ABSTRACT: Prolonged hand-transmitted vibration exposure in the workplace has been recognized for almost a century to cause neurodegenerative and vasospastic disease. Persistence of the diseased state for years after cessation of tool use is of grave concern. To understand persistence of vibration injury, the present study examined recovery of nerve conduction velocity and structural damage of myelinated axons in a rat tail vibration model. Both 7 and 14 days of vibration (4 h/day) decreased conduction velocity. The decrease correlated directly with the increased percentage of disrupted myelinated axons. The total number of myelinated axons was unchanged. During 2 months of recovery, conduction velocity returned to control level after 7-day vibration but remained decreased after 14-day vibration. The rat tail model provides insight into understanding the persistence of neural deficits in hand–arm vibration syndrome.
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PERSISTENT REDUCTION OF CONDUCTION VELOCITY AND MYELINATED AXON DAMAGE IN VIBRATED RAT TAIL NERVES

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Hand–arm vibration syndrome (HAVS) is a chronic condition caused by excessive hand-transmitted vibration.[1] The association between the use of pneumatic vibratory tools and Raynaud disease-like symptoms goes back as far as 1911, when it was described by Professor Loriga of Rome.[17] HAVS consists of a spectrum of neurovascular symptoms, including paresthesia, pain, vasospastic episodes (secondary Raynaud phenomenon), decreased strength, and decreased dexterity in the hand. These changes are intermittent and reversible at first, but with prolonged duration of vibration exposure they become more continuous and irreversible. An estimated 8 million American workers are exposed to some form of industrial vibration, and 1.5 million of these workers are exposed to hand-transmitted vibration.[24]

Histopathological changes have been seen in digital nerve biopsies of patients with HAVS, including a demyelinating neuropathy with a marked loss of nerve fibers as well as an increase in the number of Schwann cells and fibroblasts. There is destruction of the myelin sheath, an accumulation of smaller nonmyelinated axons, and perineural fibrosis.[23] Similar changes were seen in biopsies of the dorsal interosseus nerve just proximal to the wrist of patients with 17–30 years of exposure to vibratory tools. Stromberg et al.[22] concluded that vibration exposure leads to peripheral nerve demyelination that fails to regenerate and is replaced by fibrosis and intraneural edema. Vibrating tools can cause dysfunction of sensory nerve endings, including mechanoreceptors, in the finger skin.[5] Hirata and Sakakibara[13] reported that, compared to control patients, HAVS patients with an average vibration exposure of 28 years had decreased sensory nerve conduction velocities in the median, ulnar, and radial nerves of the hand.

Although human studies have focused for the most part on the long-term neurological sequelae of vibration exposure, many animal studies have investigated the structural, cellular, and molecular changes that occur in the nervous system early in the vibration injury process. The rat tail model mimics the effect of vibration exposure on the hand and digits due to the anatomical similarities of central bones closely surrounded by arteries, veins, muscles, long tendons, and mixed sensory and motor nerves. The biodynamic responses to vibration of the rat tail and human finger are very similar.[16]

Abbreviations: ANOVA, analysis of variance; HAVS, hand arm vibration syndrome; NCV, nerve conduction velocity
Key words: hand-arm vibration syndrome; occupational Raynaud disease; demyelination; nerve edema; peripheral nerve
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In the rat model, nerve conduction velocity (NCV) of rat tail nerves is significantly reduced immediately after exposure to 4 h of vibration at a constant acceleration of 50 m/s^2 and frequencies of 30, 60, or 120 Hz.[20] Rat tail cumulative vibration of 200, 500, and 800 h (56.9 m/sec^2) demonstrated that increased vibration dose correlated directly with lowered NCV.[4] Although NCV has been monitored to assess vibration-induced nerve injury, recoverability has not been examined. The present study of rat tail vibration investigated the effects of vibration exposure duration on the disruption of nerve conduction velocity and damage to myelinated axons and the ability to recover nerve function and structure.

MATERIALS AND METHODS

Animal Groups. Seventy-two male Sprague–Dawley rats (276–333 g) were randomly assigned to six groups of 12 rats per group. In each group, eight rats were vibrated, and four served as sham controls. As specified in Table 1, vibration exposure was either 7 or 14 days, and tissue acquisition occurred 0, 1, or 2 months postexposure (Table 1). Sham controls were put into the vibration setup for 7 or 14 days, except that the vibration motor was not activated. The sham controls were processed at the same timepoints as vibrated rats. Rats were housed at 22 ± 1°C and a 12/12 h dark/light cycle. Access to food and water was ad libitum. Animal treatment and husbandry procedures were approved by the Medical College of Wisconsin's Animal Care Committee and complied with the Laboratory Animal Welfare Act. Animal protocols were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee. The animal housing facilities were approved by the Association for Assessment and Accreditation of Laboratory Animal Care.

Table 1. Treatment schedule for vibrated and sham control rats.

Group	Vibrated rats number (code)	Nonvibrated number (code)	Vibration (days)	Recovery (months)
1	8 (V7R0)	4 (C7R0)	7	0
2	8 (V7R1)	4 (C7R1)	7	1
3	8 (V7R2)	4 (C7R2)	7	2
4	8 (V14R0)	4 (C14R0)	14	0
5	8 (V14R1)	4 (C14R1)	14	1
6	8 (V14R2)	4 (C14R2)	14	2

The shorthand designation for the subgroups was constructed as follows: Group 1 – "vibrated 7 days recovery zero months" (V7R0), Group 1 – "7 day sham control recovery zero months (C7R0). Using this strategy, the designations are Group 2 (V7R1) (C7R1), Group 3 (V7R2) (C7R2), Group 4 (V14R0) (C14R0), Group 5 (V14R1) (C14R1), and Group 6 (V14R2) (C14R2).

Vibration and Recovery. Rats exposed to vibration were placed individually in restraint tubes on a nonvibrating platform, and their tails were taped against a vibrating stage as described previously.[7] The sham controls were housed and restrained daily in an identical fashion. However, their tails were taped to a nonvibrating platform. Vibration was performed without anesthesia or sedation and consisted of linear vertical oscillations of 60 Hz and 5*g* acceleration (49 m/s^2, r.m.s.) for 4 h/day for either 7 or 14 consecutive days (Table 1). These vibration time periods were based on prior studies that showed significant electrophysiological and ultrastructural changes after that period of time.[4,20] Vibration was administered during the light cycle in a temperature-controlled room at 22 ± 1°C. An electromagnetic vibration accelerator, a Brüel and Kjaer (B&K) vibration motor type 4809 (Naerum, Denmark) was driven by a sine wave signal from a Simpson 420 Function Generator (Elgin, Illinois). Acceleration was set with a B&K Power Amplifier type 2706. Frequency and acceleration were set prior to beginning the study using an HP 1201B oscilloscope and a B&K 4384 accelerometer connected to a B&K Integrating Vibration Meter, type 2513. Following the final day of vibration the recovery rats were returned to their housing facility for either 1 or 2 months. (Table 1). The recovery times of 0, 1, and 2 months were chosen to establish the degree of damage immediately (0 months) and the recovery after 1 and 2 months.

Electrophysiologic Testing and Tissue Harvesting. On the last day of recovery, or within 24 h postvibration for the 0-month recovery groups, rats were deeply anesthetized with sodium pentobarbital, 30 mg/kg, intraperitoneally. Nerve conduction velocities were recorded in the ventral lateral nerve trunk of the tail at caudal segment C5 using a pair of platinum-wire hook electrodes cradling the nerve trunk (Fig. 1). Stimulating electrodes were positioned on the exposed nerve trunks 4 cm distal to the recording electrodes. A supramaximal stimulus of 10 mA was delivered to excite the nerve fibers. The arrival time or latency poststimulation of the action potential was recorded using a TECA Vickers Medical Sapphire 4 electrophysiological computer system (Vickers Medical, Sidcup Kent, UK). This latency period was used to calculate the NCV. The temperature of the recording site was maintained at 22 ± 1°C. After recording, the tail segments C5–C8 were excised to obtain nerve, artery, skin, and muscle samples for histological analysis. The rats were euthanized by anesthetic overdose and pneumothorax.

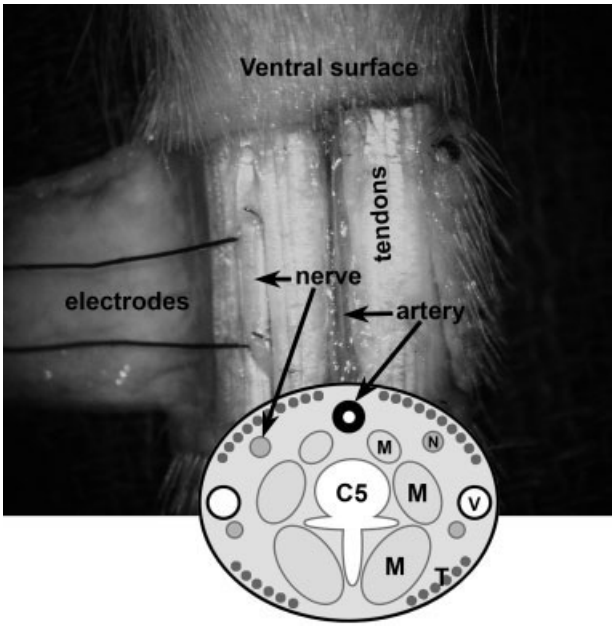

FIGURE 1. The ventral tail skin was reflected to expose the midline ventral tail artery and the ventrolateral nerve trunk cradled in a pair of platinum hook electrodes. The diagrammatic cross section of the tail segment shows the longitudinal tendons (T) below the skin, ventrolateral nerve trunks (N), large lateral veins (V), paravertebral pairs of segmental skeletal muscles (M), and the fifth caudal vertebra (C5).

Tissue Staining and Processing. After recording NCV, tail segments C5–C8 were removed individually and immersion-fixed in 4% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, at room temperature. Two hours later the tissue vials were moved to a refrigerator and stored overnight. The next morning the nerves were carefully dissected from the segments, cut into short pieces, and postfixed in 1.3% osmium tetroxide. Portions of nerves previously exposed for electrode placement were not taken for morphological analysis because of potential artifacts. Following rinsing in buffer, tissues underwent routine dehydration and infiltration for epoxy resin embedding.[10] Semithin epoxy sections (0.5 μm) of nerves were cut with glass knives and stained with Toluidine blue. Photomicrographs were taken digitally on a Zeiss microscope of whole nerve cross sections at $\times$20 and regions of nerves at $\times$40.

Morphometric Analysis. The semithin cross-section images were analyzed morphometrically using MetaVue bioimaging software (Molecular Devices, MDS Analytical Technologies, Toronto, Canada). The structure of myelinated axons was assessed after one lab member numerically coded each image to blind the operator to group identity/treatment. When all measurements were completed the code was unblinded, and the nerve data was parsed into the appropriate groups for statistical analysis.

Counting and Categorization of Axons. The total number of myelinated axons per whole nerve section was counted. The original $\times$20 image was magnified 200% on the computer screen. Beginning at the uppermost portion of the image and continuing in a clockwise fashion, each myelinated axon was counted and classified as type 1 normal, type 2 abnormal, or type 3 severely disrupted. Classification types are illustrated in Figure 2. Type 1 normal myelinated axons had uniform, densely packed myelin with zero or no more than one thickened/indented region. This thickening represented focally decompacted separation of the layers of myelin forming a wide dense region that indented the axon. Type 2 abnormal myelinated axons exhibited 2 or more regions of myelin thickening/indentation. Type 3 severely disrupted myelinated axons showed nearly complete disorganization and dark staining of the myelin that obscured the axon (Fig. 2). The total number of myelinated axons per nerve (summation of types 1, 2, and 3) and numbers of each type were used to calculate the percentages of normal, abnormal, and severely disrupted myelinated axons.

Statistical Analysis. Analysis of variance (ANOVA) and post-hoc Student–Newman–Keuls Multiple Comparisons Test were used to compare the means of the NCVs, total numbers of myelinated axons, and the percentage of myelinated axons with disrupted myelin sheaths (type 2 plus 3) for the different groups. Differences were considered significant at $P < 0.05$. Values are presented as mean $\pm$ SEM.

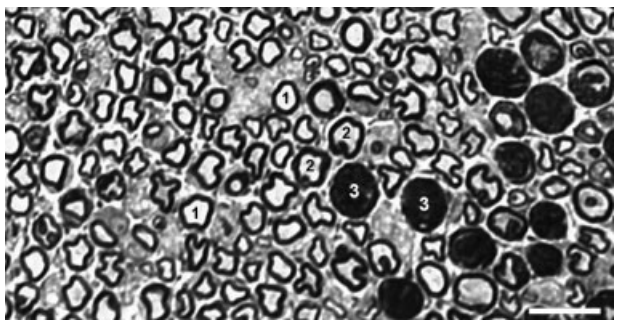

FIGURE 2. The Toluidine blue-stained cross-section of nerve fibers from a 14-day vibrated rat illustrates normal myelinated axons (type 1), abnormal (type 2), and severely disrupted (type 3) myelinated axons. Scale bar = 75 μm.

RESULTS

Nerve conduction velocities. The mean NCVs, total number of myelinated axons, and the percentage of abnormal axons of the sham controls for 7-day vibration and for 14-day vibration were not statistically different. Therefore, we combined the 12 7-day vibration sham control rats into one group (7-day vibration control), and the 12 14-day vibration sham control rats into another group (14-day vibration control). The 7- and 14-day vibrated groups were each compared to their respective control. For the 7-day vibrated rats, the 0 recovery group exhibited the largest decrease in NCV. At 1 month, NCV returned toward normal, and by 2 months NCV was not different from control (Fig. 3). Following 14 days of vibration, NCV was significantly decreased at zero recovery time, and it remained significantly decreased compared to the sham control after 1 and 2 months of recovery (Fig. 3).

Total Number of Myelinated Axons and Percentage with Disrupted Myelin. The total number of myelinated axons per nerve did not differ significantly between control and vibrated groups. The average was 1,406 ± 21 myelinated axons for caudal segment C6. Compared to the 7-day sham controls, vibrated rats examined at 0 months recovery had the greatest percentage of disrupted myelinated axons (Fig. 4). The occurrence of disrupted axons was not different from control after 1 and 2 months of recovery. Following 14-day vibration, the percentages of disrupted myelinated axons at 0, 1, and 2-month recovery were higher than control (Fig. 4). On average, the 7-day controls contained 10.6 ± 1.5%, and the 14-day controls contained 8.3 ± 1.2% disrupted myelinated axons (type 2 plus 3). Disrupted axons comprised 19.1 ± 3.1% after 7-day vibration and 16.9 ± 2.4% after 14-day vibration (Fig. 4). The type 3 severely disrupted axons were uncommon in the 7-day control (0.9 ± 0.2%) and 14-day control (0.8 ± 0.2%). Type 3 comprised small percentages of the disrupted myelinated axons in the 7-day (3.6 ± 1.6%) and 14-day (2.2 ± 0.8%) zero recovery groups.

The mean NCVs of the individual six control and six vibrated groups were plotted against the average percentages of disrupted myelinated axons in each group. The correlation coefficient was $R = 0.74$ for a polynomial curve fit (Fig. 5).

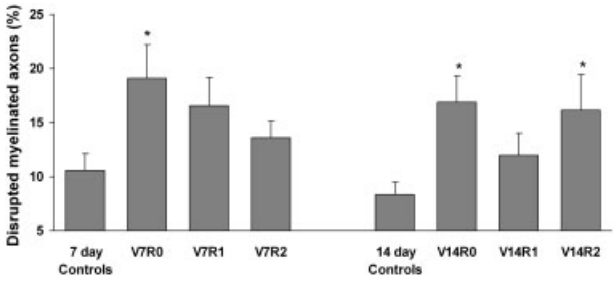

FIGURE 4. The percentage of axons with disrupted myelin was significantly (*$P < 0.05$) higher than sham control for recovery time 0 for both 7- and 14-day vibrated rats (V7R0, V14R0). The percentage of disrupted axons remained significantly high at 2 months recovery after 14-day vibration (V14R2).

DISCUSSION

This study of 7- and 14-day vibration demonstrates that the longer vibration exposure causes reduced nerve conduction velocity and myelinated axon damage that fail to recover in 2 months. For 7-day vibration, NCV is markedly reduced immediately (zero recovery), but it partially recovers after 1 month and fully recovers by 2 months. The NCV is significantly decreased after 14-day vibration (zero recovery), but

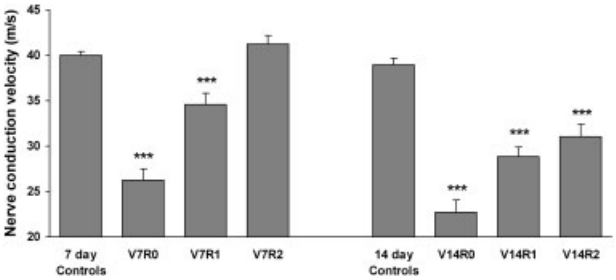

FIGURE 3. Nerve conduction velocity is shown for the 7- and 14-day sham controls, 7-day and 14-day vibrated rats recovered 0 (V7R0, V14R0), 1 (V7R1, V14R1), and 2 (V7R2, V14,R2) months postvibration. ***Vibrated is significantly less than control at $P < 0.001$.

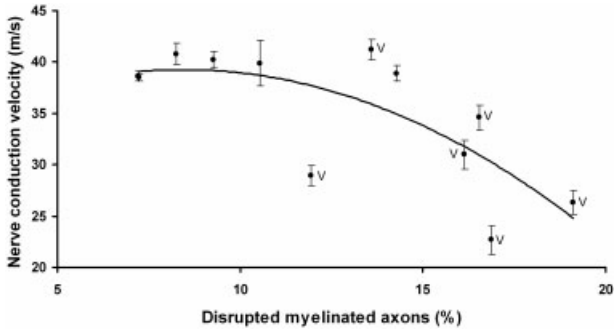

FIGURE 5. The decrease in nerve conduction velocity correlated directly with an increased in the percentage of disrupted myelinated axons. The data points for the six vibrated groups are indicated by the letter V. The six control groups are not marked. The leftmost point represents two overlapping control values.

it fails to recover at 2 months. These findings in the rat mimic human vibration injury in which nerve pathology is less likely to reverse following longer exposure to vibration in the workplace.

What structural damage to the vibrated rat tail nerves accounts for the decrease in NCV? Frank degeneration and loss of myelinated axons does not explain the decrease, because the total number of myelinated axons is unchanged. The reduction in NCV correlates directly with the increased percentage of disrupted myelinated axons. This agrees with the findings of disease and aging studies that myelin disruption is associated with decreased NCV.[3,13,22,23] In the present study, the lack of a NCV return to normal after 14-day vibration is due to persistence of disrupted myelinated axons. Repair of myelin occurs after 7-day vibration and 2-month recovery, but not following 14-day vibration. The percentages of disrupted myelinated axons are similar after 7- and 14-day vibration. This implies that the same population of myelinated axons is injured by 7- and 14-day vibration, but the magnitude of the injury per axon length increases with longer vibration. We predict that the extent of myelin disruptions per axon increases with longer vibration exposure. After 14-day vibration, the repair process is possibly overwhelmed and results in permanent damage, or the extensive injury requires longer than 2 months to repair.

Other Schwann cell pathology, such as node of Ranvier disruption during vibration, may have decreased NCV.[4] Normal myelination and survival of the axon requires a sustained trophic relationship between the myelinating Schwann cell and axon that is dependent on intact axoplasmic transport.[8,9,14] Previously, our laboratory showed reduction of retrograde axoplasmic transport following vibration.[25] Compromised axoplasmic transport may have diminished trophic interaction and prevented myelin repair during 2 months recovery.

How vibration disrupts myelinated axon structure and function in the rat tail and in humans using powered hand tools remains unclear. Direct physical damage from vibration acceleration and indirect damage secondary to restriction of blood flow are possibilities.[11] Vibration reduces tail skin blood flow and constricts the tail artery, and the tail nerves exhibit myelin disruption[10,12] and intraneural edema consistent with ischemic injury.[18,19] Ischemia produced experimentally by constricting the vasa nervorum[21] rapidly reduces nerve conduction velocity, and sustained blockage of blood flow[18,19] damages myelinated axons. Vibration white finger and slowed NCV can occur independently in forestry workers.[2] In shipyard workers, slowed sensory NCV in the hand can be increased in some nerve segments by increasing finger skin temperature systemically with exercise.[6] This reversal suggests that the nerve conduction pathology involves vascular insufficiency, because raising central body temperature, as occurs during exercise, increases blood flow and temperature in the extremities.[15] Thus, vibration may impair NCV by damaging the vasa nervorum and preventing adequate blood perfusion in the nonexercising individual. In the present study we have demonstrated that failure of recovery of NCV after 14 days of vibration correlates with persistence of damaged myelinated axons, but reduced blood perfusion is also possible.

The present study of vibration injury demonstrates a direct correlation between myelinated nerve degenerative changes and decreased NCV. The peripheral nerves are able to recover after 7-day but not after 14-day vibration exposure. The 2-month irreversible condition means that 14-day rat tail vibration can be used to test therapeutic agents that may either slow the progression toward irreversibility of injury or agents that may rescue vibration-injury conditions deemed irreversible and provide relief for long-term HAVS.

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