ABSTRACT: Long-term occupational exposure to hand–arm vibration can result in a permanent reduction in tactile sensitivity in exposed fingers and hands. Little is known about how vibration causes this reduction in sensitivity, and currently no testing procedures have been developed to monitor changes in sensory perception during ongoing exposures. We used a rat-tail model of hand–arm vibration syndrome (HAVS) to determine whether changes in sensory nerve function could be detected after acute exposure to vibration. Nerve function was assessed using the current perception threshold (CPT) method. We also determined whether changes in nerve function were associated with changes in gene transcription. Our results demonstrate that the CPT method can be used to assess sensory nerve function repeatedly in rats and can detect transient decreases in the sensitivity of Aβ nerve fibers caused by acute exposure to vibration. This decrease in Aβ fiber sensitivity was associated with a reduction in expression of nitric oxide synthase-1, and a modest increase in calcitonin gene–related peptide transcript levels in tail nerves 24 h after vibration exposure. These transient changes in sensory perception and transcript levels induced by acute vibration exposure may be indicators of more prolonged changes in peripheral nerve physiology.

Muscle Nerve **36:** 197–205, 2007

ACUTE VIBRATION REDUCES Aβ NERVE FIBER SENSITIVITY AND ALTERS GENE EXPRESSION IN THE VENTRAL TAIL NERVES OF RATS

KRISTINE KRAJNAK, PhD,[1] STACEY WAUGH, MS,[1] OLIVER WIRTH, PhD,[1] and MICHAEL L. KASHON, PhD[2]

[1] Engineering and Control Technology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, West Virginia 26505, USA
[2] Biostatistics and Epidemiology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

Accepted 18 March 2007

Occupational exposure to hand–arm vibration from the use of power tools can result in disorders of the vascular, neural, and musculoskeletal systems that are collectively referred to as hand–arm vibration syndrome (HAVS). Although the hallmark symptom of HAVS is cold-induced vasospasms that result in finger blanching and cyanosis,[19] reductions in tactile sensitivity also are common.[3] When workers with HAVS stop using hand tools, vascular symptoms often subside, but reduced tactile sensitivity is maintained or worsened.[30] The etiology of vibration-induced neuropathies is not well understood; however, even acute exposure to vibration can result in transient reductions in vibrotactile and thermotactile sensitivity in humans.[28,29] A better understanding of how vibration exposure progressively alters sensory perception and the mechanisms underlying these changes could lead to the development of more effective monitoring methods for early detection and prevention of irreversible neuropathies.

The goal of these studies was to use a rat-tail model of HAVS to characterize changes in sensory function and associated changes in gene transcription that occur in response to vibration exposure. We chose to use a rat-tail model for this study because chronic exposure to vibration produces morphological changes in the tail that are similar to the changes seen in biopsy samples collected from work-

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.
Abbreviations: ANOVA, analysis of variance; CGRP, calcitonin gene–related peptide; CGRPr, calcitonin gene–related peptide receptor; CPT, current perception threshold; DRGs, dorsal root ganglia; GDNF, glial-derived neurotrophic factor; HAVS, hand–arm vibration syndrome; IGF-1, insulin-like growth factor-1; NOS-1, nitric oxide synthase-1 (neuronal form); VTT, vibrotactile threshold test
Key words: current perception threshold; hand–arm vibration syndrome; nitric oxide synthase; sensory perception
Correspondence to: K. Krajnak; e-mail: ksk1@cdc.gov

Published online 8 May 2007 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.20804

ers with HAVS.[7,13,40] Studies using acute vibration exposures have also demonstrated that rat tails and human fingers display similar reductions in blood flow.[4,33] In addition, we recently demonstrated that the vibration frequencies that induce neural and vascular changes in our rat-tail model are the same frequencies associated with an increased risk of HAVS in workers.[3,23]

Sensory perception was assessed with the current perception threshold (CPT) test, which has been used to diagnose neuropathologies caused by various diseases and exposures, including vibration.[25] The CPT test is non-invasive and therefore can be repeated on the same animal. However, because the repeatability of this method has not been demonstrated in rat tails, we determined whether CPTs were consistent over time.

We also examined the effects of a single bout of tail vibration on sensory nerve function. The transcript levels for several genes that have been implicated in mediating both neuronal and vascular responses to vibration or other mechanical stressors also were assessed, including calcitonin gene–related peptide (CGRP), the CGRP receptor, glial-derived neurotrophic factor (GDNF), insulin-like growth factor-1 (IGF-1), nitric oxide synthase-1 (NOS-1; neuronal form), and the α1A- and α2C-adrenoreceptors.[35] Previous studies have demonstrated that GDNF, IGF-1, and CGRP expression increase in response to nerve damage.[11,17,39] Changes in these factors can occur in the nerves, myelin, or dorsal root ganglia (DRGs). Thus, if acute exposure to vibration results in damage to the nerve, an increased expression of these factors may also occur.

Vibration-induced changes in peripheral sensation may also be due to a reduction in blood flow to peripheral nerves. Acute vibration results in constriction of the rat ventral tail artery.[15] In humans, the temporary shift in vibrotactile sensitivity that occurs after acute exposure to vibration is also associated with peripheral vasoconstriction and a temporary reduction in finger blood flow.[2] In rats, this constriction is in part due to an increased sensitivity of peripheral arteries to α2C-adrenoreceptor–mediated vasoconstriction.[23] However, changes in the activity of vasodilating factors could also contribute to the vasoconstriction.[36] Therefore, we also examined the effects of vibration on the transcription of factors that mediate vasoconstriction (i.e., α1- and α2C- adrenoreceptors) and vasodilation (NOS-1, CGRP, and CGRP receptors) in peripheral nerves to determine whether a temporary change in peripheral sensation was associated with a change in factors that can affect blood flow to nerves.

METHODS

Animals. Male Sprague-Dawley CVF rats (6 weeks of age; Hilltop Lab Animals, Inc., Scottdale, Pennsylvania) were used for all exposures. All rats were maintained in a vivarium with a 12:12 light:dark cycle, a temperature range of 21.1°–22.2°C, and food and water available ad libitum at the National Institute for Occupational Safety and Health (NIOSH) facility. Rats were allowed to acclimate to the colony room for 1 week before being used in any experiments. All procedures were approved by the NIOSH Animal Care and Use Committee and were in compliance with policies of the Public Health Service.

Vibration Apparatus. The apparatus used to expose rats to vibration or control conditions was described previously.[23] Vibration was generated by V408 electromagnetic shakers and PA100E amplifiers (Ling Dynamic Systems, Royston, UK) and controlled via a closed-loop feedback system. Vibration levels were monitored with 353B15 accelerometers and 482A20 signal conditioners (PCB Piezotronics, Depew, New York). Data acquisition and vibration control were performed through PCI-MIO-16XE-10 data acquisition boards, PCI-6713 analog output boards, and custom LabView 5.0 programming (National Instruments, Austin, Texas). The exposure components were enclosed in fan-ventilated, sound-attenuating cubicles (Med Associates, St. Albans, Vermont). Constant white noise (~70 dB) generated through a speaker inside the cubicles masked extraneous noises. The ambient temperature within the chamber stayed between 22.2° and 22.7°C throughout the 4-h exposure. We did not assess core body temperature in this experiment, but in preliminary studies we measured rectal temperature at 30-min intervals during vibration and restraint conditions and found that temperature remained constant at about 35°C throughout the exposures.

The shaker platforms were aluminum, 50 mm × 25 mm (major and minor radii) elliptical platforms, which were 12.7 mm thick, tapered down to 6.35 mm at the ends, and had 14-mm-wide extensions that lengthen the entire platform from the middle ellipse to 170 mm. Each platform was mounted onto a shaker and centered directly behind the animal. The platform was fastened to the shaker, which oscillated the platform in a vertical direction.

Vibration Exposure. Prior to vibration exposure, each rat was placed in a Broome-style restrainer for 4 h/day for 3–4 days to acclimate them to restraint. The rats went into the restrainers head first, and

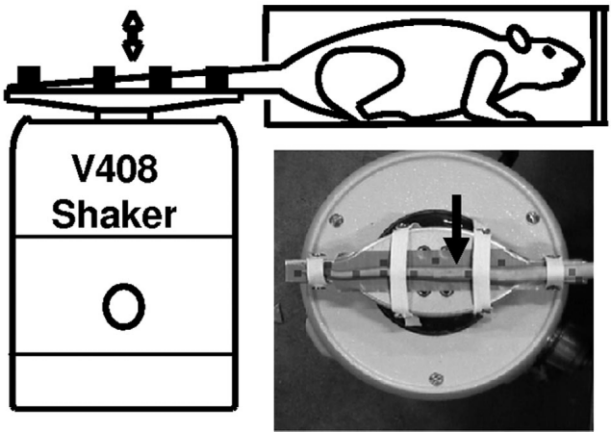

FIGURE 1. Experimental apparatus used to expose rats to tail vibration. Inset to photograph demonstrates how the tail is secured to vibrating or control platforms. CPT measures and tissues were collected from the C10 region of the tail (black arrow in the photograph).

their tails were gently threaded through a hole in the removable hatch. The hole in the hatch had been enlarged so that the rats' tails were not held in an awkward position in relation to their bodies. After 3–4 days of acclimation, rats voluntarily walked into the restrainers, did not try to back out, and remained still. Keeping the rats calm and still was critical for collecting reliable measurements of tail surface temperature and CPTs. On the day of exposure, rats were placed in their restrainers; each tail was gently placed on top of the vibration platform and four elastic straps (6.35 mm wide) were pulled over the tail and fastened over screws secured into the side of the platform (Fig. 1). Care was taken to ensure that the tail was secured to the platform without compressing the tissue. Rats were exposed to 4 h of continuous sinusoidal vibration at a frequency of 125 Hz and a constant acceleration of 49 m/s^2 rms.[15] Control animals were treated in a similar manner except that their tails were strapped to non-vibrating platforms mounted on isolation blocks.

Experiment 1: CPT Test Repeatability and Effects of Cold Exposure. Rats ($n = 4$) were acclimated to restraint as just described. On the day of testing, each rat was restrained and the surface temperature of their tail was immediately recorded using an infrared camera (IRRIS 256ST; Cincinnati Electronics, Cincinnati, Ohio). The camera was computer controlled and thermal images of the tail were digitized, saved, and analyzed using Thermosoft II software (version 1.2; EIC, Jenison, Michigan). CPT measurements were made using a Neurometer CPT/C (Neurotron, Inc., Baltimore, Maryland). To collect CPT measurements, each rat was put into a sound-attenuated chamber and its tail was cleaned with Goldtrobe electrode preparation paste and wiped with an alcohol pad. Goldtrobe electrode gel was applied to the stimulating electrode (ATE1925) and the electrode was secured to the ventral surface of the tail, just distal to the C10 tail vertebrate, using soft tape. A separate skin patch dispersion electrode (SDE44; Neurotron, Inc., Baltimore, Maryland) was then secured on the tail approximately 2 cm proximal to the stimulating electrode.

Transcutaneous electrical stimuli at three frequencies were used to test different nerve fiber types. The 2000-Hz stimulus selectively activates Aβ fibers,[22] and the 250-Hz stimulus activates Aδ and Aβ nerve fibers. However, because stimulation of the Aδ fibers results in a noxious sensation, it is likely that rats respond to Aδ activation over Aβ activation.[22] The 5-Hz frequency activates Aβ, Aδ, and C fibers.[22] However, because the responses of Aδ- and Aβ-associated DRGs to 5-Hz CPT stimulation are much lower than the responses of these DRGs to mechanical stimulation, it is unlikely that activation of Aδ and Aβ fibers results in sensation.[16] Thus, a rat's response to the 5-Hz stimulus is most likely due to C-fiber activation. The intensity of each stimulus was automatically increased in increments of 0.5 mA for the 2000-Hz stimulus, and 0.1 mA for the 250- and 5-Hz stimuli until the rat flicked its tail. The intensity that elicited the tail-flick was recorded as the CPT. CPTs were always measured in the same order: 2000, 250, and 5 Hz. After each set of frequencies was tested once, there was a 1-minute rest interval before the next set of tests was performed. The tests at each frequency were repeated three times, and the mean CPT calculated for each frequency was used for statistical analyses. After the CPT tests, rats were returned to the colony room. This protocol was repeated for an additional 3 days. All CPT tests were performed between 0900 and 1000 h.

Our preliminary studies demonstrated that tail skin temperatures are reduced after restraint or restraint plus vibration. In addition, other studies have demonstrated that exposure to vibration for 4 h results in constriction of the ventral tail artery, and an accompanying reduction in blood flow could result in a reduction in tail temperature.[9,15] Therefore, on day 5, we assessed the effects of temperature on CPT measures. Rats were restrained and tail temperatures and CPT measures were recorded. Each rat's tail was then covered with a pliable cold-pack for 5 min. After the cold exposure the tail was dried and the temperature was recorded. CPT testing was then performed again. Immediately following CPT test-

Table 1. Primers used for quantitative RT-PCR.

Transcript	Sequence (5′ to 3′)
α1A-adrenoreceptor (F)	GAATGTCCTGCGAATCCAGT
α1A-adrenoreceptor (R)	GGGATACGCACCATGTCTCT
α2C-adrenoreceptor (F)	GGGTTTCCTCATCGTTTTCA
α2C-adrenoreceptor (R)	GAAAAGGGCATGACCAGTGT
CGRP (F)	CCCAGAAGAGATCCTGCAAC
CGRP (R)	GTGGCACAAAGTTGTCCTT
CGRP receptor (F)	AGGTCCAGAGGATGAGCAGA
CGRP receptor (R)	GCTTTCAGCATCAGAACAA
HIF-1a (F)	AAGCACTAGACAAAGCTCA
HIF-1a (R)	CCATATCGTGTCCACATCA
GDNF (F)	GTACTTCGCGCTGCCAACT
GDNF (R)	GCTTTCACAGTCCAGACG
NOS-1 (F)	GAGAGGCACCCCAACTCT
NOS-1 (R)	GGAAAGAAACGCAAGGGTTC

F denotes the forward primer sequence and R denotes the reverse primer sequence.

ing, tail temperature was recorded again so that the amount of rewarming that occurred during the CPT tests could be assessed.

Experiment 2: Acute Vibration Exposure and CPTs. Rats were acclimated to restraint and CPT testing, and then exposed to a single 4-h bout of vibration ($n = 16$) or restraint control ($n = 16$). CPT tests were performed immediately before and after the exposure. Immediately after the post-exposure CPT test, half of the rats from each condition were euthanized using pentobarbital (100 mg/kg) and exsanguinated. The lumbar spinal cord, DRGs from the L2–5 regions, and ventral tail nerves were dissected, immediately frozen in liquid nitrogen, and stored at −80°C. The remaining rats were returned to their home cages and colony room. Twenty-four hours after the post-exposure CPT test, an additional CPT test was performed on the remaining rats. Immediately following this test the rats were euthanized, and tissues were collected and frozen as described previously.

Quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) was used to measure transcript levels for CGRP, the CGRP receptor, GDNF, IGF-1, NOS-1, α1-, α2A-, and α2C-adrenoreceptors, and ribosomal 18S, which served as an RNA loading control. The primer sequences for all transcripts examined are presented in Table 1.

RNA was isolated and purified as previously described.[24] First-strand cDNA was synthesized from 1 μg of total RNA using a reverse transcription system (Invitrogen, Carlsbad, California). The general protocol for performing qRT-PCR has been described previously.[24] Transcript levels from samples were measured and vibration-induced changes in transcript levels were calculated as the fold change from the time-matched restraint-control animals. Samples that did not show a single defined melt peak in the 80°C range were not included in the data set. Changes that were twofold or greater and were statistically different from each other were considered significant.

Analyses. Repeated-measures analyses of variance (ANOVAs) were used to analyze CPTs collected on days 1–4 in Experiment 1. CPTs recorded on the day of cold exposure from Experiment 1, and CPTs from Experiment 2, were analyzed using mixed-model ANOVAs, with animal added as a random variable. Transcript levels were analyzed using two-way (treatment × time) ANOVAs. Additional analyses were performed using appropriate ANOVAs, and pairwise comparisons were performed using Tukey's honestly significant difference tests. For all analyses, $P < 0.05$ was considered statistically significant. Analyses were performed using JMP version 5.0.1 or SAS/STAT version 9.1 (SAS for Windows; SAS Institute, Cary, North Carolina).

RESULTS

Experiment 1. Figure 2 shows the mean CPTs from each rat at 2000, 250, and 5 Hz over all 4 days of testing. Repeated-measures ANOVAs of mean CPTs at 2000 and 5 Hz did not reveal a significant effect of test day on CPT measures. However, at 250 Hz, repeated-measures ANOVA revealed that there was an effect of test day on CPT [$F(3, 9) = 9.23$, $P = 0.004$; Fig. 1C, D]; the CPT was higher on day 1 than on days 2–4 ($P < 0.05$). Mean coefficients of variation (CV ± SEM) across rats on days 2–4 were 5.98 ± 0.51%, 16.16 ± 1.27%, and 10.51 ± 2.30% for CPTs at 2000, 250, and 5 Hz, respectively. Thus, after acclimation to the procedure, CPTs appear to be fairly consistent at all frequencies.

On day 5, we assessed the effects of temperature on CPTs. There was an effect of time on tail temperatures [$F(2, 6) = 1322.74$, $P < 0.0001$], with temperatures in all rats being significantly lower immediately after exposure to the cold than before (Fig. 3A). Tails did rewarm while CPTs were being recorded, but post-CPT tail temperatures were still lower than temperatures recorded before cold exposure ($P < 0.05$). Although the tail temperature was significantly reduced by exposure to cold, CPTs at all three stimulation frequencies were not significantly altered by cold exposure (Fig. 3B).

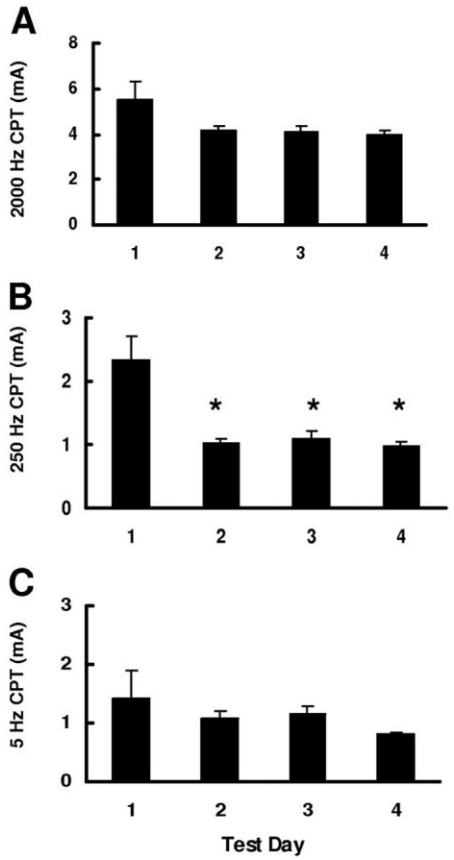

FIGURE 2. Mean CPT values (± SEM) recorded over four consecutive days at 2000 Hz **(A)**, 250 Hz **(B)**, and 5 Hz **(C)**. The 250-Hz CPT was lower at days 2–4 than at day 1 (*$P < 0.05$). However, after day 1, the CPT measures from all three frequencies were consistent.

Experiment 2. Exposure to acute restraint and vibration affected CPTs at 2000 Hz, but not at the other two frequencies (Fig. 4A–C). The ANOVA of the 2000-Hz data revealed a significant interaction between the exposure groups and time of CPT testing [$F(2, 44) = 15.78$, $P < 0.0001$]. Further analyses of this interaction demonstrated that the 2000-Hz CPT changed over time in both restraint-control [$F(2, 22) = 10.35$, $P = 0.0007$] and vibrated animals [$F(2, 22) = 9.59$, $P = 0.001$]. In the restraint-control rats, CPTs were significantly higher before the exposure than immediately or 24 h after the exposure (Fig. 4A; $P < 0.05$). Thus, following restraint exposure, rats were more sensitive to the stimulus. In contrast, in vibrated rats, 2000-Hz thresholds were significantly higher immediately after the vibration exposure than either prior to or 24 h after exposure, indicating that vibration resulted in a transient reduction in sensitivity to the 2000-Hz stimulus.

When 2000-Hz thresholds in restraint-control and vibrated rats were compared, the analyses revealed that both groups of rats displayed similar thresholds prior to and 24 h after exposures. However, immediately after exposures, vibrated rats displayed significantly higher thresholds than restraint-control rats [$F(1, 31) = 39.35$, $P < 0.0001$]. This difference in CPTs occurred because restraint-control rats become more sensitive to the stimulus, whereas vibrated rats become less sensitive.

Tail temperatures also were affected by the exposures. The ANOVA of the tail temperatures revealed a main effect of time [$F(1, 20) = 71.20$, $P < 0.0001$]; temperatures in the restraint-control and vibrated rats were similar prior to the exposures (21.9 ± 0.44°C and 22.52 ± 0.45°C, respectively), but were significantly reduced in both groups immediately following exposures (17.69 ± 0.41°C and 18.46 ± 0.41°C, respectively). By 24 h after the exposure, tail temperatures had returned to pre-exposure levels in both groups of rats.

Transcript levels in the spinal cord and DRGs were not affected by exposure to restraint or vibration. The acute exposure to vibration and restraint also did not affect IGF-1, α1-adrenoreceptor, α2C-adrenoreceptor, or CGRP-receptor transcript levels

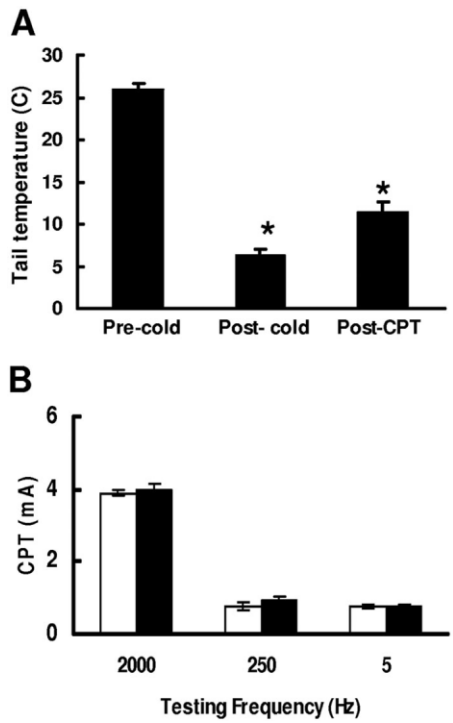

FIGURE 3. Tail surface temperatures before and immediately after exposure to cold, and after the collection of CPTs. **(A)** Cold exposure reduced tail temperatures (asterisk indicates less than pre-exposure temperature, $P < 0.05$). **(B)** CPTs recorded before (open bars) and after (filled bars) cold exposure. Exposure to cold did not alter CPT measures at any frequency.

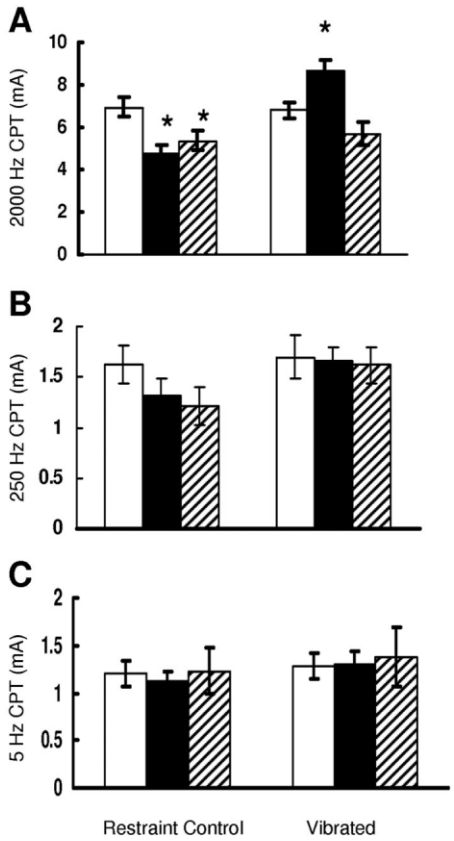

FIGURE 4. CPTs recorded before (open bars), immediately after (filled bars), and 24 h after exposure (cross-hatched bars) to vibration or restraint. **(A)** CPTs at 2000 Hz were significantly reduced immediately and 24 h after the exposure in restraint-control rats. In contrast, in vibrated rats, CPTs were significantly higher immediately following the exposure, but returned to pre-exposure levels 24 h later (asterisk indicates different from pre-exposure values, $P < 0.05$). Neither restraint nor vibration affected CPT measures at 250 Hz **(B)** or 5 Hz **(C)**.

in any tissues collected. However, in the ventral tail nerve, ANOVA revealed a main effect of condition on NOS-1 transcript levels [$F(1, 26) = 6.67$, $P = 0.02$]; NOS-1 levels were lower in rats exposed to vibration at both 1 and 24 h after the exposure (Fig. 5A). CGRP and GDNF transcript levels tended to be higher in the ventral tail nerves of vibrated rats 24 h after the exposure ($P = 0.05$ and 0.09, respectively, compared with 24-h restraint-control rats). However, without additional work to examine protein concentrations, it is difficult to determine whether these smaller changes in transcript levels are biologically relevant.

DISCUSSION

These studies demonstrate that the CPT method is reliable and can be used repeatedly to assess sensory nerve function in rat tails. CPTs at the 2000- and 5-Hz frequencies were consistent after the first day of testing both within and between subjects. The CPTs at 250 Hz also stabilized after the first day of testing. However, at 250 Hz, the intersubject variability on days 2–4 was higher than it was at the other two testing frequencies. Other studies have demonstrated that, under constant mechanical stimulation, the firing patterns of Aδ fibers in rat tails are more variable than the firing patterns of Aβ or C fibers.[16]

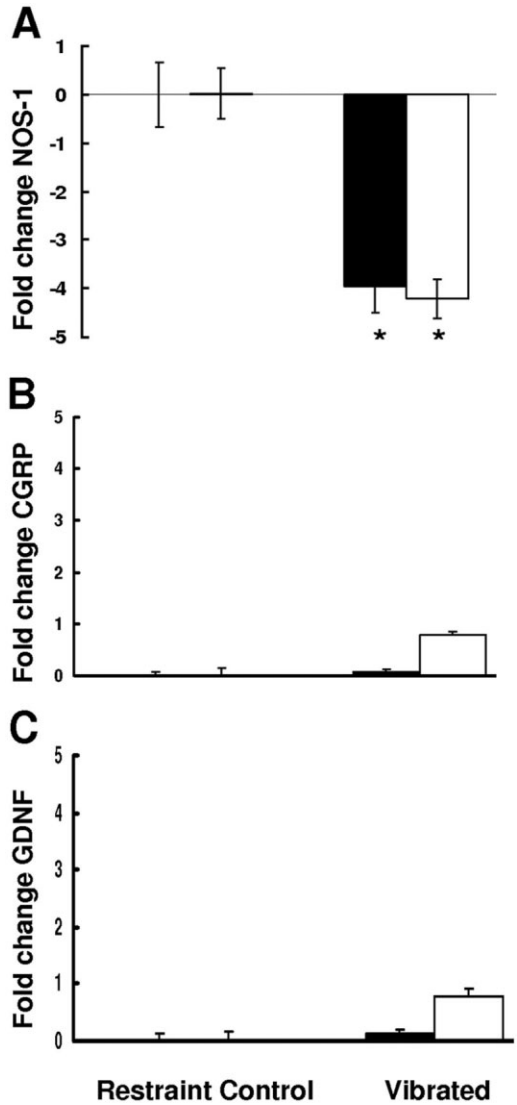

FIGURE 5. Transcript levels in the ventral tail nerves of rats collected 1 (filled bars) or 24 h (open bars) after exposure to restraint or vibration. NOS-1 transcript levels **(A)** were significantly reduced at 1 and 24 h after vibration exposure (asterisk indicates significant difference from restraint-controls, $P < 0.05$). CGRP **(B)** and GDNF **(C)** transcript levels also were higher in nerves 24 h after vibration exposure ($P = 0.05$ and 0.09, respectively, different from time-matched control).

Thus, the greater variability in 250-Hz thresholds may be due to varying discharge patterns in Aδ fibers in response to the stimulation.

Acute exposure to vibration resulted in a transient increase in CPTs at 2000 Hz, but not at 250 or 5 Hz. Thus, vibration reduced the sensitivity of the Aβ fibers to the stimulus, whereas Aδ and C fibers appeared to be unaffected. These data are consistent with human studies demonstrating that exposure to a brief bout of hand–arm vibration results in a transient reduction in Aβ-fiber sensitivity, characterized by a shift in vibrotactile sensitivity in the fingers.[27] Because the physiological effects of acute and repeated vibration exposures seem to be similar in human fingers and rat tails,[7,13,40] we conclude that the tail will serve as a good model for understanding the mechanisms leading to vibration-induced sensory loss.

The mechanisms underlying these temporary shifts in sensitivity have not been identified in animals or humans. There is evidence that exposure to a single bout of vibration results in unraveling of myelin in tail nerves of rats.[15] However, it seems unlikely that this morphological change underlies the reduced sensitivity of Aβ fibers to stimulation because the vibration-induced change in threshold is not maintained. Instead, it is likely the central nervous system rapidly adapts to the vibration and then gradually recovers after vibration is stopped.[32]

Surface tail temperatures in both restraint-control and vibrated rats were significantly decreased from pre-exposure temperatures. Surface and ambient temperatures affect the outcomes of vibrotactile threshold tests and nerve conduction velocity tests in humans.[8,21,41] However, it is unlikely that changes in CPTs were affected by ambient or surface temperatures in these studies. Experiment 1 demonstrated that reducing the surface temperature of the tail to approximately 6°C did not affect CPTs at any frequency. These data are consistent with findings from humans demonstrating that extreme differences in ambient temperature do not affect finger CPTs.[1] In addition, in Experiment 2, both groups of rats displayed a reduction in tail temperature, but vibrated rats displayed an increase in the 2000-Hz CPT while restraint-control rats displayed a reduction. Thus, it seems unlikely that temperature had a significant influence on CPTs.

A number of physiological tests have been used to diagnose HAVS in workers. For diagnostic purposes, the International Standards Committee (ISO) recommends using, and has established a protocol for administering, the vibrotactile threshold test (VTT; ISO-13091-1, 2001, and ISO-13091-2, 2003). The vibrating stimuli used in the VTT assesses Aβ and Aδ nerve fiber function.[12] This test has been used to identify vibration-induced changes in sensory perception after acute and chronic exposures to hand–arm vibration.[27,31] Other tests commonly used to diagnose HAVS include nerve conduction velocity,[5] thermal thresholds,[10] and mechanoreceptor thresholds.[35] None of these tests can be used to assess the function of the three different nerve fiber types individually, but by combining tests the function of all three different nerve populations can be assessed. The CPT technique allows all nerve fiber types to be assessed by a single test that is fairly easy to administer, making this test a more convenient monitoring tool. However, additional research needs to be done to determine whether the CPT will be able to detect progressive changes in sensory nerve function during chronic exposure to vibration, and whether these changes can be used to identify workers at risk for developing sensory dysfunction.

The vibration-induced reduction in the 2000-Hz CPT was associated with a reduction in NOS-1 expression in the ventral tail nerves, which was apparent 1 and 24 h after the exposure. NOS-1 is expressed in a number of different tissue types including DRGs, their processes, and Schwann cells.[37] A reduction in NOS-1 expression may lead to a reduction in the production of NO, which acts as a potent vasodilator.[6] Although we did not assess vascular function, other studies have demonstrated that exposure to a single bout of vibration results in vasoconstriction in tail arteries that is maintained for at least 24 h after the exposure.[15] A vibration-induced reduction in NO could be responsible in part for this prolonged vasoconstriction.

A reduction in NO also may protect nerves from damage by reducing intraneural swelling.[20] Acute exposure of rat hindlimbs or tails to vibration results in intraneural swelling in sciatic nerves and ventral tail nerves, respectively.[14,26] However, in the tail nerves, intraneural swelling occurred when skin temperatures were less than 15°C, but not when temperatures were maintained around 25°C. In our study, the skin temperature of the tail decreased to around 18.5°C. This reduction in temperature coupled with vibration may have induced intraneural edema in our animals. Additional studies examining the effects of NOS-1 reductions on NO concentrations and on vibration-induced intraneural swelling and vasodilation will determine what role the decline in NOS-1 plays in mediating vibration-induced changes in nerve function.

CGRP transcript levels also increased in ventral tail nerves 24 h after vibration exposure. CGRP transcription in DRGs is increased in response to nerve damage.[38] However, vibration did not increase CGRP transcript levels in the DRGs in this study. It is possible that a single exposure to vibration only induces a local response by the nerves and Schwann cells without affecting responses at the level of the ganglia. Increases in CGRP might also be protective by stimulating nociceptive signaling by mechanoreceptors,[34] thereby preventing overstimulation and damage of receptors and nerves.

Exposure to vibration induced an increase in IGF-1 immunoreactivity in nerves and tendons of rat hindlimbs.[17,18] However, in this study, IGF-1 transcript levels in the tail nerves were not affected by vibration exposure. A number of factors may account for the differences in these studies. First, the hindlimb studies exposed rats to vibration for 2 days, whereas our rats were exposed to a single bout of vibration. Second, vibration resulted in an increase in IGF-1 immunoreactivity that was greatest 2–3 days after the end of the exposure. We measured IGF transcript levels at 1 and 24 h after vibration exposure. Thus, we may not have seen vibration-induced changes because IGF-1 transcription may not be affected by a single exposure or because we did not sample at times when IGF-1 transcription was altered.

REFERENCES

1. Appenzeller O, Wood SC, Appenzeller T. Pentoxifylline, altitude and peripheral nerve function. Ann Sports Med 1988;4: 286–288.
2. Bovenzi M, Griffin MJ, Ruffell CM. Vascular responses to acute vibration in the fingers of normal subjects. Cent Eur J Public Health 1995;3:15–18.
3. Bovenzi M. Exposure–response relationship in the hand–arm vibration syndrome: an overview of current epidemiology research. Int Arch Occup Environ Health 1998;71:509–519.
4. Bovenzi M, Lindsell CJ, Griffin MJ. Acute vascular responses to the frequency of vibration transmitted to the hand. Occup Environ Med 2000;57:422–430.
5. Brammer AJ, Pyykko I. Vibration-induced neuropathy. Detection by nerve conduction measurements. Scand J Work Environ Health 1987;13:317–322.
6. Burnstock G, Ralevic V. New insights into the local regulation of blood flow by perivascular nerves and endothelium. Br J Plast Surg 1994;47:527–543.
7. Chang KY, Ho ST, Yu HS. Vibration induced neurophysiological and electron microscopical changes in rat peripheral nerves. Occup Environ Med 1994;51:130–135.
8. Cherniack M, Brammer AJ, Lundstrom R, Meyer J, Morse TF, Nealy G, et al. Segmental nerve conduction velocity in vibration-exposed shipyard workers. Int Arch Occup Environ Health 2004;77:159–176.
9. Curry BD, Bain JL, Yan JG, Zhang LL, Yamaguchi M, Matloub HS, et al. Vibration injury damages arterial endothelial cells. Muscle Nerve 2002;25:527–534.
10. Ekenvall L, Gemne G, Tegner R. Correspondence between neurological symptoms and outcome of quantitative sensory testing in the hand–arm vibration syndrome. Br J Indust Med 1989;46:570–574.
11. Frostick SP, Yin Q, Kemp GJ. Schwann cells, neurotrophic factors, and peripheral nerve regeneration. Microsurgery 1998;18:397–405.
12. Gescheider GA, Sklar BF, Van Doren CL, Verrillo RT. Vibrotactile forward masking: psychophysical evidence for a triplex theory of cutaneous mechanoreception. J Acoust Soc Am 1985;78:534–543.
13. Goldsmith PC, Molina FA, Bunker CB, Terenghi G, Leslie TA, Fowler CJ, et al. Cutaneous nerve fibre depletion in vibration white finger. J R Soc Med 1994;87:377–381.
14. Govindaraju SR, Curry BD, Bain JL, Riley DA. Effects of temperature on vibration-induced damage in nerves and arteries. Muscle Nerve 2006;33:415–423.
15. Govindaraju SR, Curry BD, Bain JL, Riley DA. Comparison of continuous and intermittent vibration effects on rat-tail artery and nerve. Muscle Nerve 2006;34:197–204.
16. Handwerker HO, Anton F, Reeh PW. Discharge patterns of afferent cutaneous nerve fibers from the rat's tail during prolonged noxious mechanical stimulation. Exp Brain Res 1987;65:493–504.
17. Hansson HA, Dahlin LB, Lowenadler B, Lundborg G, Paleus S, Skottner A. Transient increase in insulin-like growth factor I immunoreactivity in rat peripheral nerves exposed to vibrations. Acta Physiol Scand 1988;132:35–41.
18. Hansson HA, Dahlin LB, Lundborg G, Lowenadler B, Paleus S, Skottner A. Transiently increased insulin-like growth factor I immunoreactivity in tendons after vibration trauma. An immunohistochemical study on rats. Scand J Plast Reconstr Surg Hand Surg 1988;22:1–6.
19. Herrick AL. Pathogenesis of Raynaud's phenomenon. Rheumatology (Oxford) 2005;44:587–596.
20. Kajekar R, Moore PK, Brain SD. Essential role for nitric oxide in neurogenic inflammation in rat cutaneous microcirculation. Evidence for an endothelium-independent mechanism. Circ Res 1995;76:441–447.
21. Klinenberg E, So Y, Rempel D. Temperature effects on vibrotactile sensitivity threshold measurements: implications for carpal tunnel screening tests. J Hand Surg [Am] 1996;21:132–137.
22. Koga K, Furue H, Rashid MH, Takaki A, Katafuchi T, Yoshimura M. Selective activation of primary afferent fibers evaluated by sine-wave electrical stimulation. Mol Pain 2005;1.
23. Krajnak K, Dong RG, Flavahan S, Welcome DE, Flavahan NA. Acute vibration increases α_{2c}-adrenergic smooth muscle constriction and alters thermosensitivity of cutaneous arteries. J Appl Physiol 2006;100:1230–1237.
24. Krajnak K, Waugh S, Miller R, Baker B, Geronilla K, Alway SE, et al. Proapoptotic factor Bax is increased in satellite cells in the tibialis anterior muscles of old rats. Muscle Nerve 2006; 34:720–730.
25. Kurozawa Y, Nasu Y. Current perception thresholds in vibration-induced neuropathy. Arch Environ Health 2001;56:254–256.
26. Lundborg G, Dahlin LB, Danielsen N, Hansson HA, Necking LE, Pyykko I. Intraneural edema following exposure to vibration. Scand J Work Environ Health 1987;13:326–329.
27. Maeda S, Griffin MJ. Temporary threshold shifts in fingertip vibratory sensation from hand-transmitted vibration and repetitive shock. Br J Ind Med 1993;50:360–367.
28. Maeda S. Temporary threshold shifts in fingertip vibratory sensation from hand-transmitted vibration and repetitive shock. Nagoya J Med Sci 1994;57(suppl):185–193.
29. Miyakita T, Miura H, Futatsuka M. An experimental study of the physiological effects of chain saw operation. Br J Indust Med 1987;44:41–46.
30. Nasu Y, Ishida K. Follow-up study of patients with vibration syndrome in Japan. Scand J Work Environ Health 1986;12: 313–319.
31. Nishiyama K, Taoda K, Yamashita H, Watanabe S. Temporary threshold shift of vibratory sensation induced by a vibrating

handle and its gripping force. Int Arch Occup Environ Health 1996;69:45–52.
32. O'Mara S, Rowe M, Tarvin R. Neural mechanisms in vibrotactile adaptation. J Neurophysiol 1988;59:607–622.
33. Okada A. Physiological response of the rat to different vibration frequencies. Scand J Work Environ Health 1986;12:362–364.
34. Pare M, Elde R, Mazurkiewicz JE, Smith AM, Rice FL. The Meissner corpuscle revised: a multiafferented mechanoreceptor with nociceptor immunochemical properties. J Neurosci 2001;21:7236–7246.
35. Pyykko I. Clinical aspects of the hand–arm vibration syndrome: a review. Scand J Work Environ Health 1986:439–447.
36. Pyykko I, Gemne G. Pathophysiological aspects of peripheral circulatory disorders in the vibration syndrome. Scand J Work Environ Health 1987;13:313–316.
37. Qi W-N, Yan Z-Q, Whang PG, Zhou Q, Chen L-E, Seaber AV. Gene and protein expressions of nitric oxide synthases in ischemia-reperfused peripheral nerve of the rat. Am J Physiol 2001;281:C849–C856.
38. Ramieri G, Stella M, Calcagni M, Cellino G, Panzica GC. An immunohistochemical study on cutaneous sensory receptors after chronic median nerve compression in man. Acta Anat 1995;152:224–229.
39. Shadiack AM, Sun Y, Zigmond RE. Nerve growth factor antiserum induces axotomy-like changes in neuropeptide expression in intact sympathetic and sensory neurons. J Neurosci 2001;21:363–371.
40. Takeuchi T, Takeya M, Imanishi H. Ultrastructural changes in peripheral nerves of the fingers of three vibration-exposed persons with Raynaud's phenomenon. Scand J Work Environ Health 1988;14:31–35.
41. Verrillo RT, Bolanowski SJ Jr. The effects of skin temperature on the psychophysical responses to vibration on glabrous and hairy skin. J Acoust Soc Am 1986;80:528–532.