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Force-induced tissue compression alters circulating hormone levels and biomarkers of peripheral vascular and sensorineural dysfunction in an animal model of hand-arm vibration syndrome

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Abstract

Workers regularly using vibrating hand tools may develop a disorder referred to as hand-arm vibration syndrome (HAVS). HAVS is characterized by cold-induced vasospasms in the hands and fingers that result in blanching of the skin, loss of sensory function, pain, and reductions in manual dexterity. Exposure to vibration induces some of these symptoms. However, the soft tissues of the hands and fingers of workers are compressed as a result of the force generated when a worker grips a tool. The compression of these soft tissues might also contribute to the development of HAVS. The goal of this study was to use an established rat tail model to determine the mechanisms by which compression of the tail tissues affects (1) the ventral tail artery (VTA) and ventral tail nerves (VTN), (2) nerves and sensory receptors in the skin, (3) dorsal root ganglia (DRG), and (4) spinal cord. Tissue compression resulted in the following changes (1) circulating pituitary and steroid hormone concentrations, (2) expression of factors that modulate vascular function in the skin and tail artery, and (3) factors associated with nerve damage, DRG, and spinal cord. Some of these observed effects differed from those previously noted with vibration exposure. Based upon these findings, the effects of applied force and vibration are different. Studies examining the combination of these factors might provide data that may potentially be used to improve risk assessment and support revision of standards.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Keywords

Tissue compression; arteries; nerves; peripheral sensory system

Introduction

Workers that regularly use vibrating hand tools as part of their job are at greater risk of developing hand-arm vibration syndrome (HAVS). HAVS is characterized by cold-induced vasospasms that result in (1) blanching of the fingers and hands, (2) loss of sensation, (3) pain, and (4) reductions in manual dexterity (Griffin 2012; Griffin and Bovenzi 2001), all of which might affect a worker's ability to perform their job and their quality of life (House, Krajnak, and Jiang 2016; Bovenzi 2010a, 2010b). Vibration exposure significantly contributes to the development of these symptoms by increasing the stress and strain within exposed tissues. Subsequently, tissue stress and strain might affect functioning of blood vessels, nerves, and sensory receptors in those tissues (Dong et al. 2004, 2021; Wu et al. 2006, 2010, 2017). Vibration might also alter autonomic nervous system function (Bovenzi 1989; Harada et al. 1990; Harada, Yoshida, and Kimura 1989; Sakakibara et al. 2002). Several investigators reported that individuals exposed to vibration display changes in blood flow in response to cold challenges (Harada, Yoshida, and Kimura 1989; Sakakibara et al. 2002) or exaggerated startle responses (Gemne and Taylor 1983; Stoyneva et al. 2003), indicating that the sympathetic nervous system might display exaggerated responses to stimuli in individuals that work with vibrating hand tools.

The International Standards Organization (ISO) standard 5349–1 provides guidance to help predict if workers are at risk of developing HAVS (ISO 2001)). To date, data presented in the standard focused specifically on the effects of vibration. However, the standard states that other risk factors may also contribute, such as (1) the force applied to soft tissues when a worker is using a hand-tool, (2) posture of the worker, (3) environment that the individual is working in, and (4) various other personal health related factors.

There have been a few studies examining the effects of grip force and tissue compression on physiological responses of workers using vibrating hand-tools (Bovenzi et al. 1990; Gurram, Rakheja, and Gouw 1995; Hagberg, Morgenstern, and Kelsh 1992; McDowell et al. 2006; Nishiyama et al. 1996). These investigations demonstrated that force-induced tissue compression adversely alters blood flow and sensory functions in exposed tissues. However, because the physiological and biological effects of tissue compression have not been well characterized, additional data need to be collected to clarify the effects of compression and vibration, individually, on measures of blood flow and sensory function in exposed tissues. Previously Krajnak et al. (2024) used a rat tail model to characterize the effects of ≤ 10-days exposure to 2 or 4 Newtons (N) applied force on blood flow, vascular responsiveness to vasoconstricting and dilating factors, responsiveness to transcutaneous electrical stimulation and sensitivity to applied pressure (Randall-Selitto test (Krajnak et al. 2024)). These force levels were selected because various studies demonstrated that the grip at the fingertip of a worker using a hand tool might be between 2 and 4 N (Seo and Armstrong 2008; Wu et al. 2017). Repeated compression of tissues using 2N of force increased blood flow as measured

by laser Doppler at the beginning of each exposure, and sensitivity to acetylcholine (ACh)-induced vasodilation with exposure to both 2 and 4N applied force. Exposure also resulted in an enhanced sensitivity to transcutaneous electrical stimulation at 250 hz, and an elevated sensitivity to applied pressure using the Randall-Selitto analgesia test (Krajnak et al. 2024). These data are consistent with the hypothesis that tissue compression that occurs while gripping leads to alterations in vascular and sensorineural function (Färkkilä et al. 1986; Nishiyama and Watanabe 1981; Riedel 1995; Wu et al. 2017). However, these changes differ from those noted with vibration alone. It is noteworthy that vibration exposure alone results in a reduction in blood flow and change in the 2000 hz current perception threshold (CPT) test in the fingers of humans and tails of rats in a model of vibration-induced injury (Griffin 2012; House et al. 2009; Krajnak et al. 2010, 2012; Sakaguchi et al. 2011). Understanding how each exposure variable affects mechanisms involved in inducing HAVS is important for determining the best methods for protecting workers against developing this disorder and for determining how to revise the ISO 5349 standard.

To identify the mechanisms underlying the effects of applied force on the fingers, or finger-like structures such as the rat tail, the aim of this study was to examine the effects of 10 days of exposure to 2N or 4N applied force on transcript expression or immunostaining for various markers of oxidative stress, inflammation, tissue repair and remodeling, as well as arterial morphology (see Table 1). The transcripts and proteins that were examined were selected as these constitute markers of certain cellular pathways that were found to be affected by exposure to hand-transmitted and/or tail vibration (Krajnak et al. 2012, 2010; Waugh et al. 2016) Because these markers show changes with exposure to vibration alone (Krajnak et al. 2010, 2012, 2016; Waugh et al. 2016), measuring the same factors with exposure to applied force allows for comparisons between the effects of vibration and the effects of applied pressure. These markers were assessed in exposed skin, arteries, nerves, and other tissues involved in sensorineural function including the dorsal root ganglia (DRG) and spinal cord. Circulating hormone concentrations were also measured to determine if the exposure, or potential associated stress, may affect hormone levels. The duration of the exposure was selected based upon exposures performed in previous studies with vibration alone (Krajnak, Riley, et al. 2012). The force levels were selected based upon the reported force measured at the finger-tips in workers using hand-held vibrating tools, and based upon studies examining the influence of force-induced tissue compression on physiological responses of the vascular and sensorineural systems of the rat tail (Krajnak et al. 2024; Dong et al. 2023; Wu et al. 2017).

Methods

Animals

The tissue used in these studies was collected from animals used for physiological measures reported in another paper (Krajnak et al. 2024). Male (n = 18) Sprague-Dawley rats (Hla®(SD)CVF®, 6 weeks of age and approximately 200–230 g at arrival), were purchased from Hilltop Lab Animals, Inc. (Scottdale, PA). All rats were free of viral pathogens, parasites, mycoplasma, *Helicobacter*, and cilia-associated respiratory bacillus. Upon arrival, rats were acclimated to the AAALAC International accredited animal facilities at NIOSH

for one week. The NIOSH animal facility is a specific, pathogen-free, environmentally controlled facility. Animals were housed in ventilated micro-isolator units supplied with HEPA-filtered laminar flow air (Lab Products OneCage; Seaford, DE), Teklad Sanichip and Shepherd Specialty Paper's Alpha-Dri cellulose, tap water, and auto-claved Teklad rodent diet (Harlan Teklad; Madison, WI) available *ad libitum*. Rats were housed in pairs, and under controlled light cycle (12 hr light/12 dark cycle) and temperature (22–25 °C) conditions. One week following acclimation to the facilities, rats were randomly assigned to restraint control conditions (n = 6 rats) or to an applied force condition of 2 or 4 newtons (N; n = 6 rats at each force level. The use of animals, housing, exposures, and all other procedures performed were reviewed and approved by the Institutional Animal Care and Use Committee and are 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.

Exposure

After acclimation to the facilities, rats were acclimated to restraint for 5 days. Acclimation to restraint was performed prior to beginning the exposure. The Broome style restrainers employed for this experiment were sufficient such that animals could move but were not able to turn around or rear up onto their hind legs. Acclimation to restraint was performed by starting with 1 hr exposure in the restrainer, and then increasing the length of the exposure by 1 hr/day until the rats were acclimated to 4 hr continuous restraint. After 5 days restraint treatment the experiment commenced. Animals were exposed to compression or control conditions. The tails of rats exposed to compression were placed on the holding platform, and the pressure platform was lowered onto the middle of their tail (approximately at C12–20). The length of the loading plate acting on the tail was 53 mm. The tail contact width measured in a test with cadaver tails was 4.49 mm for 2.07 N and 5.09 mm for 4.03 N. The contact width at different forces was measured to show the compression of the tail tissues at different forces. Hence, it is estimated that the average contact pressure on the living tail was approximately 8.24 kPa for 2.07 N compression and 14.1 kPa for 4.03 N compression. Additional details regarding the characterization of the system and physical effects of pressure applied on the tail are described in (Dong et al. 2023). Once the tail and applied compression apparatus were in place, the tail was marked such that the same region was exposed each day. Each animal was exposed to control (restraint only) or applied force at 2 or 4N for 4 hr/day for 10 consecutive days.

On the morning following the last treatment, rats were anesthetized using 100–300 mg/kg (i.p.) sodium pentobarbital euthanasia solution and exsanguinated by cardiac puncture. Tails were dissected and sections of skin, nerve, VTA, DRG and spinal cord were collected and stored in cryovials with RNA-Later for qRT-PCR (Thermo fisher, Waltham, PA) at room-temperature for 72 hr and then transferred to the refrigerator for storage for up to 1 month before RNA was isolated. Another set of tissue was put into Tissue Tek (Sigma-Aldrich, St Louis, MO) in cryomolds and stored at −80°C for morphological analyses and localization of proteins using immunohistochemistry.

Serum Hormone Assays

Blood was collected via cardiac puncture during euthanasia and was centrifuged at 1250 g. Serum was collected and stored in 100 μl aliquots at −80°C. Estradiol, testosterone, progesterone, follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) were measured in duplicate serum samples (10–50 μl, depending on the assay) utilizing ELISA kits. All assay kits were purchased from Calbiotech (El Cajon, CA), and assays performed according to the manufacturer's protocol.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

qRT-PCR was performed to determine if the tissue compression induced by the application of force resulted in changes in transcript levels in the ventral tail artery (VTA), skin, DRG and spinal cord using the methods described in (Hughes et al. 2009, Krajnak, Waugh, et al. 2006; Krajnak et al. 2007). The following transcripts were measured: cytokines [interleukin (*Il*)-1β, *Il6* and tumor necrosis factor (*Tnf*)-α], hypoxia-induced factor 1 (*Hif1*), vasomo-dulating factors [neuronal, inducible and endothelial nitric oxide synthase (*nNos*, *iNos and eNOS*) respectively], factors involved in vascular remodeling [vascular endothelial growth factor (*Vegf*) and endothelin (*Et1*)], and antioxidant enzymes [cata-lase (*Cat*) and superoxide dismutase-2 (*Sod-2*), metallothionine (*Mt1*), glutathione peroxide (*Gpx*), glutathione synthase (*Gsh*)], the immediate early genes and transcriptional regulators [e transcription factor BTB and CNC homology 1(*Bach1*), *c-Fos*, cyclic-AMP response element binding protein (*Creb*)] and markers of myelination, neural injury and synapse density [myelin basic protein (*Mbp*), neuronal injury protein-2 (*Ninj2*), postsynaptic density protein 95 (*Psd95*)]. These transcripts were selected based upon their responses to vibration exposures in tail tissues in previous studies (Krajnak et al. 2010, 2012), and based upon the physiological findings in these animals (Krajnak et al. 2024).

Processing of tissue for morphology and immunohistochemistry

Skin from the exposed area of the tail, a segment of the VTA, ventral tail nerve (VTN), and the DRG from the L1–5 region of the spinal cord were processed as follows: tissue was placed on a mounting platform, frozen in Tissue Tek and 20 μm cross-sections were cut on a cryostat. A total of 5 slides/animal was collected. The first section was placed on the first slide, the second on the second slide etc. Once there was a section on each of the 5 slides, the next section was placed back onto slide 1. Sections (5–10 depending on the tissue type) were collected on slides, with each section on a slide being 100 μm in distance from the adjacent section on that slide.

Immunohistochemistry

Immunohistochemistry was performed as described by (Kiedrowski et al. 2015; Krajnak, Waugh, et al. 2006). Briefly, one slide was selected from each tissue type. Slides were thawed and tissue circled with a pen which produced a liquid proof barrier. Sections were fixed for 5 min in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), rinsed in PBS, and incubated overnight at 4°C in primary antibody at dilution of 1:1000 in PBS + 0.3% TX-100 (buffer) with 10% normal donkey or goat serum (depending on the antibody). Table 1 lists the specific antibodies used, and the tissue(s) in which they were utilized.

Slides were rinsed, incubated in the appropriate secondary antibody diluted 1:800 in buffer at room temperature for 1hr, rinsed again in PBS, and the procedure was repeated using another primary and secondary antibody. After completing the immunohisto-chemistry, slides were air dried in the dark overnight, cover slipped using Fluoromount G with DAPI (Fisher Scientific, Pittsburgh PA) and stored at 4°C until imaged and quantified. All primary antibodies were purchased from Santa Cruz Biotechnology (Dallas, Tx) and fluorescent secondary antibodies (Cy2 and Cy3) from Invitrogen Life Sciences Technologies (Eugene Oregon). The antibodies and the tissues used are presented in Table 1. Mitotracker-red staining (Thermo fisher, Waltham, MA) was also examined in the VTA using a procedure described in (Krajnak 2020).

All sections were imaged on a DP73 Olympus microscope at 20 × magnification. Photomicrographs (3–5) were taken of each tissue type. To ensure all pictures were taken using the same settings, several sections were imaged to determine the best threshold for identifying immunostaining. That threshold was then employed for photographing all sections of a particular tissue type.

Quantification of immunostaining was conducted using ImageJ (National Institutes of Health; Bethesda, MD). Photos were imported into ImageJ, set to grayscale, and a threshold was set for identifying staining. This threshold was maintained for all sections of a specific tissue type and stained for a particular protein. The area of interest was outlined and the % outlined area that was immuno-stained was measured along with the intensity of the stained area. Immuno-labeled neurons were also counted in the DRG for antigens that labeled neurons. The mean cell number or area that was immuno-stained in a particular region was then utilized for data analysis.

Data Analyses

A delta-delta ($^{\Delta\Delta}$) Ct was calculated for each transcript using β-actin as the housekeeping gene. The average change in the $^{\Delta\Delta}$ Cts (or the fold change from control) were analyzed using one-way analysis of variance (ANOVA). Pairwise comparisons were made using Tukey tests. The internal and external diameter of each section of VTA and arteries in the skin, along with measurements of vascular smooth muscle (VSM) thickness were also collected and means calculated. Average diameters and muscle thickness, and mean area of immunos-taining/section were also analyzed by 1-way ANOVAs followed by post-hoc-Tukey tests as described above. For all analyses, differences with $p < 0.05$ were considered statistically significant.

Results

Serum hormone assays

Force-induced compression of the tail resulted in changes in all hormones measured (Figure 1). Serum progesterone and testosterone levels were significantly lower after exposure to 4N compression compared to control or 2N of compression (Figure 2). Serum estradiol concentrations were significantly reduced following treatment with 2N and 4N compression compared to control conditions (Figure 1c). FSH concentrations were not markedly altered

following exposure to 2N or 4N compression (Figure 1d). It is noteworthy that, TSH concentrations were significantly higher in animals exposed to 4N compared to controls (Figure 1e).

Transcript expression in tissues

Table 2 shows fold changes (compared to controls) in transcript expression in the skin. Exposure to 2N of force did not markedly alter the expression of any transcripts in the skin. However, exposure to 4N force resulted in a significant rise in protein expression of *eNos* and reduction in *Sod2* as compared to controls. In contrast *Bach1* protein expression in the skin of animals exposed to 4N was markedly reduced as compared to animals exposed to 2N.

Table 3 presents fold changes in transcript expression in ventral tail arteries. Exposure to 4N of force significantly elevated protein expression of *Cat, Et1a* and *Hif1a* in the VTA. In contrast, the only transcript that exhibited a significant change in expression after exposure to 2N of force was *Hif1a* which was reduced in the VTA in response to tissue compression that occurred in response to exposure to 2N applied force.

Table 4 demonstrates alterations in transcript protein expression in the DRG. Treatment with 2N force resulted in a significant reduction in *Cgrp* and *Gsh* and a significant rise in *Il1β*. Exposure to 4N of force initiated a significant increase in the protein expression of *nNos* and *Psd95*. The 4N treatment also decreased *Il1β* protein expression compared to exposure to 2N compression in the DRG.

Table 5 shows transcript changes in the spinal cord. There were no marked alterations in transcript protein expression in the spinal cord in animals exposed to 2N applied force. However, treatment with 4N force resulted in a significant elevation in the protein expression of *Creb* and *nNos*. Levels of *nNos* were also significantly higher following exposure to 4N compression compared to treatment with 2N compression.

Immunostaining

Table 6 presents the average area of immunostaining in the endothelium and vascular smooth muscle (VSM) of arteries, and in sensory receptors in the skin. In the skin, the only change in immunostaining in arteries was a reduction in TNFα staining in the VSM of animals exposed to 2N force. In sensory receptors, there was an increase in eNOS immunostaining in animals exposed to either 2 or 4N force.

Table 7 displays the mean area of immunostaining in the endothelium and vascular smooth muscle of the VTA. Exposure to 2N force resulted in a reduction in iNOS staining in the endothelium of the tail artery as compared to control animals. There were no other exposure-related changes in immunostaining in the endothelium or VSM.

Table 8 shows mean area of immunostaining in the DRG and VTNs of control and exposed animals. The average number of CGRP and nitrotyrosine stained cells is presented in Figure 3. Exposure to 2 and 4N force produced an increase in synaptophysin (Figure 2a, d-f), nNOS (Figure 3, B, 3 G-3I), and iNOS (Figures 2(Figures 2c, j-l) staining in the DRG as compared

to staining in controls. The number of neurons stained for nitrotyrosine (Figure 4a-d) was also higher in animals exposed to 4N compared to animals exposed to 2N applied force.

The average area of immunostaining in the VTN is also presented in Table 8. There were no exposure-related changes immunostaining in the VTN.

Histology

The internal and external diameter of arteries from the C12 region of the tail were also measured and findings are illustrated in Figure 5D. Exposure to compression resulted in a reduction in the internal diameter of the artery (Figure 5A-5B). However, this change in the diameter was only significant with exposure to 2N (Figure 5B), but not 4N (Figure 5C). Although the thickness of the vascular smooth muscle increased with exposure to both 2 and 4N of force these findings were not statistically significant (Figure 5E).

Discussion

The ISO-5349 (ISO 2001) standard states that several factors, including compression of the tissues of the hands while gripping a tool, may contribute to the development of HAVS. Data using a model designed to examine the effects of applied force (Dong et al. 2023) demonstrated that compression in response to 2N or 4N force resulted in an increase in blood flow, an enhanced sensitivity of small myelinated fibers to electrical stimulation and an elevated sensitivity to pressure using the Randall-Selitto test (Krajnak et al. 2024). The findings reported here differ from than those seen with vibration alone (Krajnak et al. 2010, 2012, 2024) (Table 9). Overall, the results of this study demonstrated that enhanced sensitivity to pressure applied in the Randall-Selitto test was associated with elevations in protein transcript expression of markers associated with oxidative stress and inflammation in nerves, and markers of synapse remodeling in the DRG and spinal cord. Immunohistochemical changes were consistent with alterations in transcript protein expression in the sensorineural system. In the VTA, the only changes noted with exposure to 2N force were a decrease in *Hif1a* transcript protein expression and diminished TNF-α immunostaining in the vascular smooth muscle (VSM). Treatment with 4N force resulted in increases in transcript protein expression in factors associated with oxidative stress, hypoxia, endothelial cell function and vascular remodeling. The alterations in transcript and protein expression in different tissues are consistent with the physiological effects previously reported (Krajnak et al. 2024).

Changes in transcript protein expression in sections of skin from regions that were compressed were different depending upon the level of compression which was dependent upon the level of force (Dong et al. 2023). There were no marked changes in transcript protein expression with exposure to 2N applied force. However, with exposure to 4N force decrease in *Bach1* and *Sod2* expression was noted. *Bach1* was initially identified as a gene that played a role in the development of cancer (Zhang et al. 2018). However additional data demonstrated that *Bach1* might regulate the production of new blood vessels and reduce atherosclerosis due to oxidative stress (Jia et al. 2022; Yusoff et al. 2021). A fall in *Bach1* suggests that oxidative stress was reduced following exposure to 4N applied force. If diminished oxidative stress was present, this may also explain why levels of transcript

for the antioxidant *Sod2* were lowered. Expression to 4N applied force also elevated protein expression of *eNos*, *Et1a*, *Il6* and *Vegf*. Changes in the expression of these factors has been associated with vasodilation and/or oxidative stress (*eNos*), (Hughes et al. 2009; Kennedy et al. 1999; Krajnak et al. 2009; McKenna et al. 1993; Noel 2000Nakamura et al. 1996; Stoyneva et al. 2003) inflammation (*Il6* (Villar-Fincheira et al. 2021),; and vascular remodeling and angiogenesis (*Il6*, *Et1a*, *Vegf*; (Coultas, Chawengsaksophak, and Rossant 2005; McKenna et al. 1993; Noel 2000; Stoyneva et al. 2003Nakamura et al. 1996; Villar-Fincheira et al. 2021). The only change in immunostaining in arteries of the skin was a reduction in TNFα in the VSM following treatment with 2N applied force. TNFα is an inflammatory factor that can induce oxidative stress (Hartge, Unger, and Kintscher 2007; Krajnak et al. 2010, 2012). The decreased levels of TNFα may have contributed to the fact that there were no marked alterations in oxidative stress or factors involved in vascular remodeling associated with exposure to 2N.

The changes seen in response to force-induced tissue compression in the VTA are a bit different than those noted in the skin. In the skin, exposure to 4N of force increased factors involved in vascular remodeling and vasodilation (e.g., *Il6*, *eNos* and *Vegf*) but reduced expression of the antioxidant (*Sod2*). In the VTA, there was a rise in *Hif1a* transcript levels following the 4N exposure. These changes suggest that hypoxia in the tails of animals may have been induced with exposure to 4N but not 2N force. Exposure to 4N force also enhanced the expression of *Cat*, *eNos*, *Et1a and Tnfa*. in the VTA. These alterations are consistent with the postulation that tissue compression that occurs as a result of exposure to applied force may regulate vascular factors involved in inflammation, oxidative stress (Hughes et al. 2009; Simpson, Mickelson, and Lucchesi 1987), vasodilation (Hughes et al. 2009; Krajnak et al. 2009) and vascular remodeling (McKenna et al. 1993; Noel 2000Nakamura et al. 1996; Stoyneva et al. 2003). These changes such as vasodilation and increased vascular remodeling may in part be responsible for the elevation in tail blood flow following treatment with applied compression noted in the previous study (Krajnak et al. 2024).

The effects of 4N applied pressure on transcripts such as *eNos* and *Et1* in the arteries are similar to those reported in Primary Raynaud's phenomenon. The symptoms of primary Raynaud's phenomenon are similar to those noted in workers with HAVS. These symptoms include cold-induced blanching of the fingers and/or toes, which might induce pain attributed to lack of blood flow to the affected area (Cherniack 1990; Stringer and Femia 2018) Primary Raynaud's phenomenon is more frequent primarily a disorder of the smaller blood vessels in the skin that might result of endocrine changes, genetics or local dysfunction of the autonomic nervous system. HAVS is also characterized by cold-induced blanching of the fingers and/or toes (Cherniack 1990; Eger et al. 2014), but might be associated with occlusion or thrombosis of larger arteries in the finger, hands and toes, and is believe to be initiated by vibration-induced stress and on the soft tissues of the fingers (Krajnak et al. 2010; Noel 2000; Thompson and Griffin 2009). Previous investigators demonstrated that vibration reduces blood flow both immediately after an acute exposure and after repeated exposures (Krajnak et al. 2024; Krajnak, Waugh, and Sarkisian 2019; Terada et al. 2007). In this study, blood flow in animals exposed to 4N of applied force returned to pre-exposure values when the force was removed, and animals exposed to 2N

of applied force actually exhibited a rise in blood flow. This is similar to what is seen in occlusion-reperfusion studies (Headrick, Angello, and Berne 1990). Vibration applied pressure and occlusion/reperfusion have been associated with nerve damage and therefore without additional data, it is difficult to state how these factors act together to produce the sensorineural effects detected with HAVS. Future studies might examine the physiological and biological effects of vibration and applied pressure together to provide additional data regarding the etiology of vibration-induced disorders in workers.

Interestingly, morphometric measures (i.e., internal, and external diameter of ventral tail arteries and vascular smooth muscle thickness) revealed that the internal diameter of the arteries was significantly reduced and the VSM thickness was slightly increased in animals exposed to 2N, but not 4N compression. Previously Krajnak et al. (2024) reported that animals exposed to 2 or 4N of applied force exhibited a rise in blood flow over with increasing numbers of days of exposure, with the effects being more pronounced in the 2N group. The blood flow data seems inconsistent with the morphology data; An increase in blood flow should induce a vasodilation (Bovenzi, Lindsell, and Griffin 2000; Egan et al. 1996; Sakaguchi et al. 2011). The inconsistency in these findings may have been attributed to the time measures were collected. Blood flow was collected in live, non-anesthetized animals immediately before and after exposure to applied force (Krajnak et al. 2024). However, in this experiment, tail arteries were collected the morning following the final exposure. The tail artery normally does not display basal tone (Krajnak, Dong, et al. 2006). However, it is conceivable that exposure to 2N force induced an increased sensitivity to sympathetic nervous system input, thereby enhancing basal tone in the tail artery, and that this basal tone diminished the internal diameter of the artery. Because there appeared to be some hypoxia in the arteries from the 4N group as evidenced by an increase in *Hif1a* expression, it is possible that the arteries did not develop basal tone and remained dilated to enable greater blood flow and more oxygen into the VSM. Future studies comparing applied force with force plus vibration may help clarify if there is an alteration in basal tone of the artery and how co-exposure to these factors affects changes in vascular morphology, blood flow and responsiveness to vasoconstricting and dilating factors.

These changes in the expression of factors regulating vascular function might alter sensitivity to tactile stimuli. Nerves are sensitive to both applied force or compression (Cole, Steyers, and Graybill 2003; Hardy et al. 1992; Lundström 2002; Thonnard et al. 1999) and to reductions in blood blow (Loffredo et al. 2009; Lundborg et al. 1990). The alterations in transcript expression in the skin may have also been the result of changes that occurred in response to compression of other orga-nelles or tissue types in the skin. For example, there are data demonstrating that elevations in expression of in *Vegf* contribute to an enhanced sensitivity to pain after injection of the inflammatory chemical, carrageenan, in the skin (Uedaa et al. 2023). If exposure to applied force activates pathways that are similar to those induced by carragee-nan (e.g. inflammation of the nerve), a compression-induced rise in *Vegf* may have contributed to the elevated sensitivity to applied pressure during the Randall-Selitto test by acting on peripheral sensory receptors or nerves. In addition, eNOS immunolabeling was increased in sensory receptors in the skin from animals exposed to 2 and 4N applied force. An increase in blood flow within the nerve may also enhance pain

sensitivity by delivering inflammatory factors or by increasing edema (Pacurari, Waugh, and Krajnak 2019; Krajnak et al. 2007; Krajnak et al. 2012a).

Force-induced compression did not markedly alter immunolabeling for growth factors or factors indicative of myelin damage in VTNs. However, there were changes in both transcript protein expression and immunolabeling in the DRG of animals exposed to compression. Exposure to 2N compression increased expression of *Il1β* and lowered in *Cgrp* and *Gsh* transcript levels. The reduction in *Cgrp* was not associated with a change in immunostaining. However, a longer exposure may have initiated alterations in CGRP immunolabeling. For example, workers with HAVS exhibited elevated serum levels of CGRP (Tekavec et al. 2024). Our findings also demonstrated exposure to vibration at the resonant frequency increased the number of neurons immunostained for CGRP (Kiedrowski et al. 2015; Krajnak et al. 2013; Krajnak, Miller, et al. 2012; Pacurari, Waugh, and Krajnak 2019) In the DRG, and in circulating CGRP concentrations (Krajnak et al. 2016). Additional studies comparing the combined effects of vibration and compression may clarify if increases in CGRP in the circulation and DRG occur following exposure to vibration and/or with exposure to pressure.

The elevation in *Il1β* transcript levels in the DRG indicates that there may have been some inflammation following exposure to 2N applied force and this may have been associated with the reduction in transcription of the antioxidant enzyme *Gsh* aa well as rise in eNOS and iNOS immunolabeling in the DRG. Changes in *Gsh* and transcripts for *Nos* were detected in previous studies after exposure to vibration (Kiedrowski et al. 2015; Krajnak, Riley, et al. 2012; Pacurari, Waugh, and Krajnak 2019). The elevation in NOSs might promote an increase in blood flow to the DRG or enhance responses of the synapses in the DRG in response to the repeated exposure, and thereby enhance neuronal transmission (Pacurari, Waugh, and Krajnak 2019). This scenario would be consistent with the findings of this study demonstrating that exposure to 4N force results in a rise in *nNos* and *Psd95* transcript protein expression levels and BDNF and synaptophysin immunostaining in the DRG. Exposure to 4N force was also associated with a decrease in *Il1β* in the DRG as compared to levels of *Il1β* expression at 2N. Based upon the effects of force alone, one might predict that alterations in *Il1β* might be more pronounced with exposure to the greater level of force. However, it is possible that 4N force did induce and enhance inflammation, but that this occurred earlier during the exposure (e.g. after 3–5 days), and that by the time measures were collected, the levels of *Il1β* were not markedly different than control levels. Although the precise timing of the effects of different levels of applied pressure needs to be determined, changes in the expression of these transcripts and proteins suggest that there are new synapses being formed or strengthening of existing synapses in the DRG (Richner et al. 2014). Similar changes were found in the DRG with exposure to vibration (Krajnak, Miller, and Waugh 2018; Krajnak, Miller, et al. 2012; Pacurari, Waugh, and Krajnak 2019) and may in part underlie longer term changes in sensorineural function found in workers with HAVS (Krajnak, Riley, et al. 2012; House, Krajnak, and Jiang 2016; Poole and Frost; Poole, Robinson, and Frost 2020).

Changes in transcript levels with exposure to 4N force in the spinal cord are consistent with the alterations seen in the DRG. Exposure to 4N of force increased *Creb* and *nNos* transcript

levels compared to controls. *Psd95* transcript levels were higher following treatment with 4N of force compared to 2N, but transcript levels after exposure were not significantly different that those seen in controls. Exposure to both vibration and applied force, or a longer exposure, may result in changes similar to those detected in the DRG, indicating that these exposures may induce alterations in the transmission of sensory signals to the central nervous system by modulating synapses in the periphery.

Exposure to applied force might also result in an enhanced stress response. Although several studies focused on the response of the hypothalamic-pituitary-adrenal (HPA) axis (Kalil et al. 2013), noted that steroid hormones modulate the response of the HPA axis to stress. Therefore, steroid and anterior pituitary hormone concentrations were measured after exposure to force (Hueston and Deak 2020; Oettel and Mukhopadhyay 2004; Taraborrelli 2015). Measurement of steroid hormone concentrations demonstrated that exposure to 4N force significantly reduced circulating progesterone and testosterone concentrations, while exposure to either 2 or 4N of force elevated circulating estradiol concentrations. These results are consistent with findings of other studies showing that restraint stress reduces circulating testosterone levels in male bats (Alonge et al. 2023). This decrease does not appear to be due to changes in steroid synthesizing enzymes, but instead attributed to the effects of stress on the hypothalamus and pituitary (Alonge et al. 2023). There are also studies showing that psychosocial stress in humans results in reductions in testosterone and increases in estradiol in both males and females (Handa et al. 2022; Pletzer et al. 2021). In both human and animal investigations, it has been reported that stress results in a rise in circulating progesterone concentrations (Hueston and Deak 2020; Oettel and Mukhopadhyay 2004; Taraborrelli 2015). However, in this experiment progesterone was lower in animals exposed to 4N force than animals in the other two groups. The regulation and site of synthesis for progesterone during stress is unclear. Both gonadectomy and adrenalectomy did not appear to exert a marked effect on circulating progesterone levels (Kalil et al. 2013). However, progesterone in males is involved in stimulating spermatogenesis, and higher concentrations of FSH also may have contributed to diminished progesterone synthesis.

Stress also induces the synthesis of progesterone in local tissues including Schwann cells, oligoden-drocytes or astrocytes, that insulate nerve fibers and promote nerve regenerations after injury (Baulieu and Schumacher 2000; Deniselle et al. 2003; Hueston and Deak 2020; Oettel and Mukhopadhyay 2004; Taraborrelli 2015). Progesterone synthesis was not detected anywhere except in the serum, and therefore it is difficult to determine if compression affected synthesis of progesterone in these other tissues. Progesterone was also reported to affect vascular and endothelial cell function, and the reduction in progesterone levels may have contributed to enhanced blood flow noted in animals exposed to compression (Hueston and Deak 2020; Oettel and Mukhopadhyay 2004; Taraborrelli 2015). Future studies might examine markers of progesterone synthesis and progesterone receptors in non-endocrine tissues to determine if the local effects of compression on progesterone levels vary in different tissues.

Circulating TSH concentrations were also measured. TSH is released by the anterior pituitary and regulates the release of thyroid hormone, and subsequently, thyroid hormones feedback onto the pituitary and hypothalamus to reduce the release of TSH (Koivisto et

al. 2009; Mendoza and Hollenberg 2017). Therefore, it is conceivable that the increase in TSH found in animals exposed to 4N compression might be due to a decrease in thyroid hormone levels. Thyroid hormone plays a significant role in maintaining metabolism and reductions in this hormone might be associated with changes in metabolism and cardio- and peripheral vascular function (Cheng, Leonard, and Davis 2010; Mendoza and Hollenberg 2017). Thyroid hormone levels may be reduced by stress (Mendoza and Hollenberg 2017), or by alterations in circulating gonadal steroids (Harada et al. 1990; Hart, Charkoudian, and Miller 2011). In future studies comparing applied force and force plus vibration, thyroid hormone, TSH and thyroid weights might be measured to determine the effects of the exposures on thyroid hormone regulation and the regulation of other systems in the body.

Conclusions

Data demonstrated that effects of applied force and tissue compression on arteries, skin and the sensorineural system, are not the same as those previously reported after exposure to vibration only (Krajnak et al. 2010, 2012). For example, in the VTA, exposure to 10 day force-induced compression at 4N resulted in an increase in *Hif1* expression indicating that exposure induced hypoxia in the arteries. Vibration exposure alone did not result in an elevation in *Hif1* and therefore it is not likely that 10 days of vibration exposure induced hypoxia (Krajnak et al. 2010). The effects of applied force on nerves also differed from effects of vibration. Compression appeared to induce more rapid changes in sensorineural function, especially at 4N, than vibration alone (Krajnak, Miller, et al. 2012). These changes in function were not associated with major changes in morphology or immunostaining in the VTN, which is different than that noted with vibration (Krajnak, Miller, et al. 2012). However, there were prominent alterations, especially in growth factors and factors involved in synaptic remodeling in the DRG with pressure. Although some of these changes are seen with exposure to vibration, these were not as prominent (Krajnak, Miller, et al. 2012; Pacurari, Waugh, and Krajnak 2019) as the alterations found with exposure to applied force (See Table 9 for a comparison of the effects of applied force vs vibration). Based upon these data, it is clear that studies examining the combined effects of exposure to vibration and applied force are needed. Data on the effects of the combined exposure might potentially be used to improve risk assessment and support revision of the ISO-5349 standard.

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Data sharing

A link to the data will be available upon acceptance of the manuscript.

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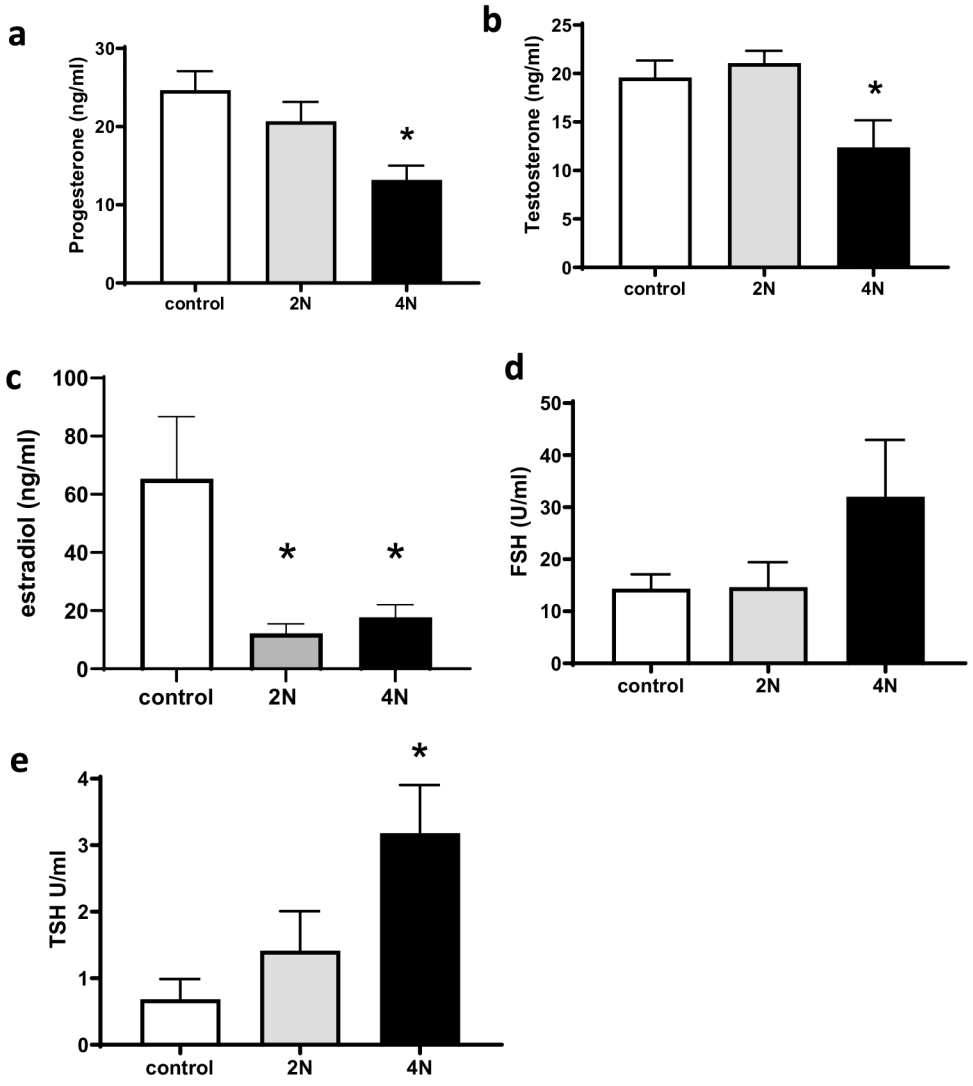

FIGURE 1.

Serum concentrations of various hormones (mean ± sem). Exposure to 4N of force resulted in a reduction in circulating progesterone (a), testosterone (b) and estrogen (c) concentrations and an increase in follicle stimulating hormone (FSH, d) and thyroid stimulating hormone (TSH, e) concentrations (n = 6/condition; *Significant difference from control, $p < 0.05$).

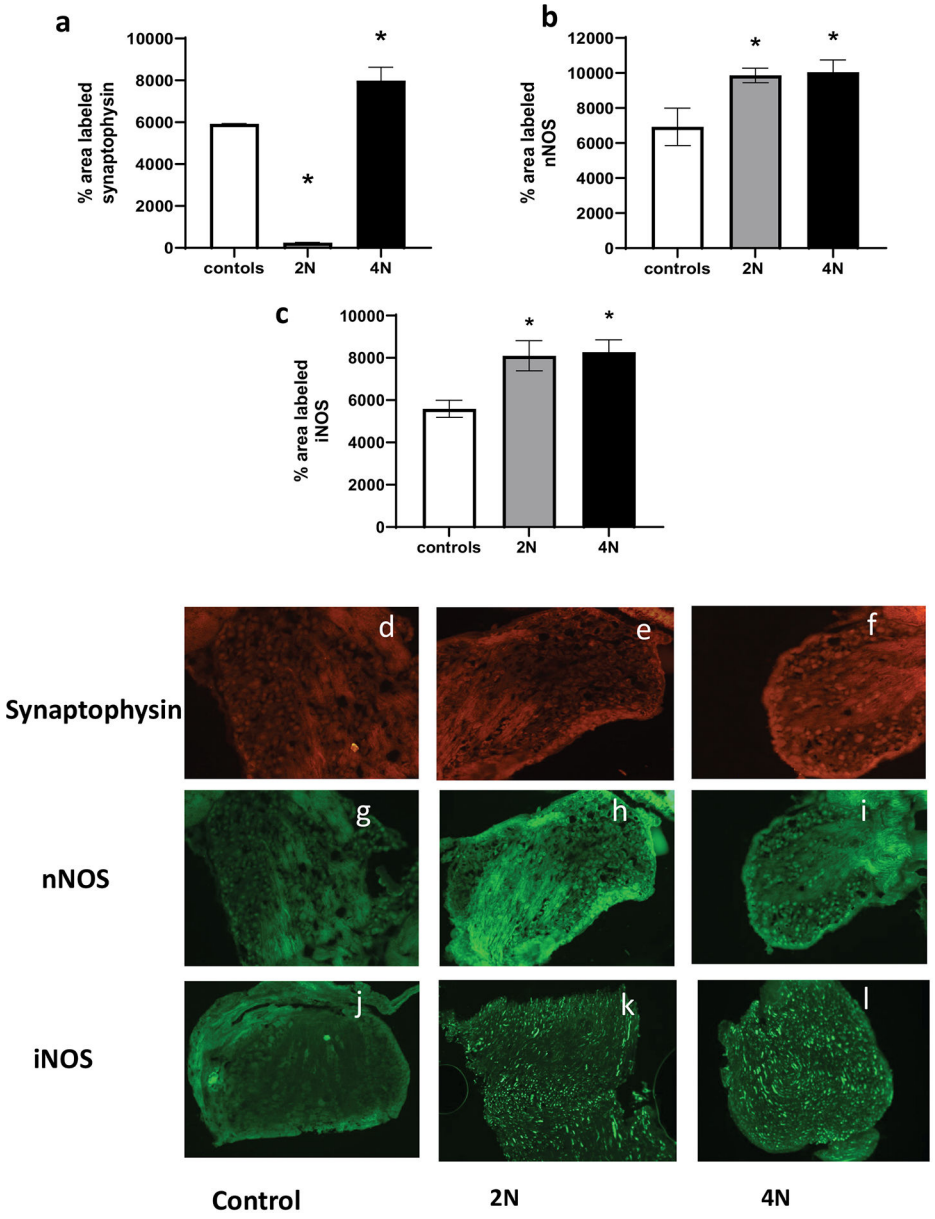

FIGURE 2.

The graphs and photomicrographs show the %area immunolabeled for synaptophysin (a, d-f), nNOS (b, g-i) and iNOS (c, j-l) with exposure to 2 or 4N in the DRG. Exposure to 2 and 4N of force resulted in increases in nNOS and iNOS in the DRG. Exposure to 2N resulted in a decrease, and the 4N exposure resulted in an increase in synaptophysin in the DRG (n = 5-6/condition;. *Significant difference from control, $p < 0.05$).

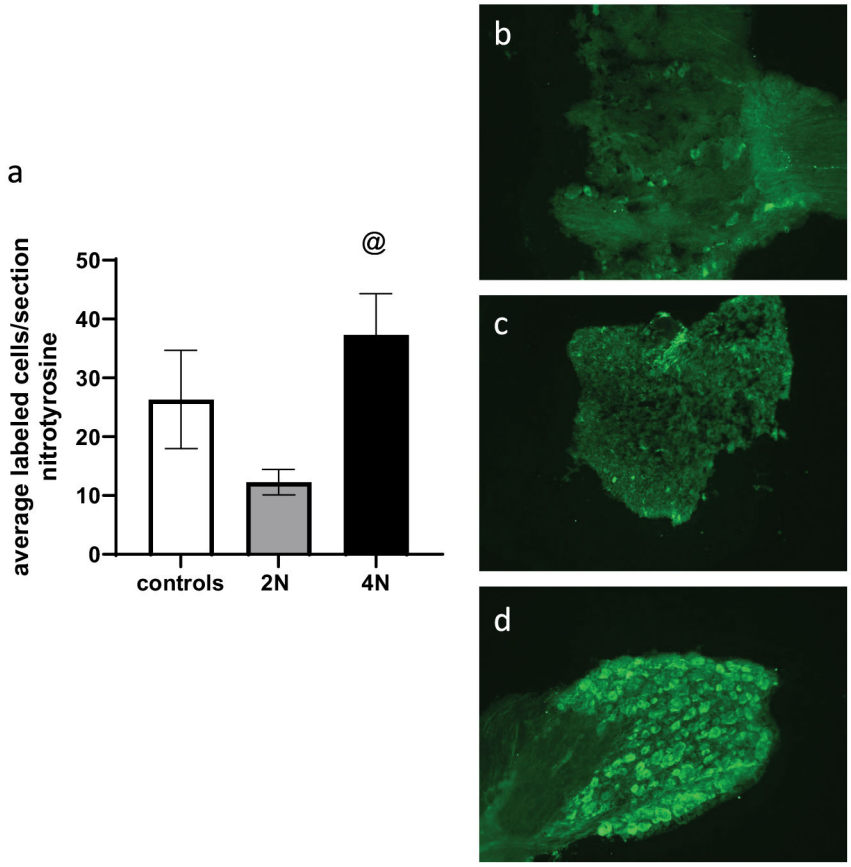

FIGURE 3.

The number of neurons immunostained for nitrotyrosine in the DRG (a) and photomicrographs from DRG from control animals (b) and animals exposed to 2N (c) or 4N (d) of course. The number of labeled neurons was greater in DRG from animals exposed to 4N of than 2N of applied force (n = 5-6/condition; @p < 0.05).

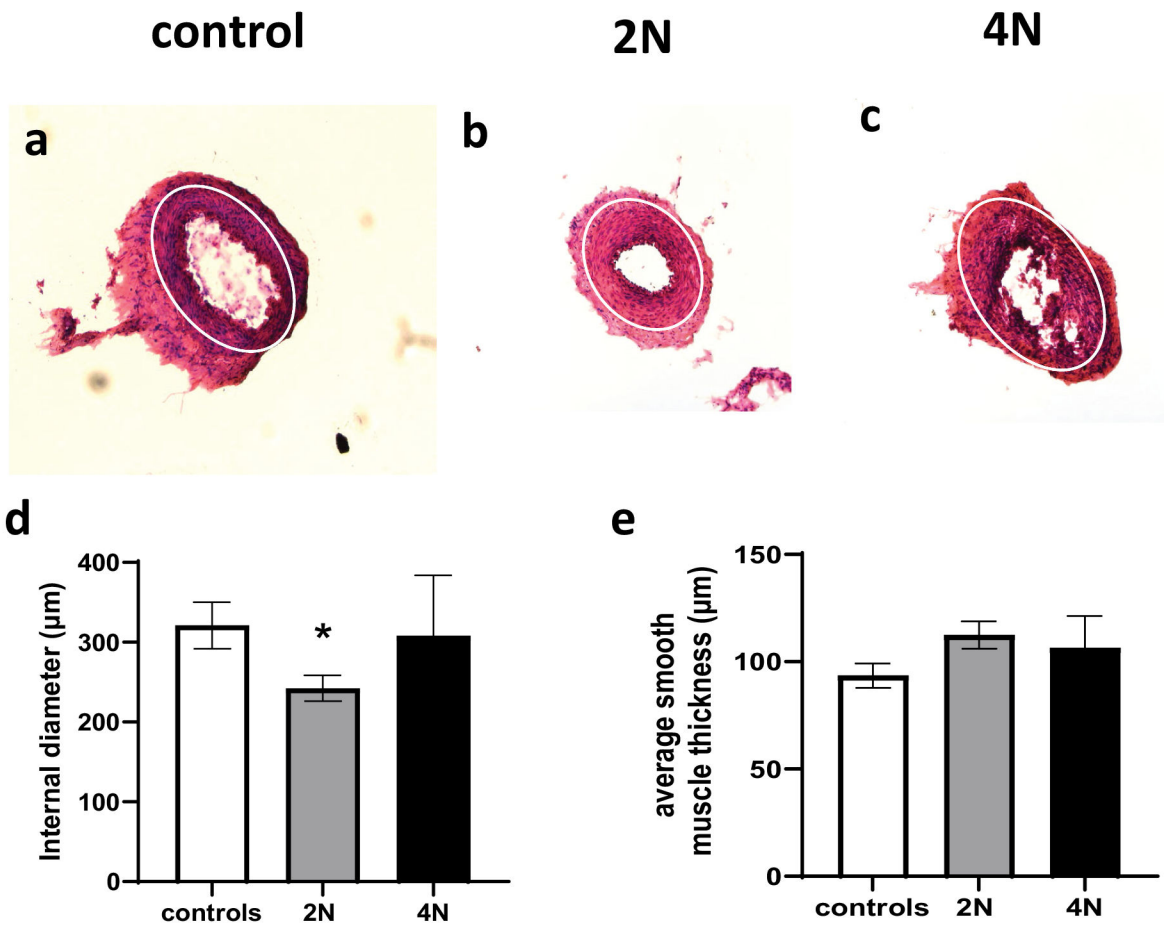

FIGURE 4.

The photomicrographs of the ventral tail artery (a–c). The circle shows the external perimeter of the vascular smooth muscle of the artery. The tissue outside the circle is connective tissue. Exposure to 2N of force resulted in a reduction in the internal diameter of the artery as compared to controls (a–c and d, n = 5-6/condition; *significant difference from control, $p < 0.05$). Although there was a slight increase in the thickness of the vascular smooth muscle with exposure to applied force, this difference was not significant (a–c and e). *Significant difference from control, $p < 0.05$).

TABLE 1.

All Antibodies Were Anti-Mouse Primary Antibodies (Santa Cruz Biotech, Dallas Tx). Used at a Dilution of 1:1000 and All Secondary Antibodies Were from Invitrogen (Invitrogen, Eugene OR)). Abbreviations: Brain-Derived Neurotrophic Factor (BDNF), Calcitonin-Gene-Related Peptide (CGRP), Cyclic Nucleotide Phosphatase (CNPase), Glial-Derived Neurotrophic Factor (GDNF), Myelin-Basic Protein (MBP), Endothelial Nitric Oxide Synthase (eNOS), Inducible Nitric Oxide Synthase (iNOS), Neuronal Nitric Oxide Synthase (nNOS), PGP 9.5 Neuronal Marker (Also Ubiquitin Carboxyl-Terminal Hydrolase L3), Tyrosine Hydroxylase (TH), Tumor Necrosis Factor (TNF)α, and Vascular Endothelial Growth Factor (VEGF)

	Skin	Ventral tail artery	Ventral tail nerve	DRG
BDNF				X
CGRP	X			X
CNPase			X	
GDNF				X
MBP	X		X	
ENOS	X	X		X
INOS	X	X		X
NNOS	X	X	X	X
Nitrotyrosine	X	X	X	X
PGP9.5	X			
Synaptophysin				X
TH				X
TNFα	X	X	X	X
VEGF	X	X		

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TABLE 2.

Transcript Levels in the Skin from Tails of Control Rats, and Tails of Rats Exposed to 2 or 4 Newtons (N) of Applied Force. β-Actin was Used as a Control and is Expressed as the Average Ct Value ± Sem. All Other Values are the Fold Change from Control of the $^{\Delta\Delta}$Ct Value. Bold * Values are Different Than Controls ($p < 0.05$), and ^ Different Than Controls ($p < 0.07$) and @ Different Than 2N ($p < 0.05$), $N = 5$-6/condition

Transcript	Control	2N applied force	4N applied force
B-actin	18.33 (0.11)	18.62 (0.12)	18.65 (0.07)
Bach	1.08 (0.19)	1.37 (0.17)	**0.69 (0.04)@**
eNOS	1.09 (0.20)	0.96 (0.19)	**1.79 (0.17)***
Et1a	1.03 (0.11)	0.97 (0.10)	**1.41 (0.16)**^
IL6	0.42 (0.21)	0.82 (0.19)	**2.63 (0.58)**^
Sod2	1.39 (0.37)	0.92 (0.26)	**0.41 (0.12)***
Tnfa	0.89 (0.13)	0.62 (0.15)	1.38 (0.36)
Vegf	1.02 (0.09)	1.59 (0.48)	**2.30 (0.39)**^

TABLE 3.

Transcript Levels in the Ventral Tail Arteries from Tails of Control Rats, and Tails of Rats Exposed to 2 or 4 Newtons (N) of Applied Force. β-Actin was Used as a Control and is Expressed as the Average Ct Value ± Sem. All Other Values are the Fold Change from Control of the $^{\Delta\Delta}$Ct Value. Bold * Values are Different Than Controls ($p < 0.05$), @ Different Than 2N ($p < 0.05$), and ^ Different Than Controls ($p < 0.07$) $N = 5$-6/condition

Transcript	Control	2N applied force	4N applied force
B-actin	20.62 (0.15)	19.76 (0.13)	20.62 (0.12)
Cat	1.25 (0.42)	1.04 (0.30)	**3.18 (0.55)***
eNOS	1.15 (0.27)	2.57 (0.73)	**3.65 (0.90)^**
Et1a	0.94 (0.25)	1.04 (0.16)	**3.11 (0.87)***
Hif1a	1.03 (0.10)	**0.67 (0.13)***	**2.24 (0.21)*@**
Tnfα	1.16 (0.29)	1.91 (0.37)	**2.30 (0.27)^**

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TABLE 4.

Transcript Levels in the DRG of Control Rats, and Tails of Rats Exposed to 2 or 4 Newtons (N) of Applied Force. β-Actin was Used as a Control and is Expressed as the Average Ct Value ± Sem. All Other Values are the Fold Change from Control of the $^{\Delta\Delta}$Ct Value. Bold * Values are Different Than Controls ($p < 0.05$), @ Different Than 2N ($p < 0.05$), and ^ Different Than Controls ($p < 0.07$), N = 5-6/condition

Transcript	Control	2N applied force	4N applied force
B-actin	20.75 (0.72)	20.17 (0.29)	19.70 (0.10)
Cgrp	1.70 (0.70)	**0.69 (0.29)***	2.90 (0.39)
Gsh	1.06 (0.16)	**0.54 (0.12)***	0.77 (0.12)
IL1β	0.84 (0.19)	**2.84 (1.04)***	**0.51 (0.11) @**
nNos	1.24 (0.36)	0.31 (0.01)	**6.22 (1.47)***
Psd95	1.84 (0.71)	0.74 (0.45)	**3.69 (0.42)***
Sod2	1.68 (0.53)	0.83 (0.30)	2.71 (0.62)^
Tnfα	0.81 (0.17)	0.71 (0.06)	2.53 (0.81)^

TABLE 5.

Transcript Levels in the Spinal Cord of Control Rats, and Tails of Rats Exposed to 2 or 4 Newtons (N) of Applied Force. β-Actin was Used as a Control and is Expressed as the Average Ct Value ± Sem. All Other Values are the Fold Change from Control of the Δδct Value. Bold * Values are Different Than Controls ($p < 0.05$), @ Different Than 2N ($p < 0.05$), and ^ Different Than 2N ($p < 0.05$, $N = 5$-6/condition)

Transcript	Control	2N applied force	4N applied force
B-actin	17.58 (0.36)	17.42 (0.08)	17.14 (0.06)
Creb	1.85 (0.03)	1.93 (0.03)	**2.00 (0.03)***
nNos	1.09 (0.17)	0.74 (0.04)	**1.57 (0.09)*@**
Psd95	1.25 (0.14)	0.87 (0.06)	**1.28 (0.18)@**

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TABLE 6.

The Average Area Immunostained (SEM), in the Endothelial Cells of Arteries, the Vascular Smooth Muscle (VSM) or Sensory Receptors in the Skin. Endothelial Nitric Oxide Synthase (eNOS) Labelling was Greater in the Sensory Receptors of Animals Exposed to 2 or 4N Applied Force. There Were No Other Effects of the Exposure on the Skin. Abbreviations: Calcitonin Gene-Related Peptide (CGRP), Inducible Nitric Oxide Synthase (iNOS), Neuronal Nitric Oxide Synthase (nNOS), Myelin Basic Protein (MBP), Protein Gene Product 9.5 (PGP9.5), Tumor Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor (VEGF). Bold * Values Different That Controls ($p < 0.05$). Missing Data Means Measures Were Not Collected from Those Regions, $N = 5$-6/condition

	Arteries		
Primary antibody	**Endothelial area**	**VSM area**	**Sensory receptors area (μm^2)**
CGRP			
control			95058 (4307)
2N			45918 (10286)
4N			48660 (5821)
eNOS			
control	6945 (2995)	18620 (5759)	639 (383)
2N	3242 (2562)	25973 (23209)	1278 (209)*
4N	9207 (3013)	24168 (6910)	1411 (147)*
iNOS			
control	7065 (1326)	124 (35)	392 (246)
2N	3039 (425)	97 (27)	107 (110)
4N	7034 (1769)	167 (18)	656 (414)
nNOS			
control	5280 (815)	16093 (3413)	122 (157)
2N	2424 (466)	6802 (784)	2315 (575)
4N	6235 (1726)	19282 (4368)	1738 (320)
MBP			
control			1368 (165)
2N			1654 (156)
4N			1665 (113)
Nitrotyrosine			
control	11346 (3700)	171 (27)	872 (169)
2N	46370 (1660)	117 (28)	976 (135)
4N	12837 (4257)	190 (29)	1186 (149)
PGP9.5			
control			57669 (4090)
2N			59615 (4538)
4N			72388 (9291)
TNFα			
control	99 (7)	64 (6)	153 (12)
2N	77 (6)	37 (5) *	116 (13)

	Arteries		
Primary antibody	**Endothelial area**	**VSM area**	**Sensory receptors area (μm^2)**
4N	103 (111)	60 (7)	144 (18)
VEGF			
control	5850 (1249)	14050 (4193)	130 (17)
2N	2626 (532)	7540 (116)	228 (49)
4N	6270 (1490)	18343 (4688)	189 (41)

TABLE 7.

The Average Area Immunostained (SEM), in the Endothelial Cells and Vascular Smooth Muscle (VSM) of the Ventail Tail Artery. Exposure to 2N of Pressure Reduced Inducible Nitric Oxide Synthase (iNOS) in the Endothelial Cells When Compared to Controls. There Were No Other Effects of the Exposure on the Ventral Tail Artery. Abbreviations: Endothelial Nitric Oxide Synthase (eNOS), Interleukin (Il)6, Inducible Nitric Oxide Synthase (iNOS), Tumor Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor (VEGF). Bold * Values Different That Controls ($p < 0.05$), $N = 5$-6/condition

	Ventral tail arteries	
Primary antibody	**Endothelial Area**	**VSM Area**
α-Actin		
control	412 (65)	1489 (127)
2N	369 (58)	1396 (161)
4N	501 (107)	2057 (477)
Endothelial		
control	419 (69)	4 (1)
2N	347 (35)	3 (0.9)
4N	426 (38)	4 (2)
eNOS		
control	552 (70)	1341 (237)
2N	456 (66)	1951 (211)
4N	741 (124)	1509 (259)
Il6		
control	406 (46)	1536 (231)
2N	308 (38)	1675 (165)
4N	580 (193)	1747 (359)
iNOS		
control	628 (89)	1595 (205)
2N	344 (56)*	1589 (215)
4N	580 (104)	1929 (362)
Mitotracker		
control	397 (71)	1161 (169)
2N	286 (49)	1472 (86)
4N	500 (137)	1332 (268)
TNFα		
control	350 (49)	2800 (582)
2N	396 (45)	2703 (380)
4N	389 (68)	2648 (416)
VEGF		
control	605 (37)	1635 (210)
2N	599 (87)	1905 (71)
4N	667 (49)	1506 (155)

TABLE 8.

The Average Area Immunostained (± SEM), in the DRG and Ventral Tail Nerve. Exposure to 2 or 4N of Pressure Resulted in an Increase in nNOS, iNOS and Synaptophysin in the DRG. Nitrotyrosine was Lower After Exposure to 2N of Applied Force Than After 4N. Bold * Values Different That Controls ($p < 0.05$), and Bold @ are Different from 2N Values ($p < 0.05$). Missing Data Means Measures for That Antibody Were Not Collected from Those Regions. Abbreviations: Brain-Derived Neurotrophic Factor (BDNF), Cyclic Nucleotide Phosphatase (CNPase), Calcitonin Gene Related Peptide (CGRP), Glial-Derived Neurotrophic Factor (GDNF), Myelin Basic Protein (MBP), Neuronal Nitric Oxide Synthase (nNOS), Inducible NOS (iNOS), Endothelial NOS (eNOS), Protein Gene Product 9.5 (PGP9.5), Proliferating Cell Nuclear Antigen (PCNA),tumor necrosis factor (TNF)-α.

Primary Antibody	Average labeled neurons/section DRG	Average labeled area/section DRG	Average labeled area/section nerve
BDNF control		7190 (1217)	3053 (717)
2N		8835 (748)	2784 (941)
4N		9713 (799)	2782 (941)
CNPase control			3054 (834)
2N			3927 (834)
4N			2684 (792)
CGRP control	25(5)	7376 (487)	2812 (433)
2N	20 (4)	7438 (760)	2294 (434)
4N	27 (4)	6503 (866)	2833 (496)
GDNF control		7857 (824)	1881 (261)
2N		7956 (903)	2933 (622)
4N		8740 (903)	1348 (264)
MBP control			2173 (662)
2N			2580 (488)
4N			2722 (715)
nitrotyrosine control	26 (8)	7421 (589)	2108 (900)
2N	12 (2)	6916 (775)	2571 (485)
4N	37 (7)@	61756 (770)	2571 (742)
nNOS control		5591 (402)	1860 (227)
2N		8097 (713)*	2850 (662)
4N		8268 (585)*	1412 (263)
iNOS control		623 (802)	3082 (824)
2N		9860 (655)*	3154 (932)

Primary Antibody	Average labeled neurons/section DRG	Average labeled area/section DRG	Average labeled area/section nerve
4N		10004 (655)*	4347 (341)
eNOS control			2960 (500)
2N			2120 (379)
4N			3171 (479)
PGP9.5 control			3220 (924)
2N			2998 (746)
4N			4696 (242)
PCNA control	8 (1)	7700 (229)	
2N	17 (4)	7908 (113)	
4N	15 (3)	7142 (131)	
TH control		7566 (901)	
2N		8160 (1217)	
4N		9071 (988)	
Synaptophysin control		5924 (541)	
2N		8762 (505)*	
4N		7988 (640)*	
TNFα control	19 (6)	7314 (248)	
2N	22 (5)	8563 (110)	
4N	18 (3)	7150 (112)	

TABLE 9.

Summary of Effects with Exposure to Applied Force or Vibration (250 hz, 49 m/sec^2). Information for This Table Came from (Krajnak et al. 2024; Krajnak et al., 2012; Krajnak et al. 201, *N*= 5-6/condition)

	2N pressure only	4N pressure only	Vibration (250 hz) only
Blood flow (laser Doppler)	↑↑	↑	↓
Phenylephrine-induced vasoconstricton	—	—	—
Acetylcholine-induced vascular re-dilation	↑↑	↑↑	↓↓
Randall-Selitto applied pressure (change in sensitivity	↑	↑	↑
CPT test (change in sensitivity) 2000 hz	—	—	↓↓
250 hz	↑↑	↑	—
Oxidative stress-mediated factors (*Cat, Sod2*, nitrotyrosine)	—	—	↑↑
Inflammatory factors (*Il1ß, Tnfα*)	—	↑	↑
Vascular remodeling factors (*Et1, Vegf, Il6*)	↑	↑↑	↑
Vasomodulating factors (*eNos, iNos, nNos*, Et1)		↑↑	↓ (*Nos*)
Neural/sensory factors (*Cgrp, Psd95, PCNA, synaptophysin*)	↑	↑	↑