A quantitative study of vibration injury to peripheral nerves—introducing a new longitudinal section analysis

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Abstract

Purpose Long-term vibrations are known to cause neurovascular diseases, which are common in workers who operate handheld power tools or motor vehicles. Understanding the neuropathology of vibration-induced nerve injury is critical to its prevention and treatment. This study aims to evaluate whether light microscopy of longitudinal nerve sections can be used as a simple yet effective method for quantifying nerve injury.

Methods The rats were split into two groups that were subjected to vibration (4 h/day) for 7 or 14 days. They were then allowed to rest for varying periods of time. Longitudinal sections of the tail nerves were examined under light microscopy. Injuries to the nerves were classified into three types, counted, tallied, and then divided by the length of the nerve being studied.

Results Both 7 and 14 days of vibration showed significant damage when no recovery time was given. After 1 month of rest, the 7-day group began to show signs of recovery, but the 14-day group did not. After 2 months of rest, the 7-day vibration group showed almost complete recovery, while the 14-day vibration group still showed significant damage when compared to the sham control groups.

Conclusion The amount of damage to the myelin sheath directly correlated with vibration duration. When vibrated for longer than 7 days, nerve recovery was limited. This study also demonstrated that light microscopy of longitudinal slices is a simple yet effective method of quantifying the nerve damage.

Keywords Nerve injury · Longitudinal section analysis · Vibration nerve injury · Peripheral nerve injury

Abbreviations

HAVS	Hand-arm vibration syndrome
WBVS	Whole body vibration syndrome
NCV	Nerve conduction velocity
TEM	Transmission electro-microscopy

Introduction

It is estimated that greater than eight million American workers and military service personnel are exposed to vibration in the workplace [1, 5, 13]. Hand-arm vibration syndrome (HAVS) or whole body vibration syndrome (WBVS) are the results of chronic and excessive vibration to the extremity or whole body. Lorigia of Rome first made the connection between vibration and Raynaud's phenomenon-like symptoms in 1911 [15]. HAVS results in a wide variety of symptoms including numbness, pain, weakness, cold sensitivity associated with secondary Raynaud's phenomenon, carpal tunnel syndrome, and impaired dexterity. HAVS symptoms, if severe enough, can significantly affect activities of daily living [2, 11]. These symptomatic changes are reversible if the vibration duration is brief but can become permanent if vibration exposure is prolonged.

Histological findings in HAVS include demyelinating neuropathy, loss of nerve fibers, and increased number of fibroblasts. Fibrosis of the perineurium is also seen, as indicated its circular thickening [3, 9]. In addition to causing injury to the myelin sheaths of axons, HAVS has also been shown to cause dysfunction of mechanoreceptors in the skin of the fingers [12]. For the most part, human studies have been concentrated on long-term

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clinical and neurological findings in HAVS. However, studies of animal models allow us to visualize the ultrastructural changes seen earlier in the disease process, enabling early detection before HAVS symptoms appear.

Prior studies have reported that nerve conduction velocity (NCV) significantly decreases after 4 h of vibration at an acceleration of 50 m/s^2 and frequencies of 30, 60, and 120 Hz [8, 10]. Thus, vibration with these constraints is sufficient to cause temporary damage to the nerve axons. Although vibration-induced injury and recoverability have been studied with NCV as a parameter, pathological change and structural recovery have not been investigated. In our study, we use the rat tail vibration model to examine vibration-induced peripheral nerve injury and demonstrate the use of light microscopy as a fast, simple, and accurate method to determine nerve injury severity. While previous studies have used electron microscopy to view ultrastructural changes, they are not able to view a large section of the nerve to examine damage to the overall profile [3, 9]. Light microscopy, on the other hand, allows us to view the entire profile of the nerve in a longitudinal section, which enables a more accurate and reliable statistical analysis. With our technique, it will be possible to quickly analyze injuries caused by new vibration tools and equipment. This will aid in the development of prevention and treatment methods for vibration-induced nerve injury.

Fig. 1 Normal sham control. The tail of a normal rat was harvested and stained with Toluidine blue to serve as normal sham control. There are minimal injuries, due to handling and preparation, which are labeled. A photomicrograph of the rat tail nerve longitudinal cross section was captured at $\times 400$ magnification. The three types of myelin abnormalities were labeled with red numbers as type 1, 2, or 3 (please refer to *Counting and Categorization of Axons* in the “Materials and Methods” section). The black lines indicate the center of the axons, from which length measurements were obtained

Materials and Methods

Animal Groups Seventy-two male Sprague–Dawley rats (276–333 g) were randomly assigned to six groups, each consisting of twelve rats. Each group was then subdivided, and eight rats were vibrated while the remaining four served as sham controls. The experimental rats were vibrated for either 7 or 14 days, and nerves were harvested at 0, 30, and 60 days after the vibration periods. Nerves were harvested from the

Fig. 2 7-day vibration harvest with Toluidine blue stain. **a** 30 days of recovery. After 7 days of vibration experiments, the rat tail was harvested after 30 days of recovery. A photomicrograph of the rat tail nerve longitudinal cross section was captured. **b** 60 days of recovery. After 7 days of vibration experiments, the rat was allowed to recover for 60 days prior to harvesting. A photomicrograph of the rat tail nerve longitudinal cross section was captured. There are fewer injuries following the 60-day recovery. All figures were captured at $\times 400$ magnification. The three types of myelin abnormalities were labeled with red numbers as type 1, 2, or 3 (please refer to *Counting and Categorization of Axons* in the “Materials and Methods” section). The black lines indicate the center of the axons, from which length measurements were obtained

sham control groups at the same time as the experimental groups. All rats were housed at 22 ± 1 °C and a 12-h light/dark cycle. Access to food and water was ad libitum. Animal treatment and husbandry procedures were approved by the College's Animal Care Committee and complied with the Laboratory Animal Welfare Act. The College's Institutional Animal Care and Use Committee approved this animal protocol. The animal housing facilities were approved by the Association for Assessment and Accreditation of Laboratory Animal Care.

Vibration and Recovery The vibration group rats were individually placed in restraint tubes on a steady platform. Their tails were then taped to a vibrating stage as described previously [9]. The sham control groups were treated the same way; however, the platform was not turned on. The experimented rats were then vibrated, without anesthesia or sedation, for 4 h/day for either 7 or 14 consecutive days, during the light cycle in a temperature-controlled room identical to the room the rats were housed in. These time periods were based on prior studies that showed significant pathological changes occurred after that time [9]. Based on the literature, linear vertical oscillations of 60 Hz were used due to the minimum speed that decreased blood flow and conduction velocities [10]. An electromagnetic vibration accelerator, a Bruel and Kjaer vibration motor type 4809 (Naerum, Denmark), was driven by a sine wave signal from a Simpson 420 Function Generator (Elgin, IL). 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. These parameters were selected to be similar to those of most vibrating hand tools. After the specified time periods of vibration, the rats were returned to the housing facility to recover for either 0, 30,

and 60 days, at which point nerves from the tail were harvested.

Tissue Harvesting, Staining, and Processing Within 24-h post-vibration, rats were deeply anesthetized with sodium pentobarbital, 30 mg/kg, intraperitoneally. Once anesthetized, the tail segments C5–C8 were removed to obtain nerve samples for histological analysis. Rats were then euthanized by means of anesthetic overdose and pneumothorax. Nerve fibers were then immersion-fixed in 4 % glutaraldehyde, 2 % paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, at room temperature. Tissues were moved to a refrigerator after 2 h and stored overnight. Nerves were then carefully dissected from the segments, cut into short pieces, and postfixed in 1.3 % osmium tetroxide. After being rinsed in buffer, the tissues underwent routine dehydration and infiltration for epoxy resin embedding. Semithin epoxy sections (0.5 μ m) of nerves were cut with glass knives and stained with Toluidine blue. Photomicrographs were taken digitally, on a Zeiss microscope, of longitudinal sections of the nerves at $\times 200$ magnification and regions of those nerves at $\times 400$.

Pathological-Morphological Analysis The semithin longitudinally sectioned images were analyzed morphometrically using Meta-View bioimaging software (Molecular Devices, MDS Analytical Technologies, Toronto, Canada). The structure of the myelinated axons was assessed after one lab member numerically coded each image to blind the operator to group identity/treatment. After completion of all measurements, the code was unblinded, and the nerve data was organized into the proper groups for statistical analysis.

Counting and Categorization of Axons Myelinated axons were then coded along the total lengths for different types of abnormalities, which were categorized into three different

Fig. 3 7-day vibration. Results of total number of abnormalities/length (μ m 10^{-2}) after 7 days of vibration with 0, 30, and 60 days of rest. The blue box on the left indicates sham control while the red box on the right indicates the rats that underwent vibration. 30- and 60-day results were not statistically significant in terms of nerve recovery when compared to sham control ($P=0.2752$ and 0.0522 , respectively)

types, beginning with the least severe. Type 1 injuries represented an inward compression of the myelin sheath. Type 2 injuries were an outward loosening of the myelin sheath, showing diamond ring-like structures. Type 3 the most severe, were complete breaks in the myelin, the myelin layers were destroyed and presented an irregular stain. This classification is according to our previous transmission electro-microscopy (TEM) study [9]. Examples of all three types can be seen in Figs. 1, 2, and 4. Four fields of each longitudinally sectioned nerve were analyzed. The mean numbers of each type injury of the four fields of each particular section was then calculated to give the most representative data. The amount and type of deformities were used to compare the damage in the 0-, 30-, and 60-day recovery groups to the sham control groups. The tail of a normal rat was harvested and stained with Toluidine blue to serve as normal sham control (Fig. 1).

Statistical Analysis The sum of each type of abnormality was divided by sum of the length of all measured axons in a given section. This insured that each longitudinal section was compared proportionately. Two sample *t* tests on both sides were then used to compare the number of injuries per unit length of the experimental groups to that of their respective control groups. Differences were considered significant at $P < 0.05$ for the 7-day vibration group. Values are presented as mean $\pm$ standard deviation.

Results

The results show that the severity of the damage to myelinated nerves increases with the exposure time. Figure 2 is a compilation of two figures that were taken following 7 days of vibration. Figure 2a which showed 30-day recovery had significantly less injuries than Fig. 2b, the 60-day recovery. The 60-day recovery time had fewer injuries; although not completely at baseline, it has substantially less injuries than day 0 recovery. This showed that with the 7-day vibration group, recovery is imamate with adequate time.

For the rats undergoing 7 days of vibration, the 0-day recovery group had the greatest quantity of damage to the myelin. There was visibly apparent recovery for the 30- and 60-day recovery groups compared to the sham control (Fig. 3), although the difference was not statistically significant in terms of nerve recovery ($P = 0.2752$ and 0.0522 , respectively).

It also shows that there is a point at which the nerve damage becomes irreversible. Figure 4 is an example of this type of injury and was taken following 14 days of vibration. Figure 4a, b is very similar in the same number of injuries following 14 days of vibration. Although some recovery did

Fig. 4 14-day vibration harvest with Toluidine blue stain. **a** 30 days of recovery after 14 days of vibration experiments, the rat tail was harvested after 30 days. A photomicrograph of the rat tail nerve longitudinal cross section was captured. **b** 60 days of recovery after 14 days of vibration experiments, the rat was allowed to recover for 60 days prior to harvesting. A photomicrograph of the rat tail nerve longitudinal cross section was captured. All figures were captured at $\times 400$ magnification. The three types of myelin abnormalities were labeled with red numbers as type 1, 2, or 3 (please refer to *Counting and Categorization of Axons* in the “Materials and Methods” section). The black lines indicate the center of the axons, from which length measurements were obtained

occur in the 30- and 60-day recovery period, it was not as substantial as the 7-day vibration rats with the same recovery period.

For the rats undergoing 14 days of vibration, as expected, the 0-day recovery group also had the greatest quantity of damage to the myelin. However, while there was minimal recovery, obvious amounts of damage remained (Fig. 5). Compared to the sham control group, the 30- and 60-day recovery groups were statistically significant ($P = 0.0411$ and 0.0019 , respectively).

Fig. 5 14-day vibration. Results of total number of abnormalities/length ($\mu\text{m } 10^{-2}$) after 14 days of vibration with 0, 30, and 60 days of rest. All results of the 14-day rats that underwent vibration were statistically significant in terms of nerve recovery when compared to sham control ($P<0.05$)

Myelin Abnormality Value To compare myelin abnormalities between different groups, we took the total number of abnormalities of the axon and divided it by the total measured length in micrometers (no. of abnormalities) / (length of axon (μm)). We then obtained the average of the abnormality values for each group and graphed them (Figs. 3 and 5). The results for the 7-day vibration groups were as follows: 0-day recovery= 9.78 ± 1.83 , 30-day recovery= 7.96 ± 1.19 , 60-day recovery= 7.72 ± 1.92 . The results for the 14-day vibration groups were as follows: 0-day recovery= 10.11 ± 1.92 , 30-day recovery= 9.87 ± 1.68 , 60-day recovery= 9.81 ± 1.54 (Table 1).

Discussion

Our study of 7- and 14-day vibration-induced nerve injury demonstrates that prolonged vibration causes increased structural damage to the nerve myelin sheath in the rat tail model. This damage eventually becomes irreversible when the duration of vibration reaches a certain threshold. Our results in the

rat tail model are identical to those seen in human finger's vibration-induced nerve injury due to occupational exposure with handheld power tools.

Previously, our laboratory has shown an association of prolonged vibration with decreased NCV [8] as well as a reduction of retrograde axoplasmic transport [4, 14]. The structural damage demonstrated by this study may be underlying neuropathological mechanism that leads to the decreases in NCV and retrograde transport. It has also been shown that one of the early findings in the disease process is vascular endothelial cell injury [6, 7].

This study demonstrates a direct correlation between the duration of vibration and the ability of the rat tail nerves to recover. This is examined using light microscopy instead of TEM in order to obtain an overall profile view of the nerves for statistical analysis. The reversibility of the injuries sustained during the 7-day vibration group demonstrates that increased rest time without vibration can lead to some nerve recovery. However, the irreversibility of the injury in the 14-day vibration groups shows that there is a threshold of injury that nerves can tolerate and still recover before permanent injury occurs, and 14 days of vibration exceeds that threshold.

Table 1 Data of 7- and 14-day vibration

7-day vibration		Sham control ($\mu\text{m } 10^{-2}$)	SD (+/-)	Vibrated ($\mu\text{m } 10^{-2}$)	SD (+/-)
0-day recovery		6.45	1.03	9.78	1.83
30-day recovery		6.69	1.4	7.96	1.19
60-day recovery		6.67	1.54	7.72	1.92
14-day vibration		Sham control ($\mu\text{m } 10^{-2}$)	SD (+/-)	Vibrated ($\mu\text{m } 10^{-2}$)	SD (+/-)
0-day recovery		6.63	1.34	10.11	1.95
30-day recovery		6.7	1.02	9.87	1.68
60-day recovery		6.61	1.16	9.81	1.54

Comparing myelin abnormalities between different groups. The total number of abnormalities of the axon divided it by the total measured length in micrometers (no. of abnormalities) / (length of axon (μm)). These numbers represent the average of the abnormality values for each group

This translates into human injuries sustained as a result of prolonged periods of vibration, which can potentially be permanent depending on the duration.

Finally, our study also demonstrates that light microscopy longitudinal section examination is a fast and accurate method to quantitatively evaluate vibration injuries and it is also convenient for statistical analysis. Compared to TEM studies, light microscopy is faster, more economical, and provides an overall profile view for convenient statistical analysis. Transverse cross-section microscopy slides can also be used for this purpose. However, vibration injuries damage the entire myelin. A transverse cross section only captures a thin segment of a long myelin, which may not contain visible damage. On the other hand, longitudinal view of the nerve enables easier detection of myelin injuries and less probability of missed detection.

In conclusion, examining longitudinal sections using light microscopy is a simple, reliable, and economic method for identifying vibration injury to nerves compared to the standard transverse cross-sectional methods. This study may aid in the design and safety improvement of power tools and devices that can cause vibration-induced nerve injury. Once a power tool or device is prototyped, designers and engineers can utilize the rat tail vibration model along with longitudinal section light microscopy to determine the severity of damage caused by vibrations. This allows a simple and cost-effective way to test, adjust, and improve the safety of the power tool or device.

Conflict of Interest John Davis declares that he has no conflict of interest.

Ziyi Wang declares that he has no conflict of interest.

Lin Ling Zhang declares that she has no conflict of interest.

Michael Agresti declares that he has no conflict of interest.

Hani S. Matloub declares that he has no conflict of interest.

Ji-Geng Yan declares that he has no conflict of interest.

Statement of Human and Animal Rights All institutional and national guidelines for the care and use of laboratory animals were followed. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of Informed Consent No human subjects were used in this scientific research.

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