ABSTRACT: Hand-arm vibration syndrome (HAVS) results from excessive exposure to hand-transmitted vibration. Whether the peripheral nerve damage characteristic of HAVS is a direct result of vibration or is secondary to vascular insufficiency remains unclear. The purpose of this study was to explore the effect of vibration exposure on axoplasmic transport in peripheral nerves and soleus motor neurons. Sciatic nerves and motor neurons from rats following two 5-h periods of vibration exposure demonstrated disruption in retrograde transport compared to normal. After 10 days of vibration (5 h/day), axoplasmic transport failed to recover within 24–48 h in most rats. This study demonstrates that disrupted axoplasmic transport is an early consequence of short-term vibration exposure. The effects of vibration on axoplasmic transport also appear to be cumulative. This study provides a new biological way to evaluate measures to prevent early vibration injury.
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VIBRATION-INDUCED DISRUPTION OF RETROGRADE AXOPLASMIC TRANSPORT IN PERIPHERAL NERVE

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Occupational vibration exposure currently affects 8–10 million American workers.[5] Excessive hand-transmitted vibration may cause the debilitating neurological, vascular and musculoskeletal problems of hand-arm vibration syndrome (HAVS),[2,23] such as Raynaud's phenomenon and carpal tunnel syndrome.

In addition to the epidemiologic importance of HAVS,[1–3,7–9,14,23,24,32,33] several authors have reported the resulting histologic damage to nerve.[1,10,14,16,34] The precise etiology, however, of the myelin and axonal damage of peripheral nerve is uncertain. The neurologic symptoms of numbness, paraesthesias, and decreased dexterity may either be a direct response of nerve to vibration or a secondary response to pathologic vascular changes. To determine the mechanism of these neurologic changes, further analysis is necessary of the cellular response of nerve tissue to vibration. Since axoplasmic transport is the foundation for survival and metabolism of the neuron and its axon, this study explored the effect of vibration on motor neurons and on axoplasmic transport in peripheral nerves of the rat hind-limb.

Abbreviations: HAVS, hand-arm vibration syndrome; HRP, horseradish peroxidase; WGA, wheat germ agglutinin
Key words: axoplasmic transport; hand-arm vibration syndrome; occupational neuropathy; vibration
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MATERIALS AND METHODS

Subjects. Thirty 1.5-month-old male Sprague-Dawley rats, weighing 180–200 g, were the subjects of this research. The design of this study received approval from the Animal Resource Center of the Medical College of Wisconsin, and institutional guidelines for the care and use of laboratory animals were carefully followed. Rats were assigned to three groups of 10 rats each: a control group, a short-term vibration group, and a long-term vibration group.

Methods. After induction of anesthesia with intraperitoneal injection of pentobarbital (3.5 mg/l00 g body weight), the left hind-limb of each rat was shaved. Aided by surgical microscope magnification, a 1-cm incision was made in the medial side of the leg to expose the soleus muscle, while preserving its neurovascular bundle. A 0.2% solution (25 μl) containing 5 μg of wheat germ agglutinin (WGA) conjugated with horseradish peroxidase (HRP) was injected into the soleus muscle.[17,18,22,27] The injection hole in the muscle was closed with one 10/0 nylon suture, and the skin incision was closed with 5/0 nylon sutures.

The rats in the control and long-term vibration groups also underwent removal of the right soleus muscle (noninjection side) to determine muscle wet weight. The rats were then secured with standard porous tape to a customized vibration device. The rats' rectal body temperature was monitored throughout testing using microelectric thermometers (Electron Digital Thermometer SH66A, Middlefield, Connecticut).

Customized Vibration Device. The vibration device consisted of two platforms, namely a vibrating platform for the hindquarters and a larger stationary platform for the remainder of the body to rest (Fig. 1). The vibrating platform was suspended by springs from four steel rods. This platform was 5 mm away from the large stationary platform so that primarily the hindquarters received direct vibration (hindlimbs and tail).

A 120-V, 60-Hz, alternating-current 15-W vibrating motor was firmly attached to the underside of the vibrating platform (a two-intensity Swedish hand massager, PNER 24K; Oster, Milwaukee, Wisconsin). A piezoelectric accelerometer (Bruel & Kjeer Model Integrating Vibration Meter, Type 2516; Milwaukee, Wisconsin) was affixed to the vibrating platform by a magnet for constant monitoring of vibration parameters. A cooling fan for the motor was placed in the vibration chamber, to maintain the temperature of the vibrating platform and exclude any thermal effects.

The vibration frequency was 60 Hz with horizontal acceleration of 4.74 *g*. The horizontal velocity was 6 cm/s, the acceleration velocity was 49 m/s per s, and the horizontal displacement was 3.0 mm. These parameters, measured over 5 h of continuous operation, were found to have little variation.

The vibration frequency was selected because sinusoidal vibration at frequencies of 35–150 Hz adequately represents the vibration produced by the power hand tools that Radwin et al. studied in the automotive industry.[24] These authors also suggest that hazardous frequencies are in the range of 40–125 Hz, with amplitude greater than 0.1 mm.[24]

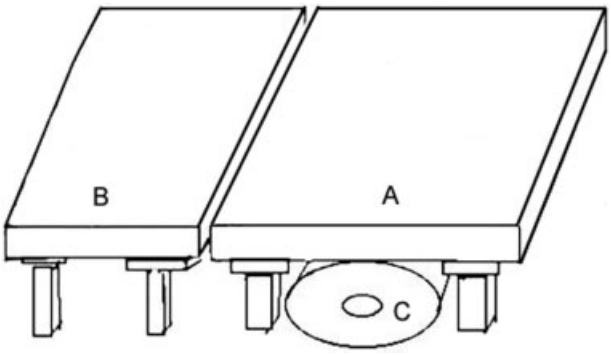

FIGURE 1. Customized vibration apparatus. The apparatus consists of a vibrating platform **(A)** separated by a 5-mm gap from a nonvibrating table **(B)**. A vibrating motor **(C)** is fixed to the underside of the vibrating platform, vibrating the hind limbs, tail, and a portion of the pelvis. The anesthetized rat is secured in a supine position by tapes across the wrist and ankles.

Nonvibrated Control Animals. These rats were not vibrated. They were attached to the vibration device two times for 5 h each time, once immediately following retrograde tracer injection, and once following 12 h free from the device. Twenty-four hours after injection, the rats were deeply anesthetized and perfused with 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) through the left ventricle. Tissue segments were harvested from the spinal cord (L3–S1), the sciatic nerve, the tibial nerve, the branch nerve to the soleus muscle, and the soleus muscle.

Short-Term Vibration Group. These rats received vibration, while anesthetized, for two 5-h sessions. One session was immediately following injection, and the other was following 12 h without vibration. After each vibration period, the rats were removed from the platform for ad-lib activity. Twenty-four hours after injection, the rats were perfused and nerve specimens were harvested in the same manner as for the control group.

Long-Term Vibration Group. These rats received 5 h/day of vibration for 10 days. Immediately following completion of vibration on day 10, the rats were anesthetized, and the soleus was injected with WGA/HRP. Following injection, the animals were returned to their cages. Two rats had 24 h of relaxation time and eight rats had 48 h of relaxation time, free from vibration, before perfusion and nerve harvesting, using the procedures described above.

Histologic Analysis. Frozen sections of the specimens from all groups, 40-μm thick, were prepared in a cryostat and reacted with the tetramethyl benzidine method to visualize HRP activity.[4,12,18,27] All reacted spinal cord, nerve, and soleus muscle tissue sections were studied by light microscopy. Reactive stained neurons and dendrites were counted through all sections of the spinal series.[18] The investigator who performed these histologic analyses was blind to the group origin of each slide.

Table 1. Summary of retrograde axonal transport in the rat nervous system.

Rat group	No. of labeled neurons (mean ± SD)	No. of stained dendrites (mean ± SD)	Tracer in nerve fibers	Deposits in the intraneural venulae
Nonvibrated control	29 ± 5	3.5 ± 1.3	Homogenous	No
Short-term vibration	0	0	Thick spots, rough line	Yes
Long-term vibration	0 in 8 rats 20 ± 3 in 3 rats	0 in 8 rats 1.1 ± 0.3 in 3 rats	Thick spots, rough line	Yes

RESULTS

The results are summarized in Table 1.

Nonvibrated Controls. Control rats displayed normal retrograde axoplasmic transport by conjugated WGA/HRP tracer (Figs. 2 and 3). Peroxidase-stained neurons were present in the anterior horn segment of every rat. Labeled neurons had dense, tiny stained particles of peroxidase reaction product distributed throughout the cell body and within the processes. Each neuron contained 2–8 stained processes, primarily dendrites, with a mean value ± SD of 3.5 ± 1.3 processes (Fig. 2). The axoplasmic tracer was labeled in the expected region of the lumbar cord for the motor nucleus of soleus.[27] The number of labeled neurons varied from 25 to 40 in each column, with a mean of 29 ± 5 neurons.

In the sciatic nerve, the entire path of axoplasmic transport, from the soleus branch to the tibial nerve to the sciatic nerve in the thigh, contained reactive nerve fibers. These presented homogenously, as a slight blue-purplish background with some fascicles/axons containing WGA-HRP staining product (Fig. 3). The average anal temperature for this group of rats was 33.1 ± 0.6°C.

Short-Term Vibration Group. In the spinal cord, no labeled neurons were present in any of the rats in this group (Fig. 2). In the peripheral nerve, abundant deposits of peroxidase staining were present in the branch nerve to the soleus muscle, the tibial nerve, and the distal portion of the sciatic nerve. The heaviest deposits were in the more distal portions. In the soleus nerve of all rats in this group, an irregularly-shaped deposit of dark purplish color was present. In the tibial nerve and distal sciatic nerve, the deposits presented as a dense rough line (Fig. 3B-1), or dispersed spots with thick deposits (Fig. 3B-2). Some dense stasis presented in the lumen of the micro-vessels of the tibial nerve (Fig. 3B-3).

Based on qualitative observation, peroxidase staining in the injected muscle was consistently more intensely bluish-purple in these vibrated rats than in the nonvibrated controls. The reactive product was primarily interstitial. The average anal temperature for this group of rats was 33.6 ± 1.2°C.

Long-Term Vibration Group. Neither of the two rats that had 24 h of relaxation before perfusion had stained neurons in the spinal cord under light microscope. Of the eight rats that had 48 h of relaxation before perfusion, only three had labeled axons. The mean number of retrograde labeled neurons in these three rats was 20 ± 3 neurons. The mean number of stained processes per neuron was 1.1 ± 0.3 processes. This was significantly fewer ($P < 0.01$, *t*-test) than in controls.

The mean number of labeled neurons was 29 neurons for controls, and 20 neurons for rats that underwent long-term vibration (Fig. 4). In vibrated nerves, a small amount of peroxidase staining was present in the interfascicular space. This deposition was not present in the control group. The average anal temperature for this group of rats was 37.2 ± 0.5°C.

Muscle Weight and Rat Behavior. The mean wet weight of the right soleus muscle for rats that underwent long-term vibration was 65 ± 6 mg. This was significantly less ($P < 0.01$) than that for controls, which was 128 ± 8 mg. The soleus muscle mean weight for the vibrated rats was only 51% of the mean weight for the controls, indicating significant ($P < 0.01$, *t*-test) atrophy.

In addition, the rats anesthetized daily and vibrated on the vibration units for 5 h/day for 10 days showed progressive weakness of the hind limbs and difficulty in walking. The nonvibrated animals (anesthetized and placed on the vibration unit for two 5-h sessions in 1 day) walked normally throughout the test period.

DISCUSSION

HAVS, prevalent among individuals with occupational vibration exposure, can cause significant functional problems due to damage to peripheral nerve.[25] Although myelin and axon disruption following vibration has been reported, the etiology of such nerve damage remains unclear.[5,11,15,25,26] Since

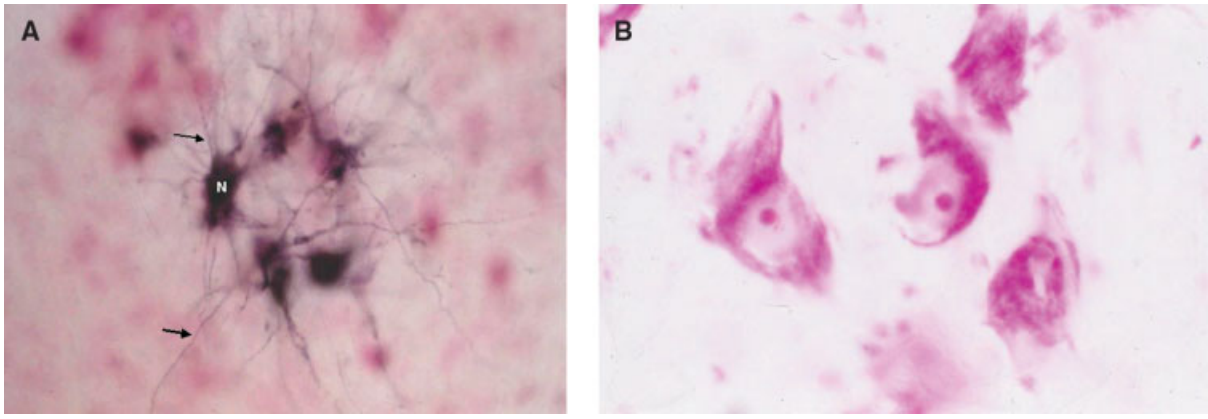

FIGURE 2. (A) WGA-HRP retrograde labeling of an α motor neuron. The motor neuron in the anterior horn of the L4 spinal cord of a nonvibrated rat exhibits peroxidase staining in the cell body (N), and numerous cell processes (arrows; original magnification, $\times$25). **(B)** No retrograde labeling occurred in the motor neurons in the L4 ventral horn of a rat vibrated for two 5-h periods (original magnification, $\times$100).

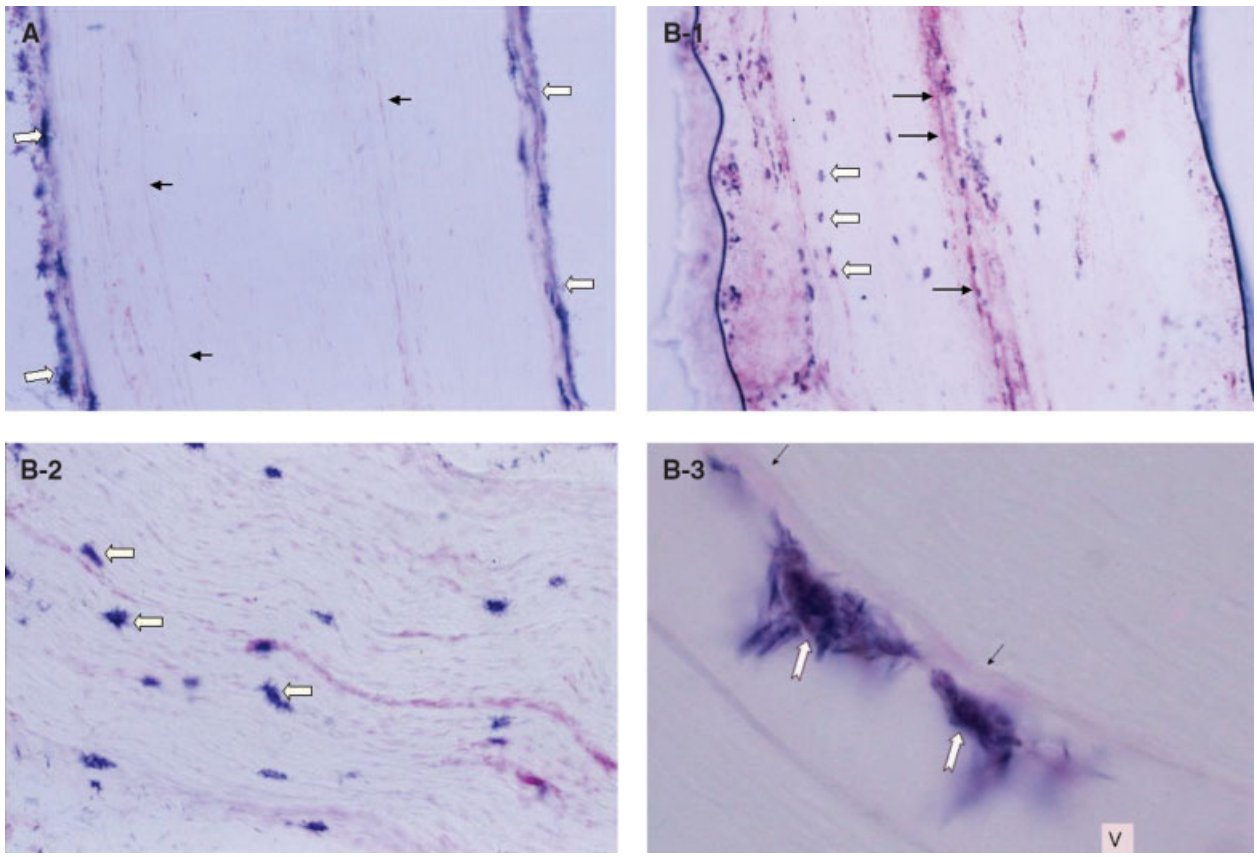

FIGURE 3. (A) A longitudinal section of the sciatic nerve from a nonvibrated rat. Axons containing retrograde tracer (black arrows) appear as fine streaks running longitudinally in the nerve. The remainder of the nerve was devoid of staining except for epineurium and perineurium staining, presumably within the venula system (white arrows; original magnification, $\times$50). **(B-I)** Retrograde tracer is localized in intense punctuate deposits (white arrows) along the length of the sciatic nerve from a rat after two 5-h periods of vibration. Obvious stasis in the vein of the sciatic nerve manifests as a dense, rough line (black arrows; original magnification, $\times$50). **(B-2)** The arrows identify these intense punctuate tracer deposits, at higher magnification, in the sciatic nerve from a rat after two 5-h periods of vibration (original magnification, $\times$150). **(B-3)** In a rat after two 5-h periods of vibration, dense deposits penetrated into the intraneural venulae of the tibial nerve (white arrows), due to vibration injury to the nerve fibers and venula walls, which were swollen and thickened (black arrows). "V" indicates the lumen (original magnification, $\times$210).

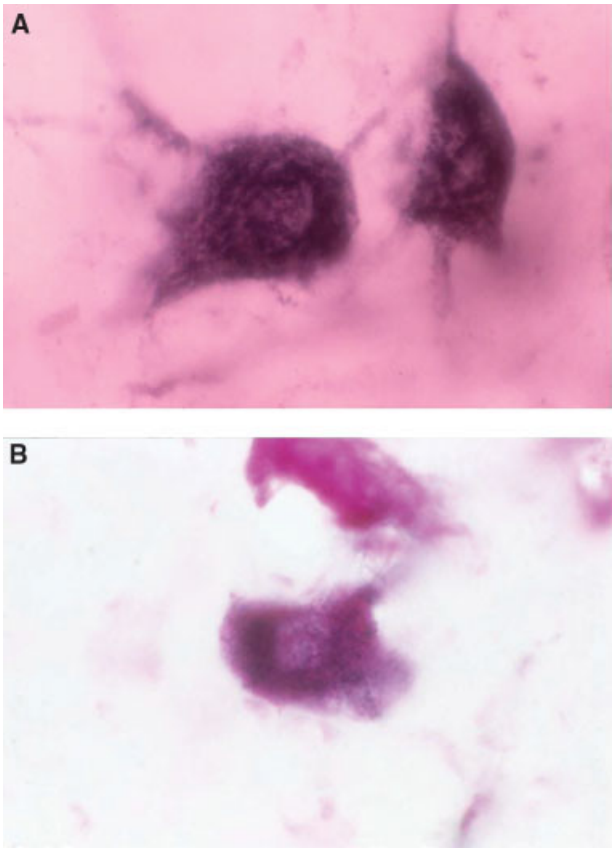

FIGURE 4. (A) Cross section of the anterior horn area of the spinal cord from a nonvibrated rat. The axons and many dendrites were distinctly labeled, with staining of each neuron and 2–8 dendrites (original magnification, ×210). **(B)** A small amount of dark retrograde reaction product is present in the motor neurons in the lumbar spinal cord of a rat vibrated 5 h/day for 10 days. Labeling of processes is much less common than in control rats (original magnification, ×210).

axoplasmic transport in peripheral axons is required for the survival of neurons, this study focused on the effect of vibration on axoplasmic flow.

Short-term exposure to vibration immediately impeded axoplasmic transport. The retrograde tracer appeared in clumps in the nerve. Retrograde transport of the WGA-HRP occurred effectively from the soleus muscle to the motor nerve cell body in the spinal cord. The reduction in transport in the vibrated rats was not due to differences in age or in the dosage of anesthetic drug compared to the nonvibrated rats. In addition, the control and test sections were reacted on the same slides to eliminate differences arising from the histochemical incubation media. Lower body temperature can slow axoplasmic transport. However, the average body temperature of the vibrated rats was approximately 0.5°C higher than that of the controls. This would enhance, rather than impede, axoplasmic transport.

Subjecting the hindquarters of anesthetized rats to x-axis, 5-g vibration for 5 h/day for 10 days caused profound deterioration of the neuromuscular system. These rats exhibited progressive loss of hindlimb motor control, muscle atrophy, and compromised soleus motor neuron axoplasmic transport. These degenerative changes are suggestive of axon damage that interrupts impulse conduction and produces frank degeneration of axons. Other animal studies of long-term vibration have demonstrated loss of axons and demyelination in limb nerves, as have finger skin biopsies in late-stage white finger disease in humans.[10,13,28,29]

Although the skeletal muscles of the rats with walking deficiencies in this study were not examined for damage, other vibration studies have found injury of muscle fibers.[19,20] Muscle weakness also occurs in late-stage HAVS.[6,8]

Compromised axonal transport in response to vibration could be secondary to vascular spasm, because such spasm is a direct result of vibration.[5,16,34] Severe ischemia due to vessel ligation reduces axonal transport,[4,31] but the reduction in blood flow to the nerve after vibration has not been determined. Rat tail vibration in a previous study resulted in only a 37% reduction in blood perfusion of the skin.[5] Whether this level of reduction in blood flow reduces axonal transport is uncertain.

Axonal transport deficits may also be a direct result of vibration. The mechanism by which vibration damages myelinated axons is unknown.[25,26] The morphological alterations resemble compression damage of peripheral nerves.[21,30] During vibration, pressure waves would need to be transmitted through the tail to compress the nerves, because the nerves are not superficial enough to be in direct contact with the vibration platform. Nerve compression impedes axoplasmic flow.[30] Vibration exposure injury may involve nerve compression in the rat hind limb. Compression may explain the increased susceptibility to carpal tunnel syndrome of workers exposed to vibration.

The results of this study indicate that the effects of vibration in disrupting axoplasmic transport are cumulative. After 2 days of vibration, although neurons were labeled, labeling was lighter than in normal controls. By the tenth day, 80% of the rats displayed no motor neuron labeling. Further study of the effect of more extended vibration exposure on axoplasmic flow would be valuable.

This study provides a useful biologic method to apply to future research on preventive measures for early vibration injury, such as glove use. Such investigations can compare the results of this study to

responses while employing preventive strategies during vibration, to evaluate the efficacy of such measures.

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