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Applied Force Alters Sensorineural and Peripheral Vascular Function in a Rat Model of Hand-Arm Vibration Syndrome

Kristine Krajnak, PhD,

Christopher Warren, MS,

Xueyan Xu, PhD,

Phillip Chapman, PhD,

Stacey Waugh, MS,

Theresa Boots, MS,

Daniel Welcome, MS,

Renguang Dong, PhD

Physical Effects Research Branch, National Institute for Occupational Safety and Health, Morgantown, West Virginia.

Abstract

Objective: This study described the effects of applied force (grip) on vascular and sensorineural function in an animal model of hand-arm vibration syndrome (HAVS).

Methods: Rat tails were exposed to 0, 2, or 4 N of applied force 4 hr/d for 10 days. Blood flow and sensitivity to transcutaneous electrical stimulation and pressure were measured.

Results: Applied force increased blood flow but reduced measures of arterial plasticity. Animals exposed to force tended to be more sensitive to 250-Hz electrical stimulation and pressure applied to the tail.

Address correspondence to: Kristine Krajnak, PhD, National Institute for Occupational Safety and Health (NIOSH), 1000 Fredrick Land, Morgantown, WV 26508 (ksk1@cdc.gov).

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Conclusions: Effects of applied force on blood flow and sensation are different than those of vibration. Studies examining co-exposures to force and vibration will provide data that can be used to determine how these factors affect risk of workers developing vascular and sensorineural dysfunction (ie, HAVS).

Keywords

hand-arm vibration syndrome; vascular; sensorineural; laser Doppler; pressure; force

Occupational exposure to hand-transmitted vibration (HTV) has been associated with the development of hand-arm vibration syndrome (HAVS). Hand-arm vibration syndrome is characterized by cold-induced finger blanching,[1–3] alterations in peripheral sensory function,[4–6] and reductions in grip strength and manual dexterity.[7–12] Based on epidemiological studies the International Organization for Standardization established a standard for the measurement and assessment of the HTV exposure, ISO-5349-1.[13] Since then, studies examined the physical, physiological, and cellular responses to vibration and have provided a better understanding of the aspects of vibration that pose the greatest risk for inducing injury (ie, specific frequencies and amplitudes of the exposure[14–20]). The findings from these studies have been used to develop an alternative method for assessing vibration-induced white finger—the hallmark of HAVS.[21]

Workers using vibrating hand tools are not only exposed to vibration but also exposed to pressure at the fingers tips and hands that is applied during tool gripping, awkward postures, and other environmental exposures such as changes in temperature. Research has demonstrated that all these factors can affect the risk of developing HAVS or other occupationally related upper limb disorders.[22–25] An international standard has been developed to describe how to measure and evaluate hand forces and contact pressures.[26] Another standard has also been proposed to help assess the contribution of the coupling hand forces to vibration health effects.[27] However, the force weighting recommended in this standard did not include an explanation of how force may the symptoms that are seen in workers with HAVS, but instead, it described how force dependency of the vibration transmissibility on the wrist and arm substructures measured in a single experiment. Therefore, this standard may not be suitable for assessing the risk of the vibration-induced white finger and other finger disorders, which are the most important components of HAVS. Data describing how applied force by itself, and in combination with vibration, contribute to the development of HAVS can be used to revise the standards that provide information that can be used to more accurately predict risk and improve the development of vibration-reducing tools and protective equipment.

The goal of this experiment was to determine how force, similar to that experienced while gripping a tool, affects peripheral vascular and sensorineural function. Peripheral vascular and sensorineural dysfunction are the most common symptoms seen in workers using vibrating, as well as other hand tools.[28–32] To determine how applied force affects vascular and sensorineural function, a rat-tail model that was developed to characterize the physiological and biological effects of vibration was modified.[33,34] Studies using the initial rat-tail model have shown that the physiological responses of the tail to vibration

are similar to the responses of the human finger[34] and that the resonant frequency is in the same range as that of the human finger.[33] The difference between the finger and the rat tail is that the tail is not as stiff as the human finger and weighs less. However, it has a similar stiffness to mass ratio as indicated by the frequency response,[33] but the restraint or pressure applied on the tail was less than that seen at the fingertips of a human gripping a hand tool.[35–37] Therefore, the amplitude of the response of the tail at the resonant frequency is greater than that of the human finger.[33] Applying force (or grip) in combination with vibration may alter the biodynamic (or physical), physiological, and biological responses of tissues to vibration.[37–40] Because applied force might also have its own effects of tissue, it is important to characterize the effects of force and vibration exposure separately and then in combination to determine the contribution of each factor to injury induced by the use of vibrating hand tools. In the current study, the rat-tail exposure system previously used was modified by adding a pressure plate to the system so that the tails of the rats could be exposed to 2 or 4 N of force, and the effects of 10 days of exposure to each level of pressure on vascular and sensorineural function were measured. This level of pressure is similar to that seen at the fingertips of many workers using a vibrating hand tool.[41]

METHODS

Animals

Male (n = 18) Sprague-Dawley rats (Hla®(SD)CVF®, approximate body weight of 200 to 230 g at arrival) were obtained from Hilltop Lab Animals, Inc (Scottdale, PA). All rats were free of viral pathogens, parasites, mycoplasma, *Heliobacter*, and cilia-associated respiratory bacillus. Upon arrival, rats were acclimated to AAALAC International accredited animal facilities at the NIOSH for 1 week. The NIOSH animal facility is specific pathogen-free, environmentally controlled, and accredited by AAALAC International. They were housed in ventilated microisolator 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 autoclaved Teklad rodent diet (Harlan Teklad, Madison, WI) ad libitum. Rats were housed in pairs and under a controlled light cycle (12-hour light/12-hour dark) and temperature (22°C–25°C) conditions. One week after acclimation to the facilities, six rats were randomly assigned to a control or to an applied force condition of 2 or 4 N groups (the number of animals/group was consistent with a power calculation that have been performed before beginning the experiment). 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 by putting animals into Broome Style restrainers for gradually longer lengths of time until the total time in the restrainer was 4 hours. The restrainers were large enough so that animals could move but they could not turn around or rear up onto their hind legs. Acclimation to restraint was performed by starting with 1 hour of exposure

in the restrainer and then increasing the length of the exposure by 1 hr/day until the rats were acclimated to 4 hours of continuous restraint. After 5 days of exposure to restraint, the experiment began, and animals were exposed to applied force or control conditions. The tails of rats exposed to applied force were gently placed on the holding vibrating platform, and the pressure platform was gently lowered onto the middle of their tail (approximately at C12–20), as shown in Figure 1. Lines were drawn on the tail to ensure that the placement of the loading plate was the same every day. The length of the loading plate acting on the tail was 53 mm. The tail contact width was measured in tests with cadaver tails; it was 4.49 mm for 2.07 N and 5.09 mm for 4.3 N.[41] Hence, it is estimated that the average contact pressure on the living tail was approximately 8 kPa for 2 N force and 14 kPa for 4 N force. In a previous study, the applied force did not change under static conditions similar to those seen here or when vibration was combined with force.[42] Additional details regarding the characterization of the system can be found in the study by Dong et al.[42] Once the tail and pressure apparatus were in place, the tail was marked so that the same region was exposed each day. The exposure was 4 hr/d for 10 consecutive days. Control rats were also placed in a restrainer but their tails were not exposed to applied force. Pre-exposure body weights were collected on days 1, 5, and 10 of the experiment. Blood flow (measured by laser Doppler) and sensitivity to applied force (measured using the Randall-Selitto test) were measured immediately before and after the exposure on days 1, 5, and 10, and nerve function was measured using the current perception threshold method immediately before and after exposures before exposures began and on days 2 and 9.

On the morning after the last exposure, rats were anesthetized using 100 to 300 mg/kg body weight sodium pentobarbital euthanasia solution and exsanguinated by cardiac puncture. Tails were dissected from rats after exsanguination and placed in cold Dulbecco's modified Eagle's medium with glucose (Invitrogen/Gibco, Carlsbad, CA).

Laser Doppler Measurement of Blood Flow

Laser Doppler measurements were made using a Periflux system 5000 and PF 450 thermostatic small angle probe (Perimed, Stockholm, Sweden). At the beginning of each day, the machine was calibrated by placing the probe into the calibration solution supplied by the manufacturer. Once calibrated, the probe was secured in the opening of a plastic holder with holes in the bottom. The rats were weighed, placed in a restrainer, and then put into a sound-attenuating chambers. Each animal tail was put into the holder that held the Doppler probe stable. The probe was placed under the C15–16 region on the ventral surface of the tail and the animal tail was then covered with a piece of foam to keep it in place during the measurement. If the rat moved its tail away from the probe, the tail was quickly repositioned so that blood flow could be measured. Laser Doppler recordings of perfusion units (PUs) were made for 5 minutes at 0.2 Hz immediately before and after exposures on days 1, 5, and 10 of the experiment.

Because rats occasionally move during the recording period, and this results either in a rapid, acute increase in the Doppler signal, or a loss of signal, data were sent toa biostatistician for smoothing. Regions were identified as motion artifact if the recorded number of PUs was greater than 200, and a loss of signal was defined as less than 2 PUs.[43]

These regions, which were out of range, were identified and running means were calculated to replace the regions with motion artifact or loss of signal. To calculate the running mean, the 10 measures before and 10 measures after motion artifact were used to calculate an average and these averages (ie, running means) were used to replace data that were identified as motion artifact or loss of signal.

Randall-Selitto Test

The Randall-Selitto pressure test was performed after blood flow was collected on days 1, 5, and 10 of the study. To perform this test, a Randall-Selitto pressure gauge was used (IITC Life Sciences, Woodland Hills, CA). This gauge looks like a pair of forceps; however, there is a pressure sensor one side of the forceps that record pressure when the forceps are closed. Before testing, the Randall-Selitto pressure gauge was set to "0." To test an animal, the flat end of the gauge was placed on the dorsal surface of the tail, in the middle of the region exposed to pressure (approximately C15). Pressure was applied using the probe side (1-mm wide probe) of the gauge. The pressure was gradually increased until the rat responded by flicking its tail or vocalizing. The pressure that elicited a response from the animal was recorded. If the pressure reached 200 g, the test was stopped because previous studies have demonstrated that applying over approximately 200 to 250 g can result in injury.[44–46] If the animal did not respond before or at 200 g of pressure, the response was recorded as 200 g. Only 3 of 18 rats had a response recorded as 200 g, and this was not on every trial. Thus, the changes in detectability of the stimulus did not go beyond the measurable range of the test. This test also was not done using repeated trials because the animals quickly exhibited a learned response to the tactile sensation of the probe and responded to the probe touching them instead of changes in pressure. Immediately after the pre-exposure test, animals were placed in the exposure chambers for their respective exposure. After the posttest, animals were placed back into their home cages and returned to the colony room.

Current Perception Threshold

The rapid Current Perception Threshold (CPT) test was performed using a Neurometer (Neurotron, CO). Three measurements were collected at each stimulation frequency (ie, 2000, 250, and 5 Hz). Current Perception Thresholds were collected before and after exposure on days 2 and 9 of the experiment. Although vibration only studies suggested that there were no lasting effects of vibration until after 3 to 5 days of exposure,[46] it appeared as if 2 N of force may have had a lasting effect on the CPTs after a single exposure. Therefore, CPTs were collected before the beginning of the study for the 4 N group and before and after exposure on days 2 and 9 of the experiment in all groups. To perform the CPT, animals were placed in their restrainer and their tail was cleaned. The dispersion electrode was placed on the proximal end of the tail, near the region exposed to the front of the force plate. The stimulating electrode was filled with electrode gel and attached more distally on the tail using Soft-Tape (Neurotron), near the far end of the location at which the force plate contacted the tail. To begin testing, the Neurometer was set to deliver a 2000-Hz stimulus. The stimulus began at 5 mA and increased 5 mA approximately every 5 seconds. When the animal responded (tail flick or vocalization), the test was stopped and the CPT (or amplitude of the stimulus) was recorded. The test was run two more times and the average of the measures was used as the CPT for that frequency. After running the

2000-Hz stimulus, the stimulating frequency was changed 250 Hz and the tail was tested for sensitivity as described previously except that the test started at 1 mA and increased by 1 mA every 5 seconds until the animal responded. After three trials, the frequency was changed to 5 Hz and the test was run in a manner identical to that described for 250 Hz. The tail was tested at all three frequencies because each frequency stimulates a different population of nerves; A-beta fibers (or large myelinated fibers) are stimulated at 2000 Hz and are sensitive to vibration and light touch, A-delta fibers (or small myelinated fibers) are stimulated at 250 Hz and are sensitive to pressure, and C-fibers are unmyelinated fibers and are sensitive to painful stimuli (mechanical and temperature). Immediately after the pre-exposure test, animals were placed in the exposure chambers for their respective exposure. After the postexposure test, animals were placed back into their home cages and returned to the colony room.

Microvessel Physiology

On the day of euthanasia, ventral tail arteries from the C18–20 region of the tail were dissected, mounted on glass pipettes in a microvessel chamber (Living System, Burlington, VT), and perfused with sodium bicarbonated supplemented HEPES buffer (130 mM NaCl, 4 mM KCl, 1.2 mM $MgSO_4$, 4 mM $NaHCO_3$, 8 mM $CaCl_2$, 10 mM HEPES, 1.80 mM KH_2PO_4, 0.03 mM EDTA) plus 10% glucose added just before use and warmed to 37°C. Arteries were pressurized to 60 mm Hg and allowed to equilibrate for approximately 1 hour. After 1-hour acclimation period, the chamber buffer was replaced with fresh HEPES bicarbonate buffer and vascular responsiveness to phenylephrine (PE)-induced vasoconstriction and acetylcholine (ACh)-induced redilation was measured. All chemicals for microvessel exposures were purchased from Sigma (Indianapolis, IN) unless otherwise noted. To assess the effects of treatment on sensitivity to α_1-adrenoreceptor–mediated vasoconstriction, PE was applied to the chamber so that changes in the concentration occurred in half-log increments (−9.0 to −5.5 M) and the internal diameter of the artery was recorded after vessels stabilized (approximately 5 minutes between concentrations). After assessing vasoconstriction, the chamber buffer containing PE, was removed and replaced with fresh, oxygenated HEPES buffer. After rinsing, arterial diameter returned to levels that were near baseline. Because ventral tail arteries usually display little basal tone, endothelial-mediated redilation was assessed after arteries were preconstricted to approximately 50% of their baseline diameters with PE. We have demonstrated that reconstricting arteries with PE does not affect subsequent responses to ACh. To assess the dilatory effects of ACh, the agonist was added cumulatively in half-log increments (−10.0 to −5.0) and changes in the internal diameter of the vessel were measured as described for PE.

Data Analyses

The average PUs were calculated for each recording, and this value was used to look at overall blood flow over the recording period. An average CPT value also was calculated at each frequency along with average pre-post exposure difference at each frequency. Three (treatment) × 3 (days of exposure) repeated measures analysis of variance (RM-ANOVA) were performed on Doppler measures to determine whether there were acute effects of the exposure on blood flow or nerve function. The CPT measurements were analyzed using a 2 (treatment) × 2 (days of exposure) RM-ANOVA for the 2 N group and a 2 (treatment)

× 3 (day of exposure) RM-ANOVA for the 4 N group. Pre-exposure averages from each day were also analyzed using 3 (treatment) × 3 (days of exposure) RM-ANOVA. This analysis was done to determine whether the exposure resulted in longer-term effects on these measures. Pre-post and pre-exposure averages from the Randall-Selitto test were also analyzed as described previously except that analyses performed were a 3 (treatment) 3 (days of exposure) RM-ANOVA. Significant findings with the RM-ANOVAs were further analyzed using one-way ANOVAs and Tukey pairwise comparisons. Differences with $P < 0.05$ were considered significantly different. Microvessel data (the internal diameter collected after PE or ACh-induced changes stabilized) was analyzed using a 3 (condition) × 10 (dose of PE or ACh) RM-ANOVA. Nonlinear regression was also used to fit a line to the dose response curves and the effective dose 50 (ED50 or the dose of the drug that induced a 50% change from baseline) was calculated for each animal and analyzed using a one-way ANOVA.

A fast Fourier transform analysis was also performed on all pre-exposure laser Doppler datasets. This was done to identify the amplitude and width of the peak that occurs 0.4 to 0.2 Hz. This peak has been associated with arterial pulse[47,48] and can be sensitivity to changes in vascular function induced by a number of diseases and exposures.[49–51] All peaks within an individual dataset (150–175 peaks per recording) were used to calculate an average peak width and height for analyses.

RESULTS

Body Weights

Body weights measured before exposure on days 1, 5, and 10 of the experiment are in Figure 2. Animals in all three groups gained weight over the experiment. The animals in the 4 N group weighed less than the animals in the other groups on day 1, and although they gained weight, their body weights remained significantly less throughout the duration of the experiment. However, the finding that all animals showed similar trends in weight gain suggests that the exposure did not affect growth or result in stress-induced reductions in food intake in animals.

Laser Doppler Blood Flow

Pre-post laser Doppler measures are presented in Figure 3A. On the first day of exposure, average PUs (averaged over the 5 minutes of measurement) increased in control animals (postexposure was greater than pre-exposure and therefore the difference was negative). Lower pre-exposure blood flow in the control rats may have been a brief stress response to animals being placed into the chamber for assessing laser Doppler because this effect was less prominent over the course of the experiment. In animals exposed to applied force, pre-exposure measures were greater than postexposure measures (therefore, the difference was a positive number). On day 5 of exposure, there was no significant differences between the groups in pre-post blood flow measures; blood flow in all groups was slightly increased postexposure as compared to pre-exposure. There also were not any pre-post exposure-related differences in blood flow between the different groups on day 10.

Figure 3B shows changes in pre-exposure blood flow on days1, 5, and10 of the study. Pre-exposure blood flow was significantly lower in control animals on days 5 and 10 of the experiment than on day 1. In the animals exposed to 2 N of force, blood flow was similar on days 1 and 5 but was increased on day 10 of the experiment. Blood flow in the animals exposed to 2 N of force was higher than blood flow in control animals. In animals exposed to 4 N of applied force, blood flow was similar on days 1 and 5 and declined slightly on day 10 of exposure. However, blood flow in the animals exposed to 4 N was greater than blood flow in the controls on days 5 and 10 of the study.

A fast Fourier transform also was performed on each pre-exposure dataset to identify the 0.4- to 0.2-Hz peaks in each animal. This frequency in the Doppler blood flow measure is indicative of pulse rate. Both the amplitude and the width of the peak were analyzed. Average peak heights in the 0.4- to 0.2 -z signal are shown in Figure 4A. There were no significant differences in peak height; however, there was a gradual reduction in the peak heights over the 10 days of the exposure in all groups. Peak width is shown in Figure 4B. Across the 10 days of the exposure, there was no change in the width of the 0.4- to 0.2-Hz peak in control animals. Animals exposed to 2 N of force had wider peak than controls or animals exposed to 4 N on days 1 and 5 of the exposure. However, on day 10, the width of the peak was significantly reduced as compared with the peak width at 1 and 5 days in the 2 N group. The group treated with 4 N of applied force displayed a significant decrease in the width of the peak at 0.2- to 0.4-Hz on day 5, and a marginal reduction in peak width on day 10 of the exposure.

Microvessel

Figures 5A and B show changes in the internal diameter of the ventral tail artery to increasing doses (−10 to −5 log) of PE, and α_{1a}-adrenoreceptor agonist. Repeated measures ANOVA did not find reveal any significant differences in PE-induced vasoconstriction between the groups of animals (A). Exposure to 2 and 4 N of applied force resulted in a gradual increase in the ED50 (B), but these increases were not significant. Figures 6A and B show redilation of the ventral tail artery in response to ACh after preconstriction with PE. Animals exposed to both 2 and 4 N of force displayed an increased sensitivity to ACh-induced redilation, especially at doses greater than $10^{-6.5}$M (A). The ED50 for ACh was also significantly lower in the arteries from animals exposed to2 N of force and marginally lower in the animals exposed to 4 N of pressure (B).

Current Perception Thresholds

Figures 7A to F show the pre-exposure CPTs at 2000-, 250-, and 5-Hz stimulation in animals exposed to 2 N (A–C) and 4 N (D–F) of force applied to the tail. For the 2 N group, there were no pre-exposure measures (day 0), so the data reported were collected on days 2 and 9 of the exposure. For the 4 N group, data were collected on day 0 and on days 2 and 9 of exposure. Exposure to 2 N of force did not affect the CPT to the 2000-Hz stimulus (Fig. 7A, Aβ fibers). The 250-Hz threshold was increased in animals exposed to 2 N applied force on day 2 as compared with controls. After 9 days of exposure, there was a reduction in the 250-Hz CPT in force-exposed animals as compared with force-exposed animals after 2 days of exposure (B, Aδ fibers). There were no significant differences in

responses to the 5-Hz stimulus after exposure to 2 N of force. In the animals exposed to 4 N, there was a gradual reduction in the 2000-Hz CPT in both control and exposed animals on days 2 and 9 as compared with pre-exposure measures (D). The 4 N exposure resulted in a significant decrease in the 250 Hz threshold on day 2 of the exposure (E); pressure exposed animals had a lower 250-Hz CPT than same-day controls and pre-exposure measures. On day 9, animals exposed to 4 N of force still had a marginal reduction in the 250-Hz CPT when compared with same-day controls (F). The 5-Hz CPT was reduced in both controls and exposed animals on day 2 as compared with pre-exposure measures, and the exposed animals had marginally lower CPTs than the exposed animals. There were no differences on day 9 of the exposure.

The pre-post differences in the CPT are presented in Figures 8A to C. There were no differences in the pre-post 2000-Hz CPT measures on days 2 and 9 of the experiment (Fig. 8A). On day 2 of the exposure, animals exposed to 2 N of pressure had a significantly higher pre-post difference at 250 Hz than controls (indicating a reduction in the 250-Hz threshold pre-exposure vs postexposure). However, 4 N of exposure resulted in a significant decrease in the 250-Hz exposure as compared with controls (suggesting an increase in the threshold). There were no significant differences in the pre-post CPT measures on day 9 of exposure. The 5-Hz CPT showed a trend that was similar to what was seen with the 250-Hz CPT (ie, reduction in the threshold with a 2 N exposure and an increase with a 4 N exposure), but these differences were not significant. There were no differences on day 9 of exposure.

Randall-Selitto Pressure Test

Responses to the Randall-Selitto test are presented in Figures 9A and B. There were no significant differences in pre-post Randall-Selitto measures. However, after 10 days of exposure, pre-exposure measures tended to be higher than postexposure measures, especially in the control and 4 N–exposed group (A). When pre-exposure measures were analyzed over the days of exposure, the analysis found that there was a significant reduction in the threshold (ie, an increase in sensitivity) in animals exposed to both 2 and 4 N of force on day 10 of exposure (B).

DISCUSSION

Workers using vibrating hand tools are exposed to both vibration transmitted from the tool to the hand of the worker, and pressure applied to the fingers and palms while gripping a tool.[52–54] These exposures result in damage and dysfunction of peripheral blood vessels and sensory nerves.[55] Although the etiology of vibration-induced disorders is still not completely understood, there are a number of studies that have described the exposure-response relationship between HTV and the development of HAVS.[18,25,55–59] However, there are few studies describing the effects of hand forces applied while gripping a tool,[15,60–62] or studies examining how combined vibration and grip exposure may affect the development of HAVS. To begin to understand how these factors may work together to affect the risk of developing HAVS, this study examined the effects of applied force on peripheral vascular and sensorineural function using modification of a rat tail model of HAVS.[33,34,45] In general, the results of this study demonstrated that the exposure to applied

force resulted in pressure-dependent changes in vascular function and blood flow. There were also pressure-dependent changes in sensorineural function. Exposure to both 2 and 4 N of force resulted in changes in the 250-Hz CPT and in sensitivity to the Randall-Selitto pressure test. The changes in blood flow and sensorineural function seen in this study are different than those seen with vibration exposure alone. Understanding the effects of each exposure separately, and in combination, on measures of peripheral vascular and sensorineural function may provide information about how each factor contributes to our knowledge regarding the etiology of HAVS and the risk of injury and dysfunction with these exposures.

In the current study, exposure to both 2 and 4 N of applied force resulted in prolonged changes in blood flow over the course of the experiment. Exposure to 2 N of force resulted in a general increase in blood flow over the 10 days of the exposure. Although exposure to 4 N also resulted in an increase in blood flow as compared with controls, blood flow gradually declined over the 10 days of exposure. These data are consistent with the microvessel data showing that there was an increased sensitivity to ACh-induced vasodilation and that arteries from animals exposed to 2 N of force were slightly more sensitive to ACh than arteries from animals exposed to 4 N of pressure. When pre-post exposure blood flow was analyzed, the results demonstrated that blood flow declined pre-post exposure on the first day of the experiment in animals exposed to pressure. However, on days 5 and 10, the difference between pre- and postexposure measures decreased, suggesting that there may have been changes in the artery that kept blood vessels dilated during the exposure. There have not been many studies examining the effects of repeated application to pressure on blood flow. However, there are a few studies that have examined the recovery of blood flow after applying pressure to the finger nails. The results of nail press test suggest that the longer a stimulus that induces a reduction in blood flow is applied, or the greater the magnitude of the stimulus, the longer it takes for blood flow to return to the compressed area.[63] The results of the current study are consistent with those seen with the nail press.

There were also reductions in the height and the width of the 0.4- to 0.2-Hz signal over days of the exposure. The 0.4- to 0.2-Hz signal has been associated with pulse rate.[43,47,48] The changes in the amplitude of the 0.4- to 0.2-Hz signal tended to decline during the exposure, but these changes were not significant. However, the width of the signal was significantly reduced after 5 and 10 days of exposure to 4 N of force and after 10 days with exposure to 2 N. Previous studies have suggested that reductions in the 0.4- to 0.2-Hz signal (either the amplitude or width of the pulse) are indicative of vascular remodeling, including a thickening of the vascular muscle, and a reduced plasticity of blood vessels.[43,47,48] It is possible that the repeated application of pressure induced changes in vascular morphology that allowed the vessels to stay open and maintain blood flow, even when pressure was applied. Although this change may be beneficial for maintaining blood flow in the short term, it may not be adaptive in the long term; a reduction in plasticity of the exposed arteries may make them less able to response to another challenge such as vibration. Therefore, the change in plasticity induced by pressure may contribute to the long-term changes in vascular function that precede the development of HAVS.[43] Additional studies will be performed to examine the effects of both applied force and vibration on changes in vascular function and morphology of blood vessels in the tail.

The effects of applied force on nerve function were less clear; exposure to pressure tended to result in an increased sensitivity of Aδ fibers (ie, 250-Hz CPT) to electrical stimulation, and this effect was most pronounced on day 9 in the 4 N group (4 N of force); acute changes in the CPT (pre-post exposure) occurred with the 250-Hz stimulus, with animals exposed to 2 N showing an increased sensitivity of the Aδ fibers to stimulation and animals exposed to 4 N showing a reduced sensitivity to electrical stimulation after exposure to pressure. Aδ fibers can carry information about pressure, pain, and temperature from the periphery to the central nervous system.[6,46] Studies in humans have demonstrated that holding a vibrating hand tool initially results in an increased sensitivity of both Aβ and Aδ fibers to stimulation.[6,64,65] It is possible that vibration, which activates Pacinian corpuscles, and the associated Aβ fibers are responsible for the shift in vibrotactile sensitivity at the higher frequencies in humans, and that pressure applied while gripping, which activates the Meissner corpuscles and Aδ fibers, affects sensitivity to applied force and the perception of vibration signals at the lower frequencies.[4,20,66,67] Because there were not CPT measure collected before beginning the experiment in the 2 N group, changes in the data collected before exposure on days 2 and 9 are probably more reliable in the 4 N exposure group. In this group, the 250-Hz threshold was lower than pre-exposure values and lower than controls on day 2 of the exposure. These data are consistent with studies that suggest that there is initially an exposure-induced increase in sensitivity to electrical stimulation due to nerve damage, but with longer-term exposure, there is more permanent nerve damage, nerve loss, and a reduction in sensitivity to stimulation.[6,68–71] Future studies will examine the effects of both vibration and applied force on responses to the CPT to try to determine the contribution of each factor to changes in sensorineural function.

The responses to applied force using the Randall-Selitto test were more robust; exposure to force at both 2 and 4 N resulted in an increased sensitivity to the pressure on day 10 of the exposure. These findings are similar to those of a number of other studies showing that exposure to vibration and/or pressure tend to induce discomfort (ie, reductions in the threshold) before inducing longer-term losses in sensitivity to tactile stimuli.[6,59,72–75] Studies examining longer exposures, or studies combining vibration and applied force, might generate the pattern of changes in sensorineural function that we have previously seen with this model (an increase in sensitivity followed by a reduction[46,76]) and in humans.[6,25,59,77]

In conclusion, the results of this study show that application of force within the range seen at the fingertips of workers using hand tools[15,61,78] affects both blood flow and sensorineural function and that the effects are dependent on the amount of pressure. The effects seen on both vascular and nerve function are different than the effects of vibration. Exposure to vibration results in a reduction in sensitivity to ACh-induced vasodilation,[79] while in this study, exposure to applied force resulted in an increase in sensitivity to ACh. The responses of the nerves to electrical stimulation were also different. Vibration induces changes in the response to the 2000-Hz stimulus (or sensitivity of the Aβ fibers[68]) and pressure seemed to have a more pronounced effect on responsiveness to the 250-Hz stimulus (or the Aδ fibers). Comparing the effects of each exposure, along with that of a combined exposure will help determine the relative risk associated with each exposure factor and how these factors together contribute to the risk of developing HAVS.

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CME Learning Objectives

After completing this enduring educational activity, the learner will be better able to:

- Discuss how force affects peripheral vascular and sensorineural function.
- Explain how applied force on blood flow and sensation are different than those of vibration suggest future research needs for examining co-exposures to force and vibration.

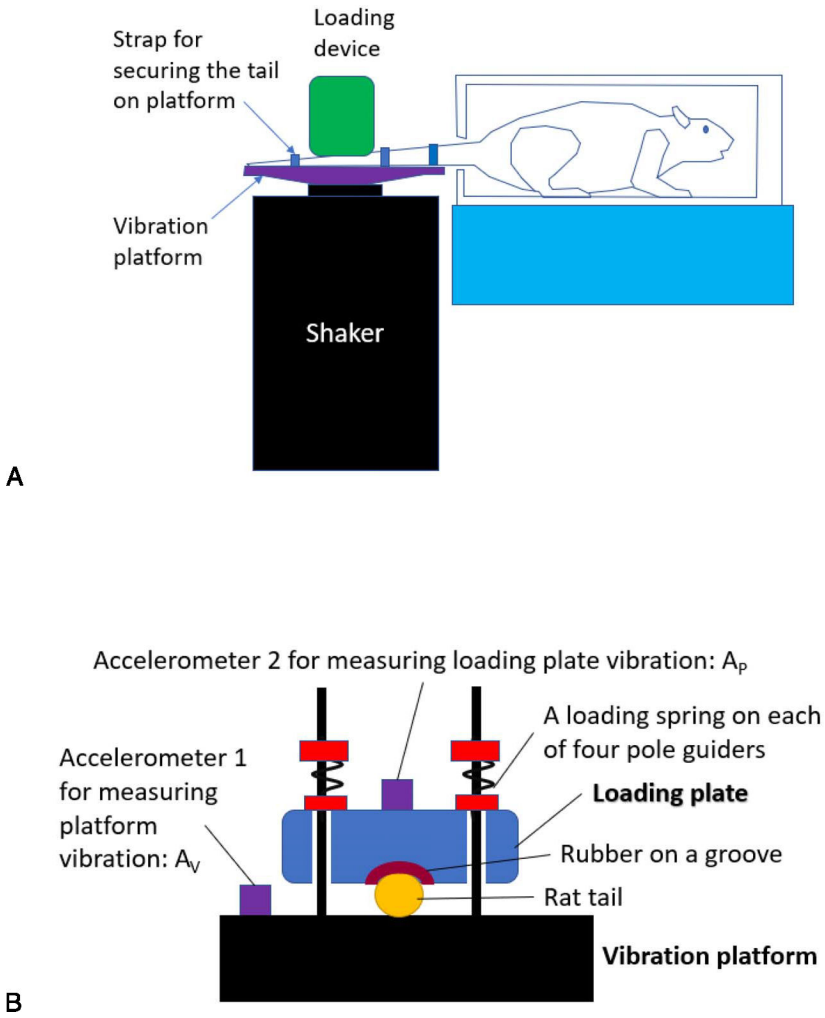

FIGURE 1.

A is a general diagram of the exposure system. B shows a more detailed diagram of both the loading and vibration platform, and the location of the loading springs used to control the level of applied force.

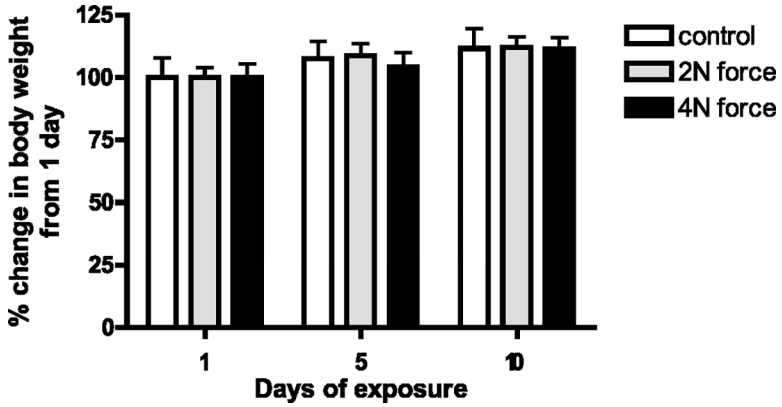

FIGURE 2.

The percent change in body weight using day 1 body weights as baseline. Although the body weights of animals in the 4 N group were less than the animals in the other groups on all days of the study, there were no differences in the % change in body weight over the course of the experiment. Therefore, animals in all groups gained weight at comparable rates during the exposure (n = 6 animals/grp, data presented as mean ± SEM).

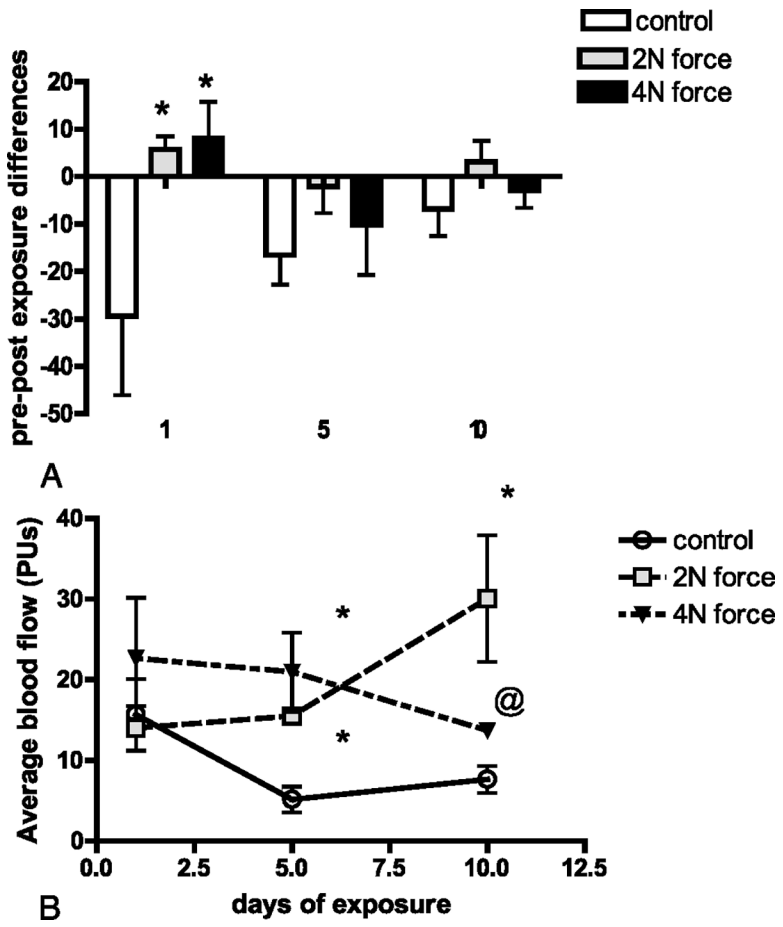

FIGURE 3.

Changes in average blood flow measured by laser Doppler. A, Pre-post exposure differences in blood flow on days 1, 5, and 10 of the experiment in controls and animals exposed to 2 or 4 N of force (n = 6 animals/grp). There were no significant pre-post exposure differences in blood flow except on day 1 where controls had higher blood flow than post- than pre-exposure (therefore, the difference was a negative number). Animals exposed to either 2 or 4 N of force displayed a reduction in blood flow and blood flow in these animals was significantly less than blood flow in controls. There were no significant pre-post blood flow changes on the other days of the experiment. B, Changes in pre-exposure blood flow over days of the experiment. On day 5 of the exposure, animals exposed to 2 and 4 N of applied force had higher pre-exposure blood flow than controls. On day 10 of the exposure, this increase in blood flow in the pressure exposed groups remained but was not as prevalent in the 4 N exposed group (All data are presented as the mean ± SEM; * different than same-day controls, $P < 0.05$; @ different than same-day control, $P < 0.06$).

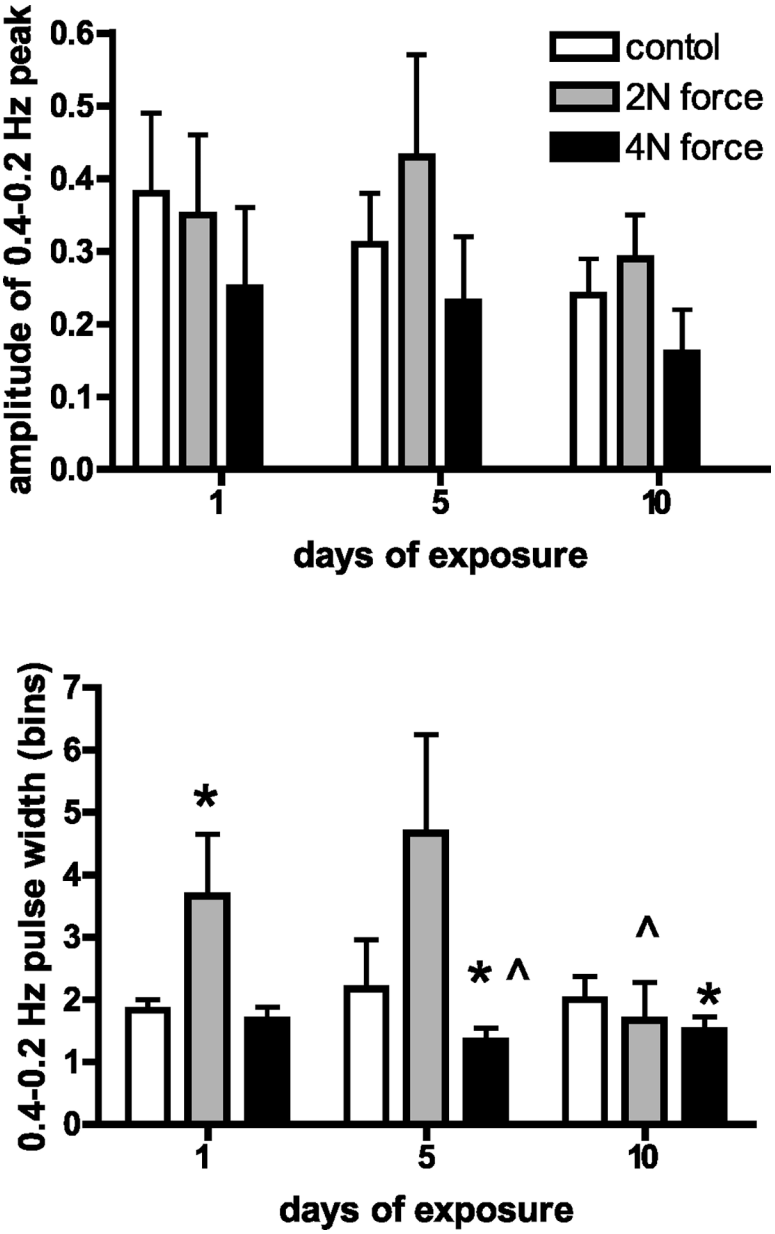

FIGURE 4.

The amplitude and width of the 0.4- to 0.2-Hz laser Doppler signal calculated by faster Fourier transform analyses on the pre-exposure data for all groups (n = 6 animals/grp). This peak is representative of arterial pulse. A, This graph shows the average amplitude of the laser Doppler signal. Although the amplitude of the signal at this frequency tended to go down with longer exposures, these differences were not significant. B, The average width of the signal at 0.4 to 0.2 Hz is plotted. Animals in the 2 N exposure had a broader pulse at 0.4 to 0.2 Hz than control animals or animals exposed to 4 N of force on day 1 of the experiment. The width of the 0.4- to 0.2-Hz signal did not change over the course of the experiment in control animals. However, the width of the 0.4- to 0.2-Hz pulse was lower in animals exposed to 4 N of force than controls on days 5 and 10 of the experiment. The width

of the 0.4- to 0.2-Hz pulse was significant reduced on day 10 as compared with on days 1 and 5 in the animals exposed to 2 N of force (all data are presented as the mean ± SEM; * different than same-day controls, $P < 0.05$; ^ different than day 1 same treatment, $P < 0.05$).

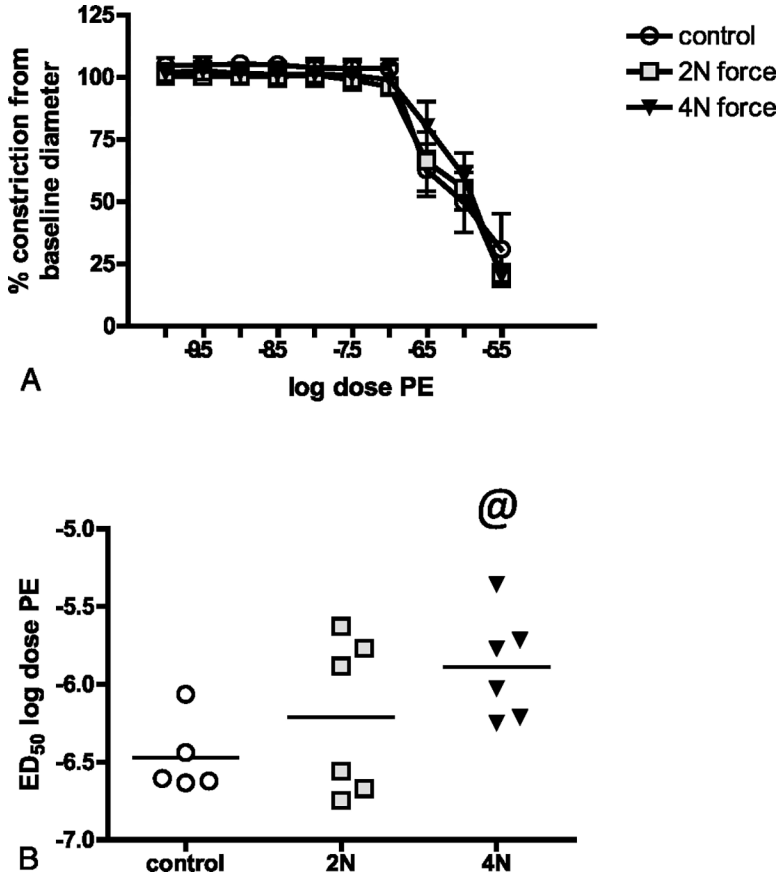

FIGURE 5.

Changes in the diameter of the ventral tail artery (& change from baseline) in response to increasing doses of PE (A: PE). There were no significant differences in PE-induced vasoconstriction between the different groups ($n = 6$ animals/grp). The average effective dose 50 (B: ED50) increased with increases in applied force (the line represents the mean value). The ED50 in arteries from animals exposed to 4 N was marginally higher than that of controls (@ $P < 0.08$). All data are presented as the mean ± SEM.

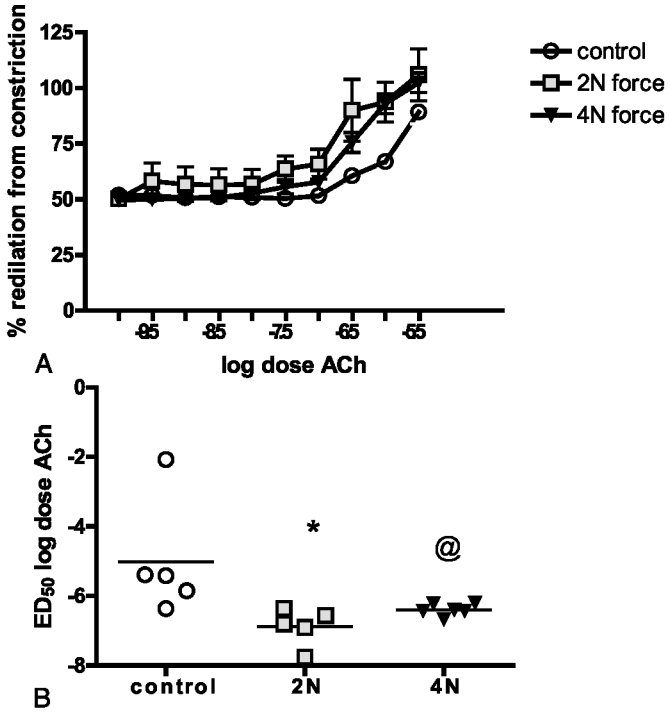

FIGURE 6.

Changes in the diameter of the ventral tail artery (after preconstriction to 50% of baseline with PE) in response to increasing doses of ACh (A: ACh). The arteries from control animals were less sensitive to ACh-induced redilation than the arteries from animals exposed to applied force. The ED50 (B) was lower in arteries from animals exposed to both 2 and 4 N of pressure ($n = 6$ animals/grp; $*P < 0.05$ and @ $P < 0.06$, different than controls). The line represents the mean value and all data are presented as the mean ± SEM.

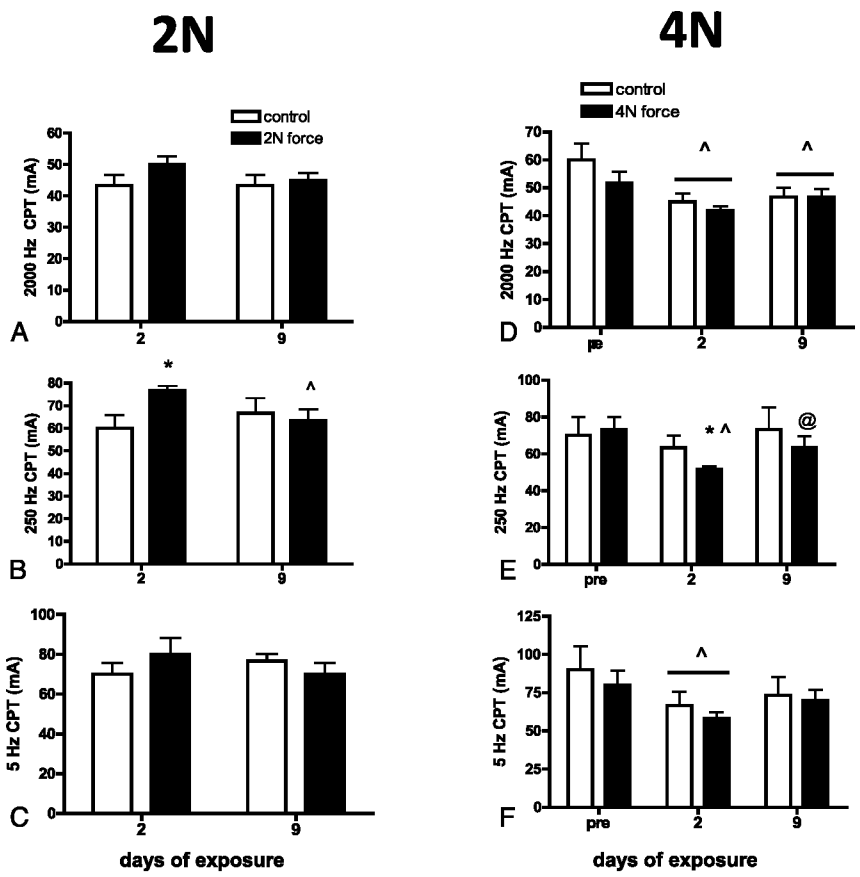

FIGURE 7.

Pre-exposure CPT measurements from the tail nerves after exposure to control, 2 N (A–C) or 4 N (D–F) of applied force ($n = 6$ animals/grp). A and C, Animals exposed to 2 N of force did not show changes in their responsiveness to the 2000- or 5-Hz electrical stimulus, respectively. B, Animals exposed to 2 N of force showed a reduced sensitivity (increased threshold) at 250 Hz after 2 days of exposure. However, after 9 days, there was a reduction in sensitivity as compared with the 2-day exposed group. D, Exposure to 4 N of force resulted in a reduced sensitivity to the 2000-Hz stimulus on days 2 and 9 of exposure in both the control and pressure-exposed group. E, Exposure to 4 N of force resulted in a reduction in sensitivity to the 250 stimulus after 5 and 10 days of exposure, but the reduction was only significant on day 5. F, Responses to the 5-Hz electrical stimulus were reduced in both control animals and animals exposed to 4 N of force on day 5 of the experiment. There were no significant differences on day 10. (* $P < 0.05$, same-day control; @ different than same-day control, $P < 0.08$; ^ different that day 1, same treatment, $P < 0.05$). All data are presented as the mean ± SEM.

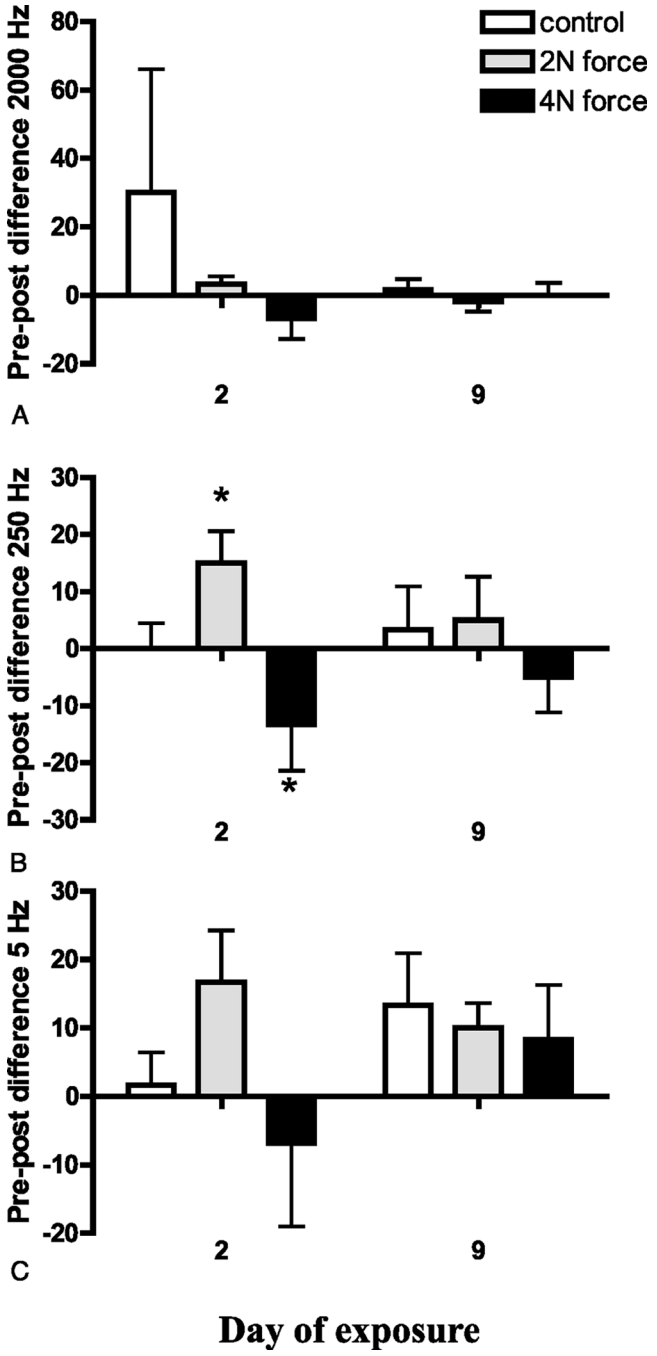

FIGURE 8.

Pre-post exposure differences in the CPT on days 2 and 9 of the experiment. A, Although there seemed to be a significant change in pre-post exposure responses at 2000 Hz in control animals (with pre-exposure CPTs being higher than postexposure), there were no significant differences in the response between controls, 2 and 4 N exposed animals (n = 6 animals/grp). There were also no significant differences on day 9 of exposure. B, On day 2 of the experiment, responses to the 250-Hz electrical stimulus, there was no pre-post exposure change in controls, pre-exposure CPTs were higher than postexposure in animals exposed

to 2 N of force, and postexposure CPTs were higher than pre-exposure CPTS in animals exposed to 4 N of force. There were no significant pre-post changes in responsiveness to 250 Hz on day 9 of the experiment. C, On day 2 of the experiment, the responses to the 5-Hz stimulus seemed to be similar to the responses to the 250-Hz stimulus; however, there were no significant differences between the groups. On day 9 of the experiment, the responses of all groups to the 5-Hz stimulus was greater pre- than postexposure. (*different than same-day control, $P < 0.05$). All data are presented as the mean ± SEM.

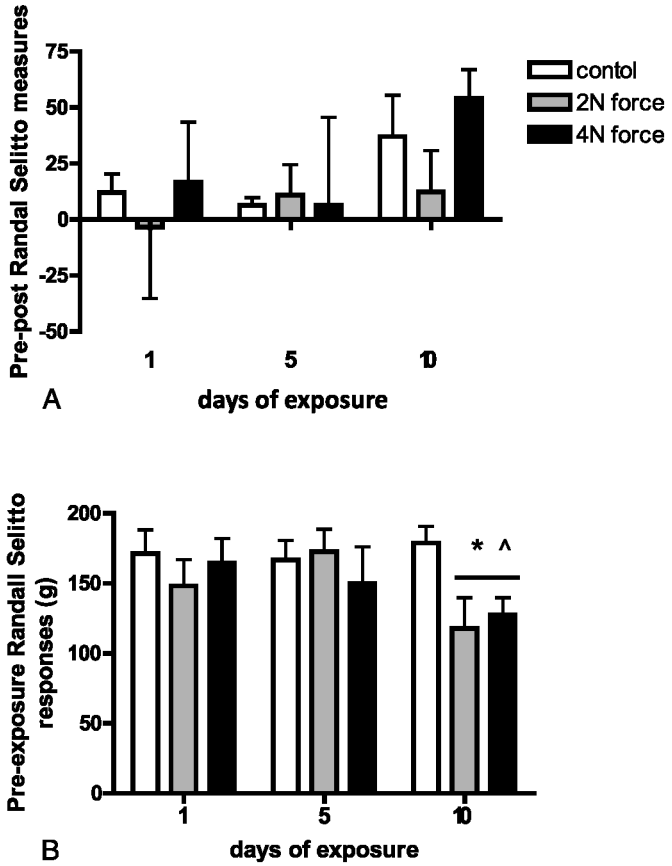

FIGURE 9.

Responses to the Randall-Selitto pressure test. A, Pre-post exposure differences in the threshold to pressure seemed to increase over time, with the postexposure threshold being lower than the pre-exposure threshold. However, these differences were not significant. B, These are the average thresholds pre-exposure on days 1, 5, and 10 of the experiment. On day 10 of exposure, animals exposed to both 2 and 4 N of force showed a reduced threshold as compared with thresholds on day 1 of the experiment and as compared with the same-day control. (* different than same-day control, $P < 0.05$; ^ different than day 1 same treatment, $P < 0.05$). All data are presented as the mean ± SEM.