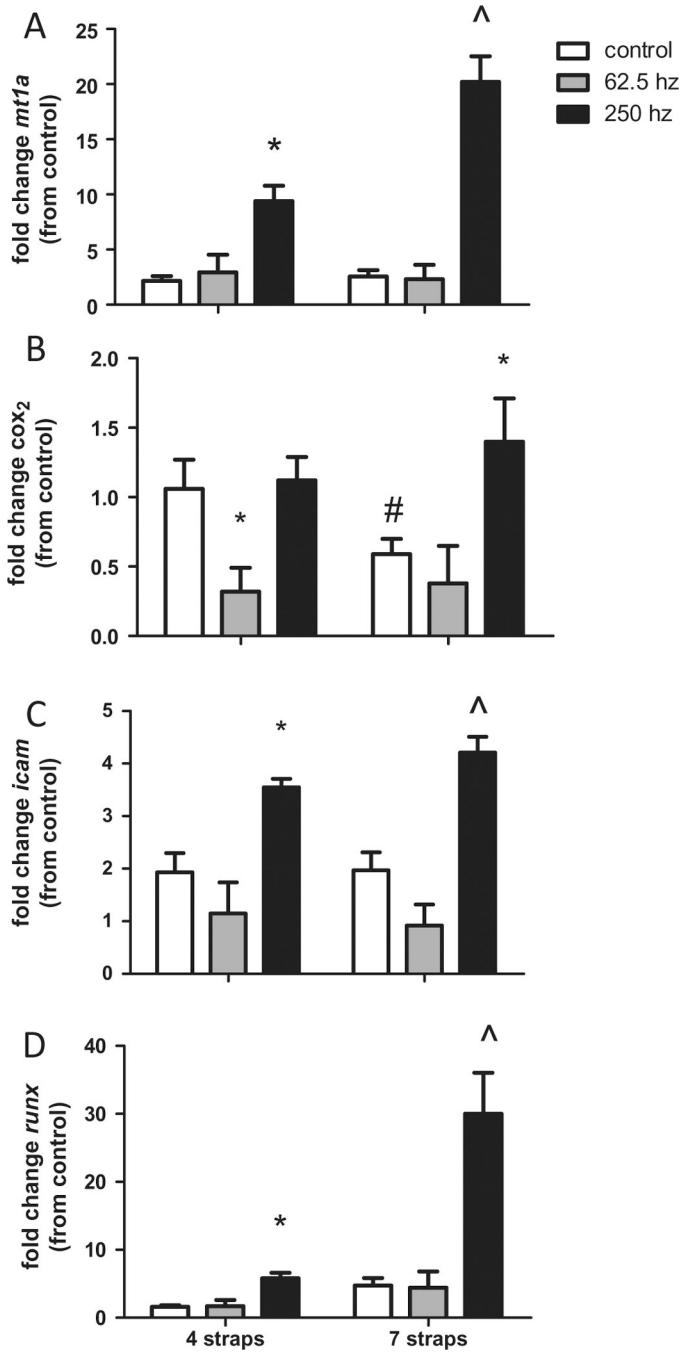

Figure 2.

Fold changes in gene expression for *mt1a* (A), *cox*$_2$ (B), *icam* (C), *and runx* (D) in ventral tail artery of rats exposed to vibration at 62.5 or 250 Hz with 4 or 7 restraint straps. With exposure to 250 Hz vibration there was a significant increase in expression of *mt1a*, *icam*, and *runx* under both restraint conditions (* different than control, ^ different than control and 4 strap restraint at the same frequency). There were also changes in *cox*$_2$ with exposure to 62.5 Hz and 4 strap restraint and 250 Hz 7 strap restraint (* $p < 0.05$ different than control, # different than 4 strap control).

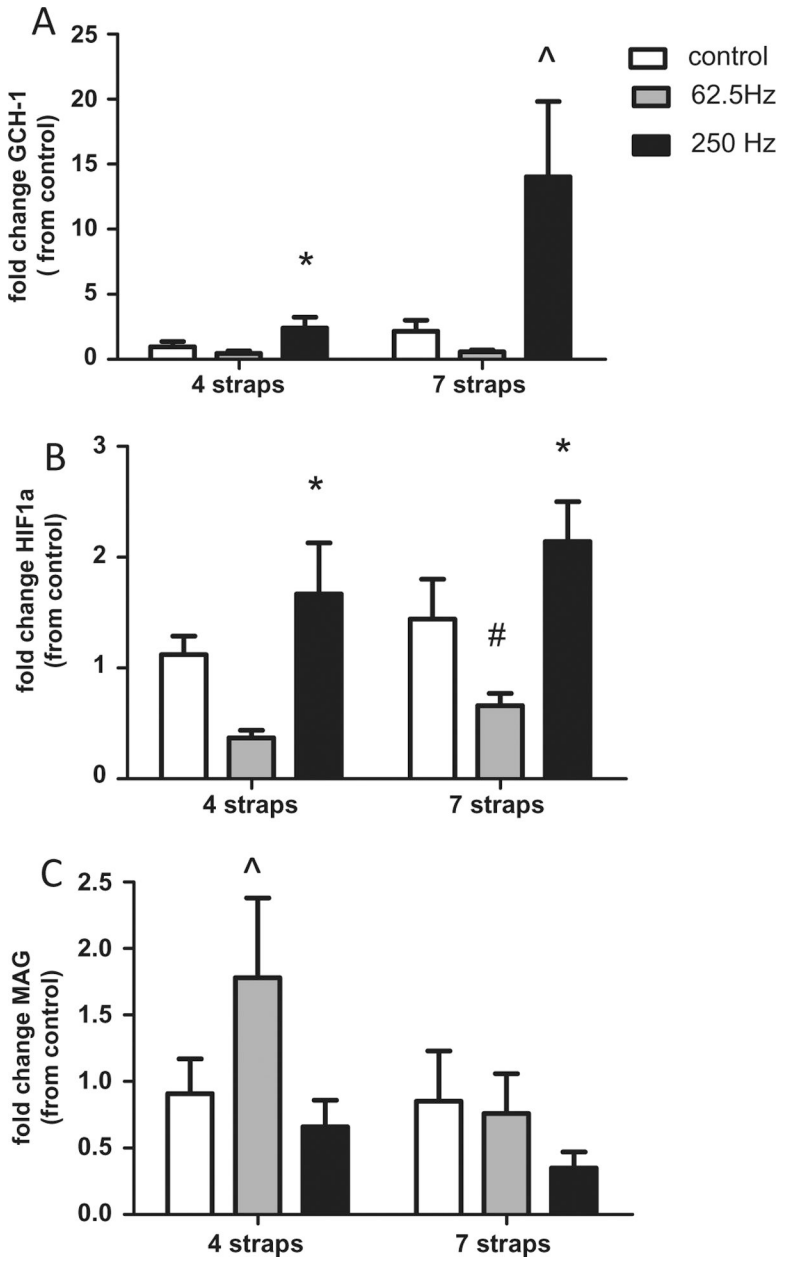

Figure 3.

Fold changes in gene expression in ventral tail nerve. Exposure to vibration at 250 Hz is resulted in an increase in expression of *cgh-1* (A), *hif1a* (B) with both 4 and 7 strap restraint (* different than control $p < 0.05$; ^ different than control and 4 strap restraint at the same frequency). *Hif1α* was reduced with exposure to 250 Hz and 7 strap restraint (# different than 4 strap control, $p < 0.05$). *Mag* increased with exposure to 62.5 Hz and 4 strap restraint (*different than control and 7 strap restraint).

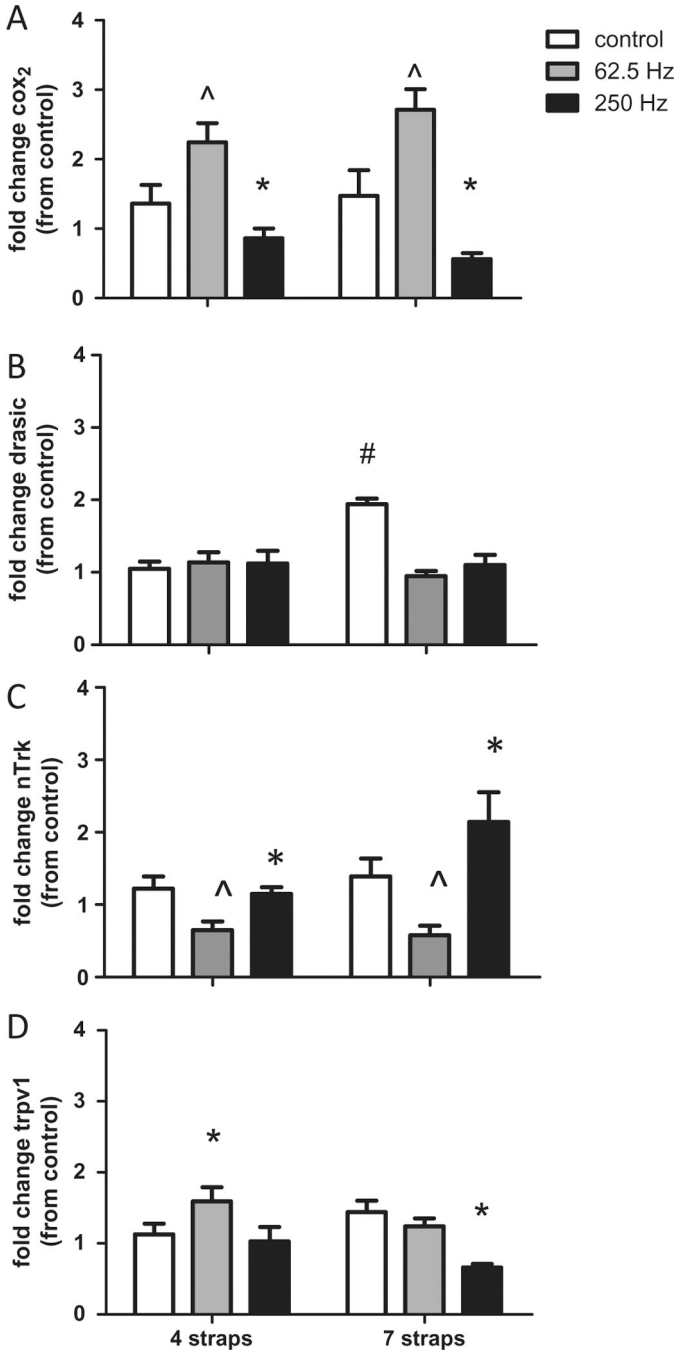

Figure 4.

Fold changes in gene transcription in DRG. Exposure to vibration at 62.5 Hz resulted in an elevation in *cox2* relative to controls or 250 Hz (A: ^ $p < 0.05$) and decrease in *nTrk* relative to controls and 250 Hz (B), regardless of the number of straps used for restraint. In contrast, cox_2 (A) was reduced and *nTrk* increased compared to controls and 62.5 Hz (* $p < 0.05$). Drasic expression in the DRG (B) was elevated in control animals with 7 strap restraint compared to 4 or 7 strap restraint and exposure to vibration at 62.5 or 250 Hz. Trpv1 expression (D) was elevated with exposure to 4 strap restraint and 62.5 Hz and reduced with

exposure to 250 Hz and 7 strap restraint (* different than control or other vibrated group, $p < 0.05$).

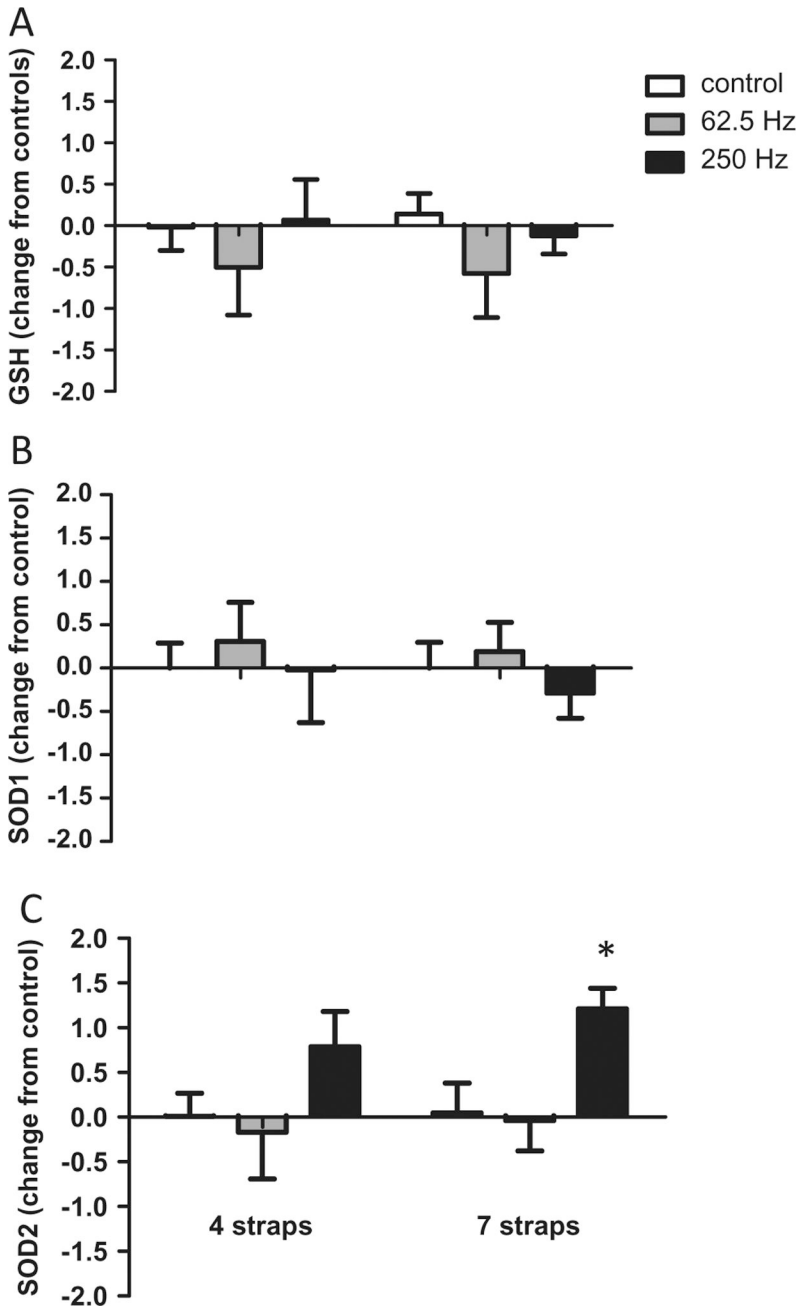

Figure 5.

Data represent change from control of anti-oxidants, GST (A), SOD1 (B) and SOD2 (C) in ventral tail nerve. The only enzyme that showed a significant difference was SOD2, where exposure to 250 Hz vibration increased concentration of enzyme when 7 straps were used for restraint (* different than controls and 62.5 Hz, $p < 0.05$).

Table 1.

This table lists the transcripts that were measured by PCR, their accession numbers and the primer sequences.

Transcript	Accession #	Forward (F) and Reverse (R) primer sequences	
COX2	S67722.1	COX-2-F	5’-ACCAACGCTGCCACAACT-3’
		COX-2-R	5’-GGTTGGAACAGCAAGGATTT-3’
DRASIC	AFO13598.1	DRASIC-F	5’-TGATGCATATGCCTGGAAAC-3’
		DRASIC-R	5’CACACACACGTGTCCTTTCG-3’
GSH	NM_012962	GSH-F	5’-GCTGGACAACGAGCGAGT-3’
		GSH-R	5’-GCTGCnCTCATCCTGCAA-3’
HIF1a	AC_000074.1	HIF1a-F	5’-AAGCACTAGACAAAGCTCACCTG-3’
		HIF1a-R	5’-CCATATCGCTGTCCACATCA-3’
iCAM	NM_012967	ICAM-F	5’-AATCTGACCTGCAGCCGGAAAG-3’
		ICAM-R	5’-GGAGCTAAAGGCAGGCACTTG-3’
MAG	NM_017190.4	MAG-F	5’-TCGCCTCACTGATACnCACG-3’
		MAG-R	5’-CTGAGTTGGGAATGTCTCCTG-3’
Mt1a	NM_138826.4	Mt1a-F	5’-CACCAGATCTCGGAATGGAC-3’
		Mt1a-R	5’-GCAGCAGCTCnCTTGCAG-3’
R18s	Ml 1188.1	18s-F	5’-AATCAGTTATGGTTCCTTTGTC-3’
		18S-R	5’-GCTCTAGAATTACCAGTTATCCAA-3’
nTRK	NM_0211589.1	nTrk-F	5’-CTCGGCTCAGTCACCTGAA-3’
		nTrk-R	5’-GCACAGTTTTCCAGGAGGAGG-3’
TrpV1	NM_031982.1	Trpv1-F	5’-GGTGTGCCTGACCTAGC-3’
		Trpv1-R	5’-CTCTTGGGGTGGGGACTC-3’
Runx	NM_017325.1	Runx1-F	5’-CCTCCTTGAACCACTCCACT-3’
		Runx1-R	5’-CTGGATCTGCCTGGCATC-3’
Sod1	NM_017050.1	Sod1-F	5’-TAAGAACATGGCGGTCCA-3’
		Sod2-R	5’-TGGACACATTGGCCACAC-3’
Sod2	NM_017051	Sod2-F	5’-TGGACAAACCTGAGCCCTAA-3’
		Sod2-R	5’-GACCCAAAGTCACGCnGATA-3’

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