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I, Brian Y Kim, hereby submit this original work as part of the requirements for the degree of Master of Science in Mechanical Engineering.

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Dependence of Duration and Frequency on Location Specific Vascular Damage in the Rat Tail Model

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Committee member: David Thompson, Ph.D.
Dependence of Duration and Frequency on Location Specific Vascular Damage in the Rat Tail Model

A thesis submitted to the
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2014

By

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Committee Chair: Dr. Rupak K. Banerjee
ABSTRACT

Hand-Arm Vibration Syndrome (HAVS) is an injury that consists of vascular and musculoskeletal disorders caused by hand-transmitted vibration exposure to industrial workers. Current International Standard Organization (ISO) guidelines may be limited in predicting damage from exposures of known vibration frequency and duration.

A rat tail model is used to investigate the effects of different frequencies and durations on vascular damage. For the experiment, 24 male Sprague-Dawley rats (250 ±15 gm) were used. Rat tails were vibrated at 125 Hz and 250 Hz (49 m/s² r.m.s.) for 1D, 5D and 20D (D: day); for 4 hr/day. Structural damage of vessels was quantified by vacuole count using toluidine blue staining. The results were analyzed using one-way mixed model ANOVA at p< 0.05 level of significance to observe the effects of frequency and duration on vacuole formation.

For the frequency effect, the structural damage increased at 125-Hz (24.94 ± 1.07) and peaked at 250-Hz (26.6 ± 1.07), evidenced by significant vacuole formation compared to the control group (10.91 ± 1.31). For the duration effect, the structural damage increased from 1D (22.75 ± 1.11) to 5D (25.0 ± 1.11) to 20D (29.58 ± 1.11), exhibiting significant vacuole formation when compared to their respective control groups (9.5 ± 1.91; 13.5 ± 1.91; 9.75 ± 1.91). Our results demonstrate that vascular damage in the form of gross-structural damage is significant at 125 Hz and 250 Hz, as well as 1D, 5D, and 20D.

A limited-scope experiment to investigate a possible spatial resonance was inconclusive in analysis using gross-average values.
ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance

HAVS – Hand Arm Vibration Syndrome

HTV – Hand transmitted vibration

IACUC – Institutional Animal Care and Use Committee

L1, L2 – Location 1 and location 2

LAMS – Laboratory Animal Medical Services

NIH – National Institutes of Health

NIOSH – National Institute for Occupational Safety and Health

PBS – Phosphate Buffered Saline

REML – Restricted Maximum Likelihood

TB – Toluidine Blue (stain)

VWF – Vibration White Finger
CHAPTER 1 - OUTLINE

1.1 Outline of the thesis

The layout of this work is described in Chapter 1. Chapter 2 detailed the background on Vibration White Finger (VWF) and the history of Hand Transmitted Vibration induced maladies. In Chapter 3, the animal experimentation method and design was outlined, with a focus on the effects of frequency and duration of vibration exposure on vacuole formation. Chapter 4 described the results obtained using the experimental set-up. First, the effect of duration and frequency were analyzed separately. Next the possibility of a spatial resonance in the artery system was explored. Chapter 5 outlined the limitations of this study as well as future work to continue this study, including suggestions on how to refine the current study and what could be done to eliminate possible misinterpretations.
CHAPTER 2 - INTRODUCTION

2.1 Background. Prolonged exposure to hand transmitted vibration (HTV) can cause a combination of vascular, neurological, and musculoskeletal abnormalities in the hand-arm system, which together is known as hand-arm vibration syndrome (HAVS) (Heaver, et al., 2011). NIOSH estimates that over 2 million workers in the United States and United Kingdom alone are at risk of developing HAVS (Bernard, et al., 1998). It is necessary to limit the exposure to HTV for workers in industries utilizing powered hand-tools; such as but not limited to construction, carpentry, plumbing, mining, and assembly. It is believed that damage in the vascular system is one of the main causes of the HAVS. Biopsy samples collected from the fingers of workers diagnosed with HAVS have shown evidence of severe vascular damage with symptoms of cold-induced vasospasms, reduced blood flow and reduced sensitivity in the hands and fingers (Kihlberg, et al., 1995).

Raynaud’s phenomenon was named after Maurice Raynaud in 1862, who identified the condition as an episodic case of vasospasm, with a painful recovery process (Bakst, et al., 2008). Even after more than 150 years, there is still much to learn about the condition; the exact pathogenesis for Raynaud’s phenomenon has not yet been identified. Raynaud’s phenomenon has two classifications: Primary Raynaud's phenomenon which is also known as Raynaud’s disease, an idiopathic condition; and Secondary Raynaud’s phenomenon, known as Raynaud’s syndrome, where there is an underlying disorder and imposed or triggered by external factors such as tool vibration. This can appear among occupational workers who have been exposed to power tool vibration for an extended period of time. This secondary Raynaud's is otherwise known as Vibration White Finger (VWF).
One major symptom of VWF suffered by occupational workers is the loss of blood from the affected extremities, which is often the tip of the finger if the worker utilizes powered hand tools. Exposure to powered hand tool vibration disrupts normal blood flow to areas near the point of interaction (i.e. the trigger-finger interface) through a combination of some or all possible factors such as mechanical shearing, vasospasm caused by nerve fibers in the vessel wall, and damage to the intraneural vasculature. These cause a lack of blood in the tissue, giving the tissue a white or blanched appearance. In advanced VWF cases, the finger may exhibit symptoms of cyanosis, showing the appearance of a blue or purple coloration of the skin or mucus membranes below. Upon cessation of vibration exposure, the blood rushes back into previously blood starved arteries and vessels which can be very painful. This condition becomes much more severe under cold and wet environmental conditions. Besides the blanched fingers, further symptoms may also include numbness and a mild to severe “pins and needles” tingling sensation in the affected area causing lack of dexterity and strength in the limb. When the conditions become severe, workers may be limited or completely unable to do their work (Griffin, 1990).

Researchers have been looking at the increase of vacuole formation as a means of observing vascular damage in the rat tail artery. This vacuole, an enclosed space where the tissue cells separate to form a membrane covered bubble, occurs within or adjacent to affected cells. Vibration injury will cause a great increase in vacuolization in artery tissue, due to tissue displacement and mechanical shearing caused by tool usage. An example photomicrograph of a toluidine blue stained artery with vacuoles visible is shown in Figure 1 below.
HAVS is a very costly and painful injury, not only does it cost those who are affected, but also their employers and government. The total cost that HAVS causes on society is in general severely under-estimated because the estimation is based on compensation claims; the additional cost due to lost personnel time, re-assignment, rehabilitation and re-integration can be more costly than the amounts lost solely in compensation claims. Detailed analysis of the total societal cost of HAVS has yet to be explored using existing literature.
In a report published by the Health and Safety Executive in Great Britain, VWF is identified as the second leading disease in many types of industry, specifically in extraction and utility supply industry (mining and non-mining) and construction industry. About 17 and 9 workers per 1000 workers are reported to have VWF in these industries respectively (IIDB, 2011). Assuming the same rate, extrapolating these numbers, VWF is estimated to affect more than 500,000 workers in the US.

2.2 Previous works relevant to this research. Because of the complexity of the physiopathology related to VWF, experimental study is essential to better understand VWF and for validating hypotheses. Vibration tests on human subjects are not only prohibitively expensive, high dosage tests are also not allowed. Because of these reasons, it is necessary to use animal tests as an analogous system model to the digital artery system. Rat tail tests have been used for this purpose because of the similarity of the mechanical biodynamic response of the rat tail to that of the human finger (Welcome, et al., 2008). Early rat vibration studies exposed the whole body or hind-quarter of rats. While such studies were successful in causing significant observable structural and functional damage of arteries and nerves, it was not possible to identify a primary mechanism of damage because of the complex and inseparable anatomical structure of blood vessels, nerves, muscles and bones in the limbs of the rat (Curry, 2002). Later research selectively vibrated the rat’s tail on a vibrating platform with the remainder at rest on an isolated, non-vibrating platform in order to observe tissue alterations in a less structurally complex system, significantly reducing confounding variables produced by whole body vibration exposure (Okada, 1986; Curry, et al., 2002). Conveniently, there also exists much anatomical similarity of the finger and the rat tail; movement of both appendages are controlled by extrinsic
muscles with long tendons and contain intrinsic skeletal muscles, mixed peripheral nerves, and a rich vascular network that exhibits similar temperature-induced vasoconstriction or vasodilation. Because of these reasons, the rat tail is considered to be the best model to obtain insights to the finger-vibration exposure.

2.3 Scope of this thesis. Even though many rat tail tests have been previously conducted, the exact damage pathogenesis of VWF through HTV still remains unclear. One area that is being investigated is the effect of the frequency of vibration excitation on VWF. It is agreed upon that the frequency of vibration excitation has an effect on the occurrence and rate onset of VWF, however methods to predict damage with information about vibration frequency and duration have yet to be refined exactly. Various frequency weighting curves were developed to consider the effect of frequency on damage to set exposure limits (Dong, et al., 2005a; Dong, et al., 2006), including the frequency weighting recommended by ISO 5349 (e.g. ISO 5349-1, 2001).

Concerns have been raised about the capability of ISO 5349 correctly predicting risk of vascular finger damage due its development being based on hand response characteristics instead of the finger. As recently found, the finger as a system has a much higher resonance frequency than the whole hand, lending to increased dynamic responses at high frequencies causing the standard to under-predict damage caused by high frequency excitation (Dong, et al., 2005b; Dong, et al., 2006, Dong, et al., 2010). Many researchers argue that ISO 5349 under-estimates the risk of VWF as a whole (Lundström, et al., 1989; Lundström, et al., 1999; Bovenzi, et al., 2006). Since this standard is utilized by regulatory agencies to enforce a limitation for worker’s
exposure to vibration, further investigation into the appropriateness of this frequency weighting is urgent. This study focuses on developing a method for conducting a rat tail vibration test to quantify the frequency effect of vibration exposure of rat tails, with a purpose to fill a gap in the current knowledge. This study goal directly continues a previous rat tail vibration study conducted by the TEM lab focusing on the effects of input frequency on vascular damage (Goenka et al., 2013). Also of interest is the effect of duration of vibration on cumulative vascular damage exhibited by the rat tail.

Another area of interest of this study is investigating the possibility of a spatial resonance excited by a vibration input. Spatial resonance was proposed by Pattnaik and Kim as a possible mechanism that causes hand-arm vascular disorders (Pattnaik and Kim, 2011). In their study, a theoretical wave propagation model an artery with a linearly varying diameter was created. The response of the artery system to a harmonically varying wave in blood was found to cause a spatial resonance response in the artery system. A large displacement amplitude occurs at a specific segment of the artery at the location that is determined by the frequency of the input wave. Consequently, it was hypothesized that repeated and/or prolonged exposure to vibration excitation by the same type of tool, that is vibration of the same type of frequency contents, will consistently excite the same sections of artery with a large amplitude. This was proposed as a possible mechanism to the contribution of symptoms of VWF. In this study, damages in two sections of different locations of rat tail were compared to investigate possible differences.
CHAPTER 3 - METHODS

To evaluate the effect of duration and frequency of vibration on vacuole formation, an animal experiment model using rats was developed. Vacuole formation counts obtained by light microscopy in different categories of duration and frequency of excitation were used to evaluate the structural damage to the artery.

3.1 Animal Groups. The animal protocol developed for this study was approved by the Institute for Animal Care and Usage Committee (IACUC) at the University of Cincinnati (UC) prior to experimentation. All procedures included in the protocol were found to be in compliance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Table 1: Experimental design for vibration experiment

<table>
<thead>
<tr>
<th>Vibration Frequency</th>
<th>1D</th>
<th>5D</th>
<th>20D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n=2</td>
<td>n=2</td>
<td>n=2</td>
</tr>
<tr>
<td>125Hz</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
</tr>
<tr>
<td>250Hz</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
</tr>
</tbody>
</table>

Twenty-four male Sprague-Dawley rats with weights ranging from 235 to 250 grams were supplied by Harlan Laboratories in Wisconsin for vibration exposure with rats randomly assigned in groups to observe the effect of both vibration frequency (non-vibrated control, 125 Hz, 250 Hz) and duration (1, 5 and 20 days) of vibration on vascular damage. This experimental design is shown in Table 1, with n being the number of rats in the group and m=6 being the number of readings taken off each slide for each rat.
When not exposed to vibration for experimentation, the rats were housed in standard cages provided by the Laboratory for Animal Medicine and Services in a colony room with a regulated temperature of 25 ± 1°C and 12:12 hour light: dark cycle at the LAMS facility in Kettering Laboratories on UC’s medical campus, where they were provided with the standard rodent diet and tap water. The rats were then left for two weeks before experimentation to acclimate to the LAMS environment.

3.2 Vibration Protocol. The rats were exposed to vibration frequencies of 125 and 250 Hz, for 4 hours/day with a consistent starting and ending time for 1, 5 and 20 days, with three rats in each group for every combination of time and frequency. For exposure the rats were placed in Broom style restrainers with their bodies resting on a non-vibrating support isolated from transmitted heat and vibration, with their tails strapped affixed to a circular steel platform. When affixing the three rat tails to the vibrating platform, the tails were secured with duct tape in a symmetrical loading pattern as seen in Figure 2.

The platform was then excited by a 1-DOF mechanical shaker oriented vertically (model V203; Ling Dynamic Systems, Herts, UK), which was driven by a two-channel in, one-channel out, function generator/oscilloscope combination (model HP 35660A; HP Inc, Palo Alto, CA) outputting a sinusoidal signal amplified by a power amplifier. An accelerometer was affixed to the vibrating platform to ensure constant acceleration excitation of 49 m/s² RMS and connected to one input on the oscilloscope.
Figure 2: Vibrating platform loading

The oscilloscope display was split to monitor the real time input voltage of the function generator output as well as the accelerometer response to allow the technician to correct for drifting acceleration values that may occur during a four hour exposure shift. A simplified diagram of the instrument chain and experimental setup is shown in Figure 3 below.

Figure 3: Experimental set up diagram for rat tail vibration study

Rats were then returned to their respective cages and housed in the colony room after vibration exposure. The metal plate to which the rat tails are restrained was designed to have natural frequencies that are sufficiently away from the experimental vibration frequencies of 125 and 250 Hz in order to ensure a uniform vibration amplitude and phase across the excitation
plate. The circular metal plate’s closest natural frequencies to experimental operating frequencies are lower than 80 Hz and higher than 1000 Hz, ensuring that there will be uniform, rigid body movement in only the axial direction with only negligible modal behavior when operating at experimental frequencies of 125 and 250 Hz.

3.3 Artery Tissue Processing. After completing vibration exposure the rats were euthanized three hours from their last scheduled vibration exposure using carbon dioxide and diaphragm perforation, per standard laboratory procedures. The rat tail was then separated from the body at the first vertebrate after the ischium and then the surrounding skin was removed to expose the muscles and tendons. This section was fixed in 4% paraformaldehyde for 24 hours and stored in 30% sucrose-PBS buffer at 4°C until they were ready to be prepared for artery tissue isolation. Figure 4 shows a typical rat tail section with partially removed skin.

Figure 4: Image showing rat tail with skin partially removed
When sectioning, the artery is cut from the resin block to obtain an axial cross section of the artery. After staining and cover-slippering, the layers of tissue from the outer adventitia to the lumen space inside that together represent the structure of the artery can be observed under a light microscope. The anatomical structure of an artery is shown below in Figure 5.

**Figure 5:** Structural diagram of an artery

After fixation and storage, the rat tail artery was isolated manually from the remaining tissue. For each rat, two 1.5 cm long artery samples centered at 60 mm and 150 mm away from the rat’s body were harvested to observe a possible location effect on damage. Each sample was then cut in half, with one half of the tissue sample fixed in glutaraldehyde, post fixed in 1.3% osmium tetroxide and then plastic embedded using Epon (Resin LX112, Polysciences Inc.) shown in Figure 6, to be further used for vacuolization analysis through TB staining.
Figure 6: Sample of embedded arteries in Epon resin block

The other was treated with 30% sucrose in PBS for 72 hours and frozen embedded for future immunohistochemistry studies to be analyzed for evidence of biochemical damage occurring in the artery. The location of harvested samples is outlined in Figure 7 below.

Figure 7: Diagram showing location of harvested artery samples

3.4 Vacuole Count. The plastic-embedded artery was sliced into semi-thin sections (1μm) with multiple sequential sections affixed to glass slides, and subjected to toluidine blue staining for counting vacuoles (2-12μm in size) under a light microscope. The artery sections were stained with 1% toluidine blue for two minutes, then rinsed with deionized water and cover-slipped. The slides were then observed at X40 objective magnification and sections were selected for imaging based on the quality of the sample stitching images together if necessary through Adobe Photoshop® (Version CS2; Adobe Systems Inc. San Jose, CA). The total number
of vacuoles visible in the smooth muscle cell portion of the artery cross-section was then counted and recorded for three sections on each slide.

3.5 Statistical Analysis. One-way mixed-model analysis of variance (ANOVA) utilizing a restricted maximum likelihood (REML) approach was used to validate the main effects of frequency of vibration (combined days of vibration groups together; 1D, 5D, 10D) on structural damage through vacuole count. For vacuole count, the data points were split into groups as shown in Table 1. In all the analyses, the animal was treated as the random effect while frequency and duration were treated as fixed effects. For unbalanced data sets, a linear mixed model (LMM) restricted maximum likelihood (REML) method was utilized under the same parameters (Chang and Wolfinger, 1995). Data analysis was performed using SAS 9.1.3 (SAS Institute Inc, NC) with p < 0.05 used as the probability level to accept statistical significance. All values are reported as mean ± SE.
CHAPTER 4 - RESULTS

There were three aims for the results of this study; observe the effect of vibration exposure duration, the effect of vibration exposure frequency, and the possibility of a location effect on vascular damage relating to frequency input.

4.1 Effect of vibration exposure duration. The effect of vibration exposure duration on vacuole formation was explored through the exposure results of the groups outlined in Table 2.1. The vacuoles were counted after staining the slides with toluidine blue (TB) stain and imaging at X40 objective magnification. Example photomicrographs of 1D, 5D, 20D (D: day) groups exposed to 125 Hz vibration and a control sample are shown in Figure 8.

Figure 8: Example photomicrographs of TB stained arteries.
A: 125-Hz 1D, B: 125-Hz 5D, C: 125-Hz 20D, D: control sample
Vacuole counts from toluidine blue stained slides were averaged and split into duration of vibration categories (1D, 5D, 20D) as well as their respective controls (1D-c, 5D-c, 20D-c). The resulting differences in vacuole counts between each day group and their respective controls were analyzed for statistical significance, utilizing a linear mixed model (LMM) with a restricted maximum likelihood (REML) approach for analysis (Chang and Wolfinger, 1995). Figure 9 shows a bar plot summarizing the effect of the duration of vibration on vacuole count revealed through toluidine blue staining.

![Bar Plot](image)

**Figure 9:** Vacuole count plot: effect of days of vibration for both 125-Hz and 250-Hz vs. respective controls

The vacuole count was statistically significant (p < 0.05) for each day group compared to their respective controls for the 1D vibrated group (22.75 ± 1.11) compared to 1D control group (9.5 ± 1.91), for the 5D vibrated group (25.0 ± 1.11) compared to the 5D control group (13.5 ± 1.91), and for the 20D vibrated group (29.58 ± 1.11) compared to the 20D control group (9.75 ± 1.91).
1.91). This exhibits a statistically significant increase in vacuolization when exposed to vibration when compared to control for each day category.

Interestingly, the data suggests that as the duration of vibration exposure increases, there is a cumulative damage effect exhibited by increased vacuolization. The averaged vacuole count for each vibrated group increases as the duration of vibration increases; the increase from 5D to 20D shows a possibility of statistical significance \( (p < 0.05) \). Overall, the 20 day vibrated group exhibited the highest value of vacuoles among the groups investigated. These results support the hypothesis that prolonged exposure to vibration causes structural damage to rat tails.

4.2 Effect of exposure frequency. Effect of vibration exposure frequency was studied based on the exposure results of the groups outlined in Table 2.1. Combined vacuole counts of 1D, 5D, and 20D groups that were averaged into groups by excitation frequency (groups of control, 125-Hz, and 250-Hz). The resulting differences in vacuole counts between each exposure frequency and the control group were analyzed for significance, utilizing a linear mixed model (LMM) with a restricted maximum likelihood (REML) approach for analysis. Figure 10 shows a bar plot summarizing the effect of vibration frequency on vacuole count revealed through the results of the toluidine blue staining.
Figure 10 – Vacuole count plot: effect of vibration frequency vs. non-vibrated rats

The difference in the vacuole count was found to be statistically significant (p < 0.05) for each frequency group compared to the non-vibrated control group, i.e.: for the 125-Hz vibrated group (24.94 ± 1.07) compared to the control group (10.91 ± 1.31) and for the 250-Hz vibrated group (26.6 ± 1.07) compared to the control group (10.91 ± 1.31). Like with duration of vibration, the averaged vacuole count for each group increases as the frequency of vibration increases from control (10.91 ± 1.31) to 125-Hz (24.94 ± 1.07) to 250-Hz (26.6 ± 1.07).

Overall, the 250-Hz vibrated group exhibited the highest count of vacuoles among the groups investigated. The difference of the mean values of the control group and the mean value of the 125 Hz vibrated group was 14.03 ± 1.69. Similarly, the difference between the mean values of control group and 250 Hz vibrated group was 15.69 ± 1.69. These differences were
comparatively larger in comparison to the difference between the mean values of 125 Hz and 250 Hz vibrated groups (1.67 ± 1.51).

The increase in vacuolization in the vibrated samples (125-Hz and 250-Hz) was found to be statistically significant when compared to the control. Among the vibrated samples, the 250-Hz group was shown to have a greater increase in vacuolization from the control group than the 125-Hz group. This supports the hypothesis that the rat tail damage due to vibration exposure is dependent on frequencies.

4.3 Location Effect. A theoretical study was conducted at the University of Cincinnati on a spatial resonance phenomenon occurring in arteries whose radius change gradually (Pattnaik and Kim, 2011). In their study, a wave propagation model of a small artery with a non-uniform radius was created. The wave guide model include an elastic tube of varying diameter that models the artery wall and incompressible fluid that models the blood. The model revealed that vibration input can cause a spatial resonance in the artery system. Harmonic disturbance input in a form of artery wall vibration or fluid pressure results in a highly localized maximum wall deflection at the location that corresponds to the local resonance frequency determined by the radius and thickness of the artery. The following presents the results obtained in Table 2.

<table>
<thead>
<tr>
<th>Location</th>
<th>Location 1</th>
<th>Location 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.5 ± 1.9</td>
<td>12.3 ± 1.9</td>
<td>0.2934</td>
</tr>
<tr>
<td>125 Hz</td>
<td>24.4 ± 1.5</td>
<td>25.4 ± 1.5</td>
<td>0.6479</td>
</tr>
<tr>
<td>250 Hz</td>
<td>26.7 ± 1.5</td>
<td>26.6 ± 1.5</td>
<td>0.9595</td>
</tr>
<tr>
<td>1D</td>
<td>22.7 ± 1.6</td>
<td>22.8 ± 1.6</td>
<td>0.9424</td>
</tr>
<tr>
<td>5D</td>
<td>24.8 ± 1.6</td>
<td>25.2 ± 1.6</td>
<td>0.885</td>
</tr>
<tr>
<td>20D</td>
<td>29.2 ± 1.6</td>
<td>30.0 ± 1.6</td>
<td>0.7179</td>
</tr>
</tbody>
</table>
In the subject of rat tail vibration experimentation, it was hypothesized that the spatial resonance effect may be observed through an increase in vacuole formation at different locations of the rat tail. To observe this effect, two locations on the rat tail were harvested and analyzed; Location 1 is 60 mm and location 2 is 160 mm away from the rat body. It was assumed that the radius of the rattail artery at these two locations would be different, thus having different resonance frequencies. This step was completed for each animal, 24 pairs of locations in all were harvested.

Figure 11 shows a bar plot that compares vacuole counts at two locations revealed through the results of the toluidine blue staining.

![Vacuole count plot: effect of location on vibrated samples vs. respective control](image)

**Figure 11:** Vacuole count plot: effect of location on vibrated samples vs. respective control

The groups were combined into either a control or vibrated group for each location, location 1 and location 2. Statistical significance was found (p<0.05) comparing vacuole counts
for the vibrated samples, location 1 (25.56 ± 1.08) and location 2 (26.61 ± 1.08) to their respective control groups, location 1 control (9.5 ± 1.89) and location 2 control (12.33 ± 1.89). Both relationships were found to be statistically significant (p<0.05) using an LMM (REML) model. For both locations, a meaningful difference in the increase in vacuole count in samples exposed to vibration from the respective control groups was observed. At location 1, the percent increase was 169% while location 2 exhibited a percent increase of was 118%.

Overall, location 2 showed a slightly higher mean vacuole count value (26.61 ± 1.08) among the vibrated samples and the highest overall. For each location, there was a significant increase in vacuole count from their respective controls. Location 2 exhibited a slightly higher average vacuole count than location 1, but the difference among the two locations was not found to be statistically significant.

Figure 12 shows a bar plot summarizing the effect of location when comparing the vacuole counts by groups split by vibration-frequency categories (non-vibrated control group included).
Figure 12: Vacuole count plot: effect of location on frequency of vibration compared to respective controls

For location 1 groups, statistical significance was found \((p < 0.05)\) comparing vacuole counts for the vibrated groups, 125-Hz \((24.44 \pm 1.54)\) and 250-Hz \((26.67 \pm 1.54)\), to the location 1 control group \((9.50 \pm 1.88)\). Location 2 groups exhibited a similar outcome, with statistical significance found \((p < 0.05)\) when comparing vacuole counts for the vibrated groups, 125-Hz \((25.44 \pm 1.54)\) and 250-Hz \((26.56 \pm 1.54)\), to the location 2 control group \((12.33 \pm 1.88)\). For both locations, a meaningful difference in the increase in vacuole count in samples exposed to vibration from the respective control groups was observed. For location 1, the 125-Hz group exhibited a 157% increase and the 250-Hz group exhibited a 180% increase in vacuolization. For location 2, the 125-Hz group exhibited a 106% increase and the 250-Hz group exhibited a 107% increase in vacuolization.
Overall, both location groups exhibited an increase in vacuole count as the vibration frequency increased. Vacuole count at location 2 at 125 Hz was higher than that at location 1 compared to 250 Hz, but the difference was not found to be statistically significant.

Figure 13 shows a bar plot summarizing the effect of location when comparing the vacuole counts by groups split by vibration duration categories (control group included).

![Bar plot showing vacuole count by location and duration](image)

**Figure 13 – Vacuole count plot: effect of location on duration of vibration compared to respective controls**

For location 1 groups, statistical significance was found ($p < 0.05$) comparing vacuole counts for the location 1 control group (9.50 ± 1.62) to each duration group - 1D (22.67 ± 1.62), 5D (24.83 ± 1.62), and 20D (29.17 ± 1.62). The location 2 groups exhibited a similar pattern, with statistical significance found ($p < 0.05$) when comparing vacuole counts for the location 2
control group (12.33 ± 1.62) to each duration group - 1D (22.83 ± 1.62), 5D (25.17 ± 1.62), and 20D (30.0 ± 1.62).

Overall, both location groups exhibited an increase in vacuole count as the vibration frequency increased. For both locations, a meaningful difference in the increase in vacuole count in samples exposed to vibration from the respective control groups was observed. For location 1, the 1D group exhibited a 138% increase, the 5D group a 161% increase, and the 20D group a 197% increase in vacuolization. For location 2, the 1D group exhibited a 80% increase, the 5D group a 104% increase, and the 20D group a 143% increase in vacuolization.

This analysis is consistent with the findings of the previous section 3.1 Effect of duration on vibration. The duration of vibration was again found to have a significant cumulative effect on structural damage indicated by increased vacuolization for both locations.

Figure 14 shows a bar plot comparing vacuole formation in location 1 and location 2.
Figure 14 – Vacuole count plot: comparing controls of location 1 and location 2

Vacuole counts for location 1 (21.54 ± 1.63) were slightly lower than those for location 2 (22.58 ± 1.63). Location 2 samples exhibited slightly more vacuolization than those from location 1 on average. There were statistically meaningful differences when the comparison was made based on the percent increase of the vacuole counts of the vibrated group from the control group.

Figure 15 shows a bar plot summarizing the effect of location when comparing the percent increase of vacuole counts from control between location 1 and location 2.
Figure 15 – Percent increase plot: vacuole count in vibrated samples comparing location 1 and location 2

Location 1 consistently showed higher increase of vacuole counts, which may indicate existence of the spatial resonance in the vicinity of location 1 at both frequencies. The percent increase values decrease in a consistent manner from location 1 to location 2. The location 2 frequency groups exhibit a smaller range of vacuole count values in terms of percent increase due to the larger control value in location 2 compared to location 1.

The results shown in Figure 15 display an observable difference between the percent difference of vacuole count values for location 1 and location 2. This supports the hypothesis of a radially dependent resonance response of the artery system.

It is believed that more spatial resolution along the length of the rat tail will be necessary to prove existence of the spatial resonance. A new study may be designed to use more sections.
harvested at more locations with two or three more separated frequencies; for example, 6 or 7 locations along the rat tail with 50 Hz, 125 Hz, 250 Hz.
CHAPTER 5 - DISCUSSION/CONCLUSION

Vibration-induced large-scale structural damage to the rat tail artery was studied through the observation of vacuole formation via toluidine blue staining. Our results show that the structural damage increase was statistically significant for every vibrated animal group compared to their respective control (non-vibrated) group. A general increase in vacuole formation was found for increasing duration from 1D to 5D to 20D, as well as increasing excitation frequency from 125-Hz to 250-Hz.

5.1 Dependency of structural damage on vibration duration. The increase in vacuole formation exhibited when increasing duration of exposure suggests evidence of cumulative damage. This is in agreement with the current group’s previous rat tail vibration study results (Goenka, et al., 2013).

5.2 Dependency of structural damage on vibration frequency. The increase in vacuole formation that was found with increasing excitation frequency was found to be in contrast with a previous study (Krajnak, et al., 2006). However, in their study a consistent axial location was harvested and analyzed (C7 vertebrae) as opposed to harvesting consistent radial cross section tail portions for analysis, which may explain the difference in result. In another study (Curry, 2005), a single 4-hour exposure to 125-Hz vibration (49 m/sec², root mean squared) showed a significant increase in vacuolization, which is consistent with our result under the same parameters.
5.3 Location dependency, and possible evidence of resonance. Furthermore, in Curry’s findings of the biodynamic response of the rat tail tissue, a location dependent maximum transmissibility was found for both 125-Hz (manifesting towards the middle of the tail) and 250-Hz (towards the end of the tail). Our results showed no statistical significance on the effect of location with the gross vacuole averages, but when investigating the data based on percent change, there is a noticeable trend for higher increase from the control sample in vacuole formation in the smaller radius (Location 1) compared to the larger (Location 2).

5.4 Limitations. One of the focuses of this study was to explore the possibility of a spatial resonance within the artery system. The amount of locations chosen (2) was decided in order to keep the resource cost manageable while still allowing for trends to be suggested within the data. Due to the limited scope, there were no conclusive results for this topic using gross vacuole averages; however, when investigating the percent difference change there was an observable difference in values between location 1 and location 2. Also, vibration in this experiment was kept to an acceleration of 49m/s^2 root mean squared, which changed the amplitude considerably when moving from 125-Hz to 250-Hz. As such, change in amplitude will have an inseparable effect from frequency on vibration damage within this experimental set-up. While there are no agreed upon methods for energy equivalence in differing experiments utilizing variable-amplitude, fixed-frequency vibration, further exploration into amplitude effects is still desirable.

5.5 Proposed Future Work. In order to further explore the possibility of a spatial resonance occurring within the rat tail system, a similar rat tail vibration experiment may be
designed. In order to explore the notion that there may be an axial-location dependent damage response to vibration input, greater spatial resolution is required. To accomplish this, a greater number of segments (i.e. 6 or 7) of rat tail artery may be harvested and exposed to the tissue processing and toluidine blue staining procedures, as suggested in Figure 16.

![Figure 16](image)

**Figure 16** – Suggested sample points for future spatial resonance experiment, as well as illustrated theorized amplitude-response curves for 125 and 250 Hz

An increase of sample points would alleviate concerns of harvesting tissue where the damage caused by the frequencies in question would be similar in certain locations. Using more data points would allow for differentiation between the responses to the two frequencies, and may result in an increase of vacuole formation from respective control, exhibiting a similar shape to a theoretical amplitude-response curve that a rat tail may exhibit.
REFERENCES


