



Somatosensory Stimulus Apparatus for Rodents

UW BME Design (400)

December 12th, 2018

Clients: Aaron Suminski, PhD, UW - Madison Department of Surgery;
Aaron Dingle, PhD, UW Department of Biomedical Engineering

Advisor: Jeremy Rogers, PhD, UW - Madison Department of Surgery

Team Members:

Stephan Blanz – Team Leader
John Beckman – Communicator
Royal Oakes – BWIG/BPAG
Rick Guo – BSAC

Abstract

Currently, no methods exist to test the efficacy of sciatic nerve repair on rodent models based on responsiveness to hindlimb somatosensory stimulation. The proposed device produces somatosensory stimulations through vibrations induced on the hindlimb of a conscious rat. By isolating the stimulation to either left or right hindlimb, the subject is presented a two-alternative forced choice (2AFC). A healthy rat is trained to conditionally respond to the stimulus by indicating on which limb the stimulus was felt. Once the rat is trained, the nerve is surgically deafferented and repaired. During recovery the rat is presented with the same 2AFC at various time points to determine the length of recovery of sensory function.

Table Of Contents

Table Of Contents	2
Introduction	3
Impact	3
Problem Statement	3
Current Devices/Methods	3
Research	4
Relevant Physiology and Biology Research	4
Design and Prototype Production Research	5
Client Information	5
Design Specifications	5
Preliminary Designs	6
Preliminary Design Evaluation	8
Design Matrix	8
Fabrication and Development Process	10
Materials	10
Methods	10
Final Prototype	11
Testing	12
Results	13
Discussion	14
Conclusions	14
Future work	14
References	15
Appendix	16
A. Preliminary Design Specifications	16
B. Design Matrix Full Description	18
C. MATLAB and Arduino Code	20

Introduction

Impact

Allowing patients to fully recover following a severed nerve is extremely challenging. The clients of this project are studying surgical methodologies as well as pre and post-operative care to repair the damaged nerves in the Peripheral Nervous System (PNS). Their work is focused on analyzing the success of these surgical procedures to determine how effective the treatment was in repairing the neurological connection that was lost, or damaged. This work could translate into surgical procedures to help humans who have had their neurological connections damaged or severed, regain the control and sensations that they may have lost otherwise. The human body already has the ability to regrow certain nerve connections in the PNS specifically. This regrowth starts through a process called Wallerian Degeneration, and is followed by axon regeneration. This process is only possible in the PNS and if the distal portion of the axon is not too far from the cell body [1]. In a scenario where the Wallerian Degeneration leaves the axon too far to reattach to the cell body, a surgeon can come in and help to guide the regrowing axon to the cell body by removing the damaged neurological tissue and scar tissue in the area. If this solution can be shown to have a significant positive impact on the regeneration of nerve connections in rats, it could one day be implemented to help regrow human nerves as well.

Problem Statement

The two clients for this project are currently researching surgical nerve repair in rats, more specifically repair of the Sciatic nerve. This is the nerve that innervates the buttocks into the leg and foot. This nerve is highly important in connecting the muscles of the leg and foot to the spinal cord and brain [2]. Thus, after surgical repair of this nerve, the success of the surgery must be assessed by stimulating this nerve, and observing the rats ability to distinguish the individual stimuli in each hindlimb. The clients are looking for a non-invasive, non-harmful way to stimulate this nerve in rats having undergone sciatic nerve repair surgery to determine the effectiveness of the surgery.

Current Devices/Methods

A device that can independently stimulate a rat's hind limbs does not currently exist on the market. However, there are some devices that have been used in past studies to apply vibrations to rats.

In one study, standard housing cages were affixed to a vibrational shaker (V408 Shaker, Brüel and Kjær North America, Duluth, GA) (Figure 1) [3]. Rats were introduced to the cage and vibrations were applied to the cage. This design does not satisfy the clients requirements because the vibration was applied to all four limbs at once, and the clients want to stimulate only one hindlimb at a time.

Aside from vibrationally stimulating the hindlimbs of rats, our device will also have to house rats for the duration of testing. There are



Fig 1. A LDS V408 Permanent Magnet Shaker from Brüel & Kjær. Image taken from <https://www.bksv.com>

several products on the market which are used to house rats during behavioral testing.

First, the Rat Passive Avoidance Cage from Coulbourn Instruments (Figure 2) is used to condition rats to stay in certain areas. The cage (20"W × 10"D × 12"H) is divided into two rooms separated by a door: a dark room and a light room. The dark room has opaque walls, preventing outside lights from entering the room while the light room has clear plexiglass walls that allow ambient light to enter the room. The rat is naturally inclined to enter the dark room, however by providing an electric stimulus, the rat can be conditioned to remain in the light room. This product can give us a starting place for determining the size of our design.

Second, the Rat Touchscreen Chamber by Lafayette Instrument Company can be used for multiple applications (Figure 3). The cage features a touchscreen with which the rat can interact, and several slots that can be used for pellet dispensers, levers, lights, and other stimulatory devices. This device can inform us on how to collect information from the mouse during training. We may want to use a touchscreen, or to use lasers to determine if the rat get the desired response to the given stimulation.



Fig 2. A rat passive avoidance cage by Coulbourn Instruments. Image taken from <https://www.coulbourn.com>

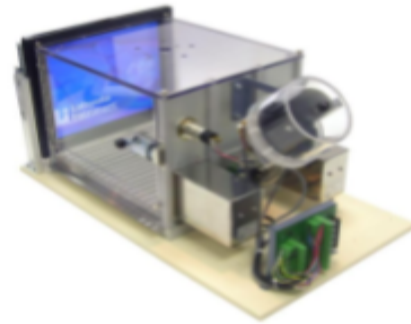


Fig 3. A Rat Touch Screen Chamber from Lafayette Instrument Company. Image taken from <http://lafayette neuroscience.com>

Background

Research

Relevant Physiology and Biology Research

Damage to nerves in adult mammals have very different regeneration possibilities depending on where in the nervous system they occur. A damaged nerve in the Central Nervous System (CNS), is almost completely incapable of regenerating due to the neuroglia in the CNS. Astrocytes in the CNS, after a nerve is damaged, form a barrier around the damaged nerves, which turns into astroglial scar tissue. In addition to the blockage of nutrients due to the astrocytes, and astroglial scar tissue, the myelin of the CNS releases growth-inhibiting proteins which block any regrowth of a nerve in the CNS [1]. However, in the PNS, damaged nerve cells undergo Wallerian Degeneration, a process which protects a portion of the damage peripheral nerve with Myelin, while the debris of the damaged section is cleared by macrophages. Wallerian degeneration is followed by axon regeneration, in which growth-promoting factors are released by the myelin and cell body, and help to guide the regrowing axon back to the cell body [3]. There are situations in which the nerve is unable to reach the cell body it was originally attached to, in these situations surgical modifications

can be used to lead the axon back to the cell body. This occurs through the removal of much of the scar tissue blocking the nerves path back to the cell body. Additionally, if the gap between the axon and cell body is too large, a non-essential nerve will be sacrificed from elsewhere in the body. Although these procedures are well documented, there currently exists no viable way to test the effectiveness of these surgeries. The clients of this project would like to stimulate these repaired nerves in rats using a vibrational stimulus.

Vibrational stimulation has been tested in different ways to study the effects it has on different rodents. In one study looking at behavioral changes in female mice when subjected to different frequencies of vibration showed that the mice exhibited the most noticeable transient responses at frequencies between 70-100 Hz., and at an acceleration of 1.0 m/s^2 [4]. Another study which looked at the vibrational frequency threshold for mice and rats found that rats, after being tested at a range from 0 - 600 Hz., would attenuate with vibrations around 50 Hz., whereas a humans resonant frequency is between 9-16 Hz [5]. This was an expected result since rats are smaller than humans their resonant frequency was expected to be significantly higher than that of a humans. These different studies will be used to help determine the range of vibrational frequencies that will be available to the clients during their testing.

Design and Prototype Production Research

Initially, the team's research focused on finding a component which could provide an isolated, user-defined vibrational stimulus to the hindlimb of a rodent. The various devices and device specific results of this research can be found under [Preliminary Designs](#) and [Preliminary Design Evaluation](#).

Another future interest will be the hardware and software used to interface between the scientist selecting a desired frequency of stimulation and the motor itself. An Arduino™ Uno Baseboard may be used for this.

A further future focus of design research is the rodent enclosure. The enclosure should be made out of a non-porous material for sanitation purposes. It should also create a watertight seal between the bottom of the enclosure and the platform through which the rodent experiences vibrations to it's hindlimbs. Additionally, it should ensure that any mechanical or electrical components housed underneath the enclosure are not subjected to stresses, strains or biological waste created by the rodents.

Client Information

Dr. Aaron Suminski is a scientist with the University of Wisconsin, Department of Biomedical Engineering. He collaborates with Dr. Aaron Dingle, a post-doctoral researcher, with the University of Wisconsin, Department of Surgery, Poore lab. The Poore lab's research focus is clinical and experimental microsurgery, with an emphasis on peripheral nerve regeneration and repair. Together they are researching the effectiveness of various treatments and methods of nerve repair.

Design Specifications

According the Project Design Specifications (Appendix A), the experiments are usually conducted in a laboratory. The design should be able to set up on a benchtop and weigh less than 12

kilograms for the convenience of the clients. Since the experiment only includes 1 rodent at a time, the size of the cage should be approximately 30 cm x 30 cm x 30 cm. Moreover, the device should be waterproof and sanitizable because the rodents may urinate during the experiment.

The main purpose of the clients' experiment is to check whether the rodent can receive the stimulation through its left hindlimb or right hindlimb. The client believes that vibration will be the safest and most humane stimulus. Thus, the device should be able to stimulate one of the rodent's legs using vibration. Other parts of the rodent's body, such as belly and the other foot, should not be stimulated. The vibration should have controllable intensity and frequency. A computer can be used to adjust these parameters. For the safety of the rodents, the device should not generate intense vibration that injures the rodents.

Preliminary Designs



Figure 4. Linear actuator capable of one-dimensional linear movement [6]. The linear actuator allows for adjustable amplitude, and potentially adjustable frequency.

The device shown above (Figure 4) is a linear actuator with its dimensions. It can produce a simple linear motion using a rod and screw. When it is fully extended, the full length of this linear actuator can reach 252.5 mm. It can be used to generate the vibration of interest by programming how far the rod extends and how long the delay is. The cost of this device is 69.99 USD.

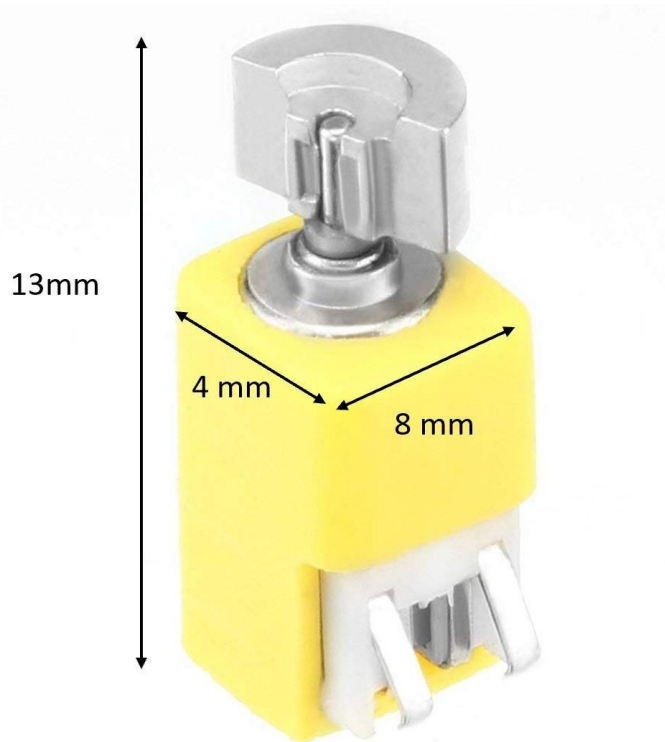


Figure 5. Micro Vibration Motor for Cell Phone. Motor has a fixed frequency of vibration that is achieved by rotating an unbalanced weight at the head of the unit [7].

The cell phone micro vibration motor (Figure 5), has a body size of 4 mm x 8 mm(D*H) and a total length of 13mm. Its head has a unbalanced weight. By spinning the head, the motor can generate vibration. It can only generate vibrations with fixed frequency. Thus, to create a vibrational platform with a controllable frequency, three of such motors are needed to be placed under one platform, with frequencies of 150 Hz, 87 Hz and 58 Hz. Frequencies other than those three are unreachable. The cost of such motors is 1 to 3 USD each.

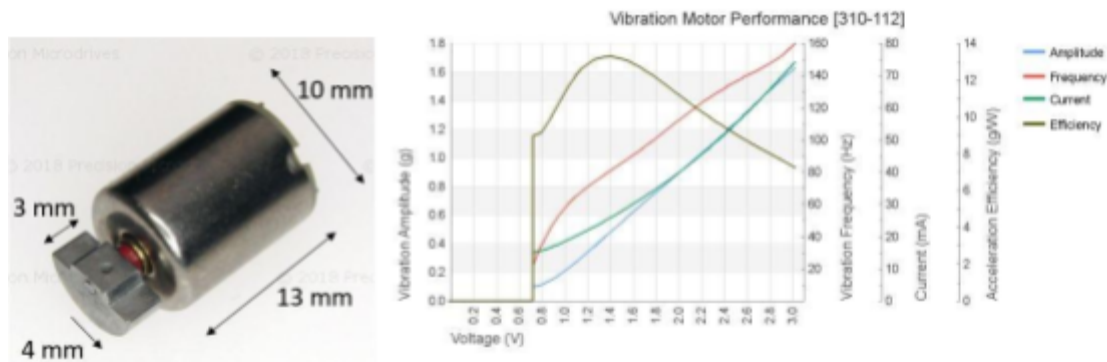





Figure 6a. (left). Precision vibration motor with variable frequency. Motor has a variable frequency of vibration that is achieved by rotating a semi-circular weight at the head of the unit. **Figure 6b.** (right). Frequency vs Voltage plot supplied by manufacturer [8].

The precision vibration motor is another unbalanced weight vibration motor (Figure 6a). Its body is cylindrical with a diameter of 10 mm and a full length of 13 mm. It has a unbalanced head, working similarly as the cell phone micro vibration motor. Different from the cell phone micro vibration motor, the frequency of the vibration generated by this motor can be adjusted by changing the input current. The frequency can range from 30 Hz to 150 Hz (Figure 6b). Thus, only one of such motor will be enough to build a vibrational platform. The cost of this motor is 26.25 USD.

Preliminary Design Evaluation

Design Matrix

Table 1. Design Matrix used in selecting a motor to create a surface vibration. The design matrix considered various categories ranging from “segregation of stimulation” to “cost” and assigned each category a weight based on importance to the project. Three devices, a linear actuator [6], a micro vibration motor [7] and a precision vibration motor [8] were evaluated on a range of 1 through 5 in each criteria. This evaluation was multiplied by the weight of the criteria. The product with the highest total sum of weighted criteria was chosen to be incorporated in the design.

		Linear Actuator		Micro Vibration Motor		Precision Vibration Motor	
							
Criteria	(Weight)						
Segregation of stimulation	25	5/5	25	4/5	20	4/5	20
Frequency range	20	3/5	12	3/5	12	5/5	20
Ease of integration	15	3/5	9	2/5	6	3/5	9
Size	15	3/5	9	4/5	12	5/5	15
Ease of achieving desired effect	5	5/5	5	3/5	3	4/5	4
Cost	5	3/5	3	5/5	5	4/5	4
Total	85		63		58		72

The team and client prioritized the development of a component which would create a variable, user-defined vibration. Other design aspects of the project will be evaluated at a later point. For a detailed description of design matrix criterion see [Appendix B](#) .

Design criteria were chosen and weighted based on engineering practicality, as well as the desires of the client. Fundamental to this project is the segregation of stimulation - the ability to only stimulate one hindlimb. All devices could perform this task. However, the linear actuator could be programmed to precisely deliver changes in length, with user defined periods and delays. The vibrational motors relied on the circular movement of a weight to create a vibration, which would thereafter have to be transferred to a platform; introducing variability and potential sources of error.

Additionally, the devices had to operate within a desired frequency range. The manufacturer of the precision vibration motor's provided a voltage vs frequency diagram covering the desired ranges, as well as a voltage vs amplitude diagram. The linear actuator could produce vibrations on the lower range of the spectrum. The micro vibration motor could only vibrate at a specific frequency. This would have meant purchasing multiple micro vibration motors with various rotations per minute (rpm) and turning them on and off separately.

Ease of integration considered the difficulty of engineering an interface between the device and the rodent. The linear movement of the linear actuator is a more simple interface than integrating a part that relies on circular movement.

The final product would have to be sized to fit on a lab-bench, or compact enough to easily be moved between lab spaces and animal housing spaces. Therefore, the individual components should be as small as possible. The order of components from smallest to largest is: precision vibration motor, micro vibration motors, and linear actuator. The micro vibration motors are smaller, however, since multiple are needed to achieve the desired effect they earn a lower size rating.

Ease of achieving desired effect was a subjective measurement of the ease in which the device could be programmed or modified to vibrate at the desired frequency. All devices would be able to vibrate within the desired range, however the linear actuator would be the easiest to program.

Finally, cost was the last metric on which devices were compared. The micro vibration motors were cheapest and the linear actuator was the most expensive option with the precision motor fitting in the middle.

Proposed Final Design:

Precision Microdrives™ Model No. 310-112 outperformed the other options of creating a vibrational stimulus and was therefore selected as the motor in the proposed final design. It operates within a frequency range of 30 - 150 Hz, with an input of 0.75 - 3.0 V.

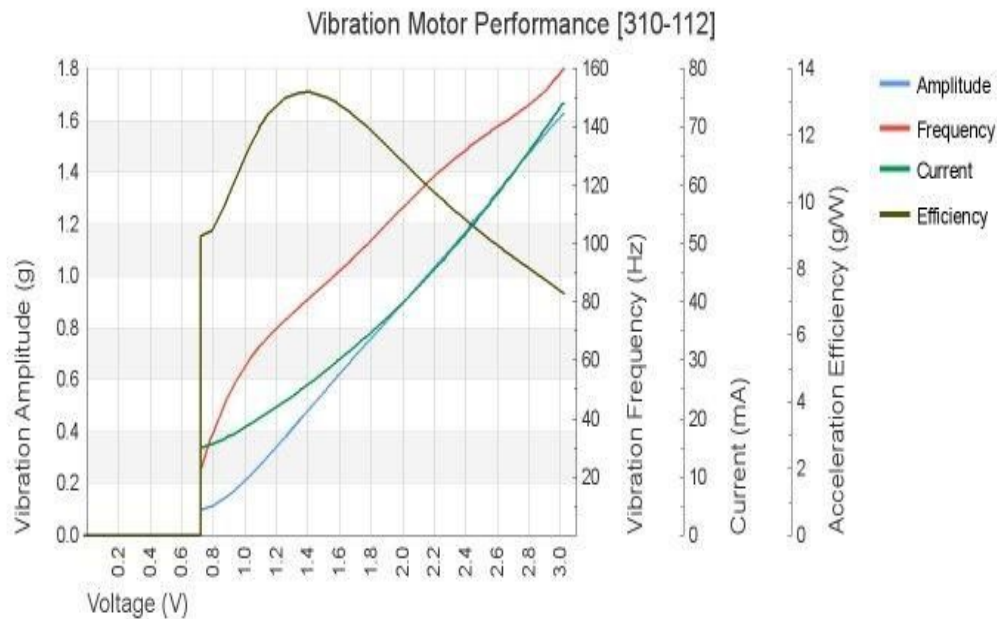


Figure 7. Precision Microdrives™ Model No. 310-112. The motor chosen for use in the proposed final design. The motor’s adjustable frequency, as well as small size and low cost made it the preferred source of vibrational stimulation.

Fabrication and Development Process

Materials

A plexiglass enclosure was used to house the rodent. This allowed easy cleaning and being able to view the animal during the procedure. Currently, the vibrational platform is 3D printed SLA using a Form 2 3D printer. A rubber adhesive pad covers the platform cutouts and provides adhesion for the platform. Future iterations of this prototype will ensure material is both waterproof and easy to sanitize.

Methods

The goal of this project is to stimulate the sciatic nerve of a rat through vibration. The vibration will be generated by a vibrational motor. Circuit and Arduino code will be developed to control the motor so that it can generate a vibration at desired frequency and amplitude. The motor itself could be attached to the bottom of the platform to cause the vibration of platform. Two of such platforms will be integrated into the bottom of a cage that is large enough to accommodate one rat for researchers to do experiments. The size and the position of the platforms depend on the average

size of the rats. The whole cage and the platforms will be made from waterproof materials since the rats may urinate during the experiments.

The rats will be trained to position itself with one foot standing on one platform and one foot on the other. There will be 3 holes on the side of the cage. The rats will be trained to use these holes to tell whether they feel the vibration and which foot. If they feel the vibration through their left foot, they will put their nose into the left hole, and right hole for right foot. If the rat is not sure or feel nothing, they will put their noses into the middle hole. Researchers can use this method to determine the function of the reconnected sciatic nerve of the rats.

Final Prototype

The final prototype consists of a plexiglass rodent enclosure with two platform cut-outs designed to accommodate the hindlimbs of a rearing rat. The cut-outs are covered with a foam adhesive, underneath which the platform is attached to. The platform itself houses both the LRA and the ERM motor (Figure 8.)

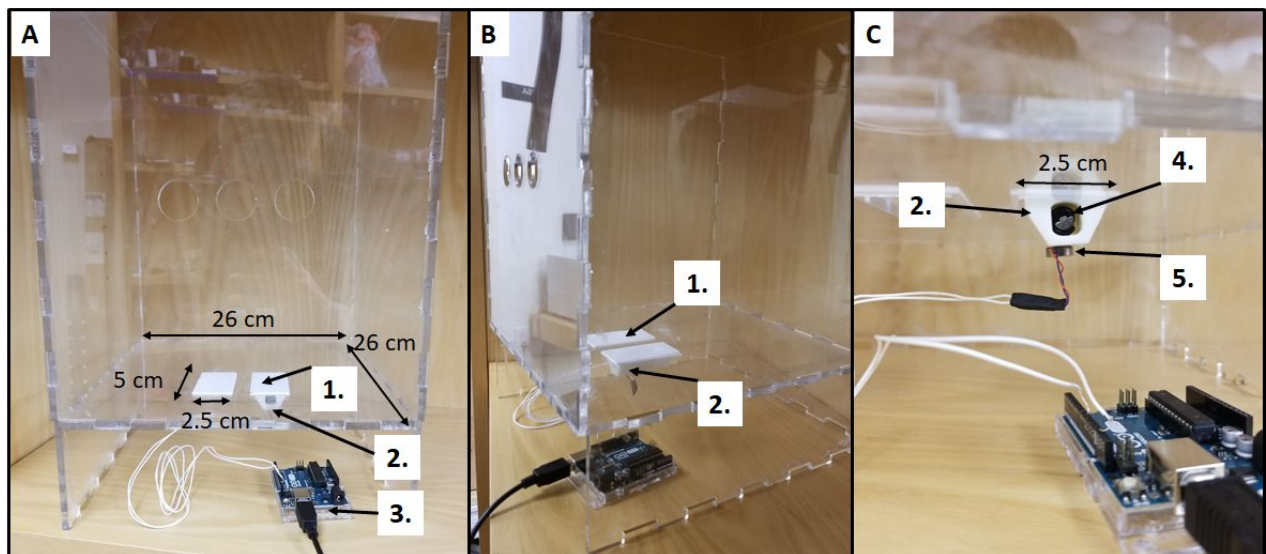


Figure 8. Rodent enclosure from various angles and enlargements. **(A)** The rodent enclosure in a front-on view. Visible are the two white foam covered cutouts on which a rodent will be trained to place its hind limbs while rearing (1.), and the platform housing the vibrational motors (2.) An Arduino microcontroller controls the vibrational frequency of the motors (3.) **(B)** Angled view displaying the cut-outs (1.) and motor(2.) **(C)** Detailed front view featuring the platform housing the vibrational motors (2.), the ERM cylindrical motor(4.) and the LRA coin motor (5).

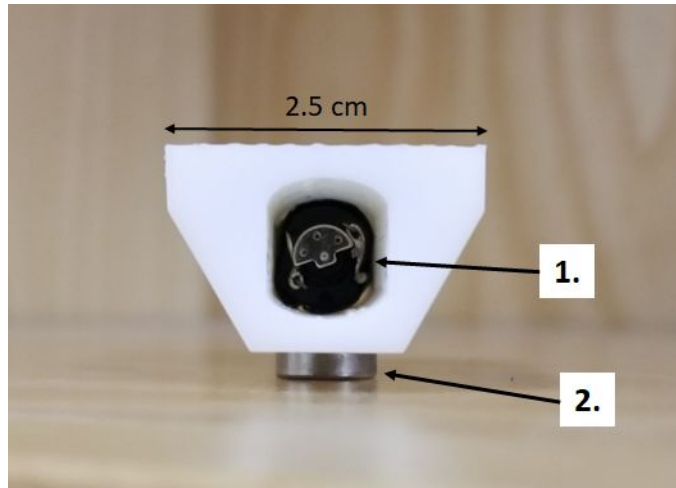


Figure 9. Close-up of the platform housing the vibrational motors. The ERM cylindrical motor (1.) is nestled in the middle of the platform. The off-centered rotating mass responsible for the vibrations is visible from this angle. The LRA coin motor (2.) is attached to the bottom of the platform. Together these motors cover the prescribed frequency range.

Testing

An experiment was conducted to determine the response of the linear resonant actuator (LRA) motor and eccentric rotating mass (ERM) motor under different conditions. Each of the two motors were placed into three different positions with an accelerometer (Figure 8). The direct placement configuration was used to determine the motor's response to different voltages, since there may be discrepancies in actual performance from the datasheet. The dampened configuration was used to determine what the rodent might perceive from the leg being stimulated by the motor during use of the cage. The off-target configuration was used to determine what the rodent might perceive from the leg not being stimulated during use of the cage.

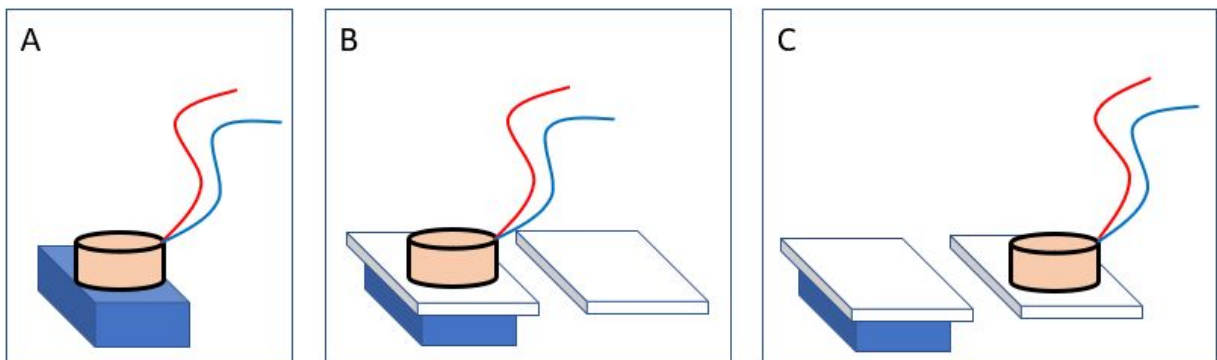


Figure 10. The configurations of the accelerometer (blue) and the motor (beige) used in our experiment. (A) The direct placement configuration. (B) The dampened configuration. There is a foam pad (white) separating the accelerometer and the motor. (C) The off-target stimulation configuration. The motor is placed on the adjacent foam pad to the accelerometer.

In each configuration, a motor was vibrated at various voltages. The voltages tested in each motor spanned the range specified in the motor’s datasheet at 0.5 V increments. Each motor was powered using a PWM signal with a max voltage of 5 V. Thus, a 2.5 V signal in this experiment corresponds to a duty cycle of 50% and a 3.5 V signal corresponds to a 70% duty cycle.

Data was collected from the accelerometer using an Arduino UNO and was sent to MATLAB for processing. First, the motor was powered and 128 samples of the acceleration vector were collected from the accelerometer at 1 kHz. This number of samples was chosen due to memory limitations of the Arduino UNO. Second, the data was imported to MATLAB. Third, the magnitude of the acceleration vector was computed. Fourth, the Fourier transform of the accelerometer magnitude was computed using the MATLAB fast Fourier transform [9]. This method was used rather than sending the data one sample at a time because literature suggests that our method leads to more accurate data [10].

Results

Table 2. The results from the Fourier Transforms run on the different different motors at the different locations. The motor dampened by platform results are the most relevant to the frequencies that will be transferred to the legs of the rats during testing. The results from the Transforms show that the motors can achieve frequencies of approximately 75 Hz and 100 - 150 Hz.

	LRA	ERM
Direct Placement of accelerometer on motor	50 Hz, 150 Hz	60 Hz, 175 Hz
Motor dampened by platform Accelerometer placed on platform	100 - 150 Hz	75, 125 Hz
Amplitude of off-target vibrations felt on adjacent pad *	25%	10% - 25%
* Based on selected prominent frequencies		

The table above concludes the results from FFT testing. When the motors are directly placed on the accelerometer, the measured frequency generated by ERM (60 Hz, 175 Hz) is slightly higher than that generated by LRA (50 Hz, 150 Hz). When the motors are dampened by the platform, the range of frequencies generated by both motors shrinks (100-150 Hz for LRA and 75-125 Hz for ERM). Moreover, both of the motors show an acceptable off-target vibration with an amplitude less than 25% of the amplitude obtained from the dampened motor test.

Beside frequency, another important factor that describes how an object vibrates is amplitude. In this case, the amplitude of device’s vibration is directly correlated to the force generated by the Micro Vibration Motor with a eccentric disk. Since the inner structure of LRA is not accessible, only the force generated by ERM can be calculated, using the formula below:

$$F = m r \omega^2$$

In this formula, m is mass of eccentric disk, obtained from a micro scale. r is the radius of the orbit that the eccentric disk spins on. This is obtained by calculating the distance between the spinning rod and the center of mass of the eccentric disk. ω is the angular velocity calculated from the motor’s speed in round per second. Finally, F is for force generated by the motor and the result is 3.73e-4 N.

With force generated by the motor, the acceleration of the device can be determined using Newton's Second Law of Motion, shown below:

$$F = m a$$

F is the generated force and m is the weight of whole device, including the motor and a pedal. The weight of these parts can be obtained using a micro scale. The calculated acceleration of the device is 0.02243 m/s^2 , which is $2.289 \times 10^{-3} \text{ G}$.

Discussion

Engaging a rodent by applying a vibrational stimulus is a far more humane method of stimulation than subjecting the animal to a painful stimulus. Conventionally, rodents have been stimulated by the use of heat, cold or electric shock. One focus of this research was to develop an ethical and effective method of stimulation. The current prototype is able to emit vibrations at 75, 100 and 125 Hz. The amplitude at these vibrational frequencies is limited by the weight of the platform. Furthermore, the amplitude in an ERM motor is coupled to the rotational velocity. It would be beneficial to create a device that can overcome these limitations due to the current state of scientific knowledge in vibrotactile stimulation of rodents. At the date of publication no known studies exist examining frequency and amplitude independently as a method for hindlimb somatosensation in rodents. Given these limitations, such a device, would lead to novel findings and help further research in multiple fields. This information would additionally benefit the project by identifying ideal stimulation parameters, as well as establish threshold parameters. Threshold parameters will be needed to ensure that the stimulation at one site does not cause off-target stimulation and/or perception at an adjacent site.

Currently, our data is only preliminary. A greater sample size will be needed to establish confidence intervals for the frequencies and amplitudes the device can produce. Additionally, the statistical power of the experiment should, by definition, be calculated prior to engaging in further data collection to ensure our tests correctly reject the statistical null hypothesis which states that vibrational frequencies are the same at various voltages.

Conclusions

With the currently available methods of testing sciatic nerve function, specifically after nerve repair, the main limitations come in the form of pain. Utilizing pain as a somatosensory reflex can have negative effects on the rats in the tests. For this reason the client was interested in using vibration as the stimulus, such that the rats were not injured during assessment of the nerve repair. Until animal testing can take place, the best determination of an appropriate stimulating frequency comes from the Rothshenker study, of approximately 50 Hz. The motors used do not quite meet these specifications. However, using the precision microdrives motor, or the voice coil in headphones these frequencies may be attainable.

Future work

After communicating with the client regarding the inefficiencies of the current motors; the decision was made to continue testing the frequency of additional versions of the same motors. After

verifying that each of the motors vibrates at the expected frequency, the current motors will be used in the next phase of testing. This next phase will include animal testing at different frequencies as well as variable amplitudes to determine the most effective frequencies and amplitudes to stimulate the somatic nerves in the hindlimbs. Once the most effective frequencies and amplitudes have been established, the results will be shared with the client as well as the prototype. However, if the current limitations of the motors prove to be ineffective at stimulating the hindlimbs, two other options will be considered.

Depending on the success of the initial animal tests, there are two alternative options that will be fulfilled. First, the team will attempt to again order the original motor from precision microdrives. At the same time, the utilization of a pair of earbud voice coils will be used to stimulate the somatic nerves in the rat. This method will require a standoff to be built so that the membrane is not in contact with the rat's foot except when it is vibrating. This alternate method of stimulation would allow for simple and accurate control of both frequency and amplitude. Frequency would be controlled by sending a sinusoidal sound wave to the voice coil, and amplitude by changing the volume.

The final step in the process of designing a prototype for the client will be designing a GUI or graphical user interface. A GUI will most likely be created utilizing matlab. This is the final step, and will only be attempted once the testing and verification steps have been completed.

References

- [1] E. Huebner and S.M. Strittmatter, "Axon Regeneration in the Peripheral and Central Nervous Systems," N.p., 27, Mar. 2010. Web.
- [2] S. G. Yeomans, "Sciatic Nerve and Sciatica." [Online]. Available: <https://www.spine-health.com/conditions/sciatica/sciatic-nerve-and-sciatica>. [Accessed: 07-Oct-2018].
- [3] A. M. Garner, J. N. Norton, W. L. Kinard, G. E. Kissling, and R. P. Reynolds, "Vibration-induced Behavioral Responses and Response Threshold in Female C57BL/6 Mice," *J. Am. Assoc. Lab. Anim. Sci.*, vol. 57, no. 5, pp. 447–455, Sep. 2018.
- [4] S. Rotshenker, "Wallerian degeneration: the innate-immune response to traumatic nerve injury.," *J. Neuroinflammation* , vol. 8, p. 109, Aug. 2011.
- [5] K. N. Rabey, Y. Li, J. N. Norton, R. P. Reynolds, and D. Schmitt, "Vibrating Frequency Thresholds in Mice and Rats: Implications for the Effects of Vibrations on Animal Health," *Ann. Biomed. Eng.*, vol. 43, no. 8, pp. 1957–1964, Aug. 2015.
- [6] Servocity, "100 mm Stroke 4 lb Thrust Light Duty Linear Servo." [Online]. Available: <https://www.servocity.com/100-mm-stroke-4-lb-thrust-light-duty-linear-servo>. [Accessed: 10-Oct-2018].
- [7] "Amazon.com: uxcell DC 3.7V 9000RPM 90mA 4mm x 8mm Yellow Micro Vibration Motor for Cell Phone: Toys & Games." [Online]. Available: <https://www.amazon.com/uxcell-9000RPM-Yellow-Micro-Vibration/dp/B072L7SRRS>. [Accessed: 10-Oct-2018].

- [8] Precision Microdrives, "10mm Vibration Motor - 13mm Type - Precision Microdrives." [Online]. Available: <https://www.precisionmicrodrives.com/product/310-112-10mm-vibration-motor-13mm-type>. [Accessed: 10-Oct-2018].
- [9] "Fast Fourier transform - MATLAB fft." [Online]. Available: <https://www.mathworks.com/help/matlab/ref/fft.html>. [Accessed: 12-Dec-2018].
- [10] A. Hjort, M. Holmberg, V. Ziemann, and K. Gajewski, "Measuring mechanical vibrations using an Arduino as a slave I/O to an EPICS control system," 2015.

Appendix

A. Preliminary Design Specifications

Function:

Peripheral nerve injuries are common, debilitating and costly. Approximately 2.8% - 5% of all trauma patients in the US sustain such an injury. Many peripheral nerve injuries are a result of amputations, which affect an estimated 185,000 people in the US each year. Prosthetics are continually improving, but a large issue that remains is the patient's lack of tactile perception. Many researchers, including Dr. Aaron Dingle, are designing devices to solve this problem. The functional outcome of these devices can be assessed in humans by asking the patient questions, but this technique is not an option in animal models. Rats are commonly used as animal models as a precursor to human subject testing. In order to receive functional outcome data from rats, a healthy rat can be trained to respond in a certain way to a somatosensory stimulus. A peripheral nerve can then be surgically cut and the novel device implanted. The device can then be used to apply what should be recognized as the same somatosensory stimulus the rat was trained with. Observations on the percent of correct reactions can be used to determine success.

This project aims to design the somatosensory stimulation device used to train the rats. The device should be able to apply a graded stimulus to at least two limbs individually. The device will consist of a cage or cage insert as well as a microcontroller to control the stimulus grade.

Client requirements:

- Must house one rat

- Must be able to individually stimulate each hind limb of the rat
- Must be completely controlled by a computer (no user input other than to start the device and configure settings)
- Must be sterilizable
- Mouse must be visible while in the cage

Design requirements:

1. Physical and Operational Characteristics

- Performance requirements:* Multiple experiments may be run per day. The device should be capable of repeated use and cleaning between trials.
- Safety:* The device should not harm the animals in any way.
- Accuracy and Reliability:* The device should accurately and reliably produce a graded stimulation of the user's choice.
- Life in Service:* Trials would last approximately 15 minutes in duration.
- Shelf Life:* A 10 year shelf life is preferred.
- Operating Environment:* Room temperature (25°C) and pressure (1 atm).
- Ergonomics:* The rodent should have enough room to rear on its hind legs and interact with the forward facing wall.
- Size:* No smaller than 30 cm x 30 cm x 60 cm. No larger than 120 cm x 120 cm x 120 cm
- Weight:* No heavier than 11 kg.
- Materials:* Plexiglass.
- Aesthetics, Appearance, and Finish:* Must be able to observe test subject through wall material.

2. Production Characteristics

- Quantity:* One unit.

b. *Target Product Cost*: < \$100

3. Miscellaneous

a. *Standards and Specifications*: The device should be approved by the UW-Madison Research Animal Resources and Compliance (RARC) center.

b. *Competition*: None

B. Design Matrix Full Description

Each motor was rated according to the following criteria (weights in parentheses):

1. **Segregation of Stimulation** (25): The degree to which the vibrational stimulation is localized to one hindlimb of the rat. A score of 5 would imply that any vibration applied to one hind-leg platform would be undetectable in the other hind-leg platform. A score of 3 would imply that when vibration is applied to one hind-leg platform there is a measurable vibration in the other hind-leg platform between 0 and 0.1 times the amplitude of the applied vibration. A score of 1 would imply that when a vibration is applied at one hind-leg platform there is a measurable vibration at the other hind-leg platform between 0.5 and 1 times the applied vibration.
2. **Frequency Range** (20): How closely the vibration range of the motor matches the desired vibration range. A score of 5 would imply that the motor has a range larger than but including 70 Hz to 100 Hz. A score of 3 would imply that the motor has a range of 70 Hz to 100 Hz but has limited resolution within this range. A score of 1 would imply the motor does not produce vibrations within the 70 Hz to 100 Hz range.
3. **Ease of Integration** (15): The ease of creating an interface between the motor and the rat. A score of 5 would imply that the interface could be designed within two weeks. A score of 3 would imply the interface could be designed within a month. A score of 1 would imply that it would take more than one month to design the interface.
4. **Size** (15): The design must be able to fit on a laboratory bench and hold one adult rat. The cage has a minimum size of 30 cm × 30 cm × 60 cm. A score of 5 would imply the motor could be used with a cage of dimensions 30 cm × 30 cm × 60 cm. A score of 3 would imply the motor could be used with a cage of dimensions 35 cm × 35 cm × 60 cm. A score of 1 would imply the motor requires a cage larger than 40 cm × 40 cm × 60 cm.
5. **Ease of Achieving Desired Effect** (5): The ease of controlling the motor. We will need to create code that will manipulate the motor. A score of 5 would imply that there is a sufficient amount of documentation on how to control the motor. A score of 3 would imply that there is some documentation on how to control the motor. A score of 1 would imply that there is little to no documentation on how to control the motor.
6. **Safety** (5): The safety of the design. The design should not harm or cause discomfort for the user or rat. A score of 5 would imply that there is little to no chance that the rat or user will

be harmed. A score of 3 would imply that there is some chance that the rat or user will be harmed. A score of 1 would imply that there is a high likelihood that the rat or user will be harmed.

C. MATLAB and Arduino Code

Accel_read.ino

```
#include <MsTimer2.h>

#define BUFF_SIZE 384

/* The function that will read data from the arduino. */
void getData();

/* The analog inputs. */
int xin = A0;
int yin = A1;
int zin = A2;

/* Buffer to hold values from accelerometer. */
unsigned short buff[BUFF_SIZE];

/* A count of the number of elements in buff. */
unsigned short cc = 0;

/* The variables to control the motor. */
int motor = 9;
int power = 252;

void setup() {
    analogWrite(motor, power);

    Serial.begin(9600);    // Start serial communication.

    pinMode(xin, INPUT);
    pinMode(yin, INPUT);
    pinMode(zin, INPUT);

    Serial.println("D");

    MsTimer2::set(1, getData);
    MsTimer2::start();
}

void loop() {
    int matCmd = 0;

    if (cc >= BUFF_SIZE) {
```

```

    MsTimer2::stop();
}

// If matlab needs more data, restart the program.
matCmd = Serial.read();
if (matCmd == 49) {
    Serial.println('1');

    for (int i = 0; i < BUFF_SIZE; i++) {
        Serial.println(buff[i]);
    }

    cc = 0;
    MsTimer2::start();
}
}

void getData() {
    buff[cc++] = analogRead(xin);
    buff[cc++] = analogRead(yin);
    buff[cc++] = analogRead(zin);

    if (cc >= BUFF_SIZE) {
        MsTimer2::stop();
    }
}
}

```

MatlabSerialArduino.m

```

clear
close all
clc

% The number of samples in a single package from the Arduino.
PACKAGE_SIZE = 128;
% The number of packages recieved from the Arduino.
PACKAGE_NUM = 1;
% The number of values in each sample from the Arduino.
SAMP_DIM = 3;

xyzData = zeros(PACKAGE_SIZE, SAMP_DIM);
xData = zeros(PACKAGE_SIZE * PACKAGE_NUM, 1);
xDataFFT = zeros(PACKAGE_SIZE * PACKAGE_NUM, 1);

```

```

t = 1:PACKAGE_SIZE;
mag = zeros(PACKAGE_SIZE * PACKAGE_NUM, 1);
magFFT = zeros(PACKAGE_SIZE * PACKAGE_NUM, 1);

% Initialize communication with Arduino.
try
    s = serial('COM5', 'BaudRate', 9600);
    fopen(s);
    fscanf(s); % Get the first byte to acknowledge Arduino.
catch
    warning('Could not establish connection with Arduino.');
```

```

    fclose(s);
end

% Get data from Arduino a certain number of times.
for n = 1:PACKAGE_NUM
    try
        % Send a signal to the Arduino to get another data set.
        fwrite(s, '1', 'uint16');

        fscanf(s);

        % Read data from arduino.
        for i = 1:PACKAGE_SIZE
            for j = 1:SAMP_DIM
                val = fscanf(s);
                xyzData(i,j) = str2double(val([1:length(val) - 2]));
            end
        end

        % Get the magnitude of the acceleration vectors.
        for i = 1:PACKAGE_SIZE
            mag(((n-1)*PACKAGE_SIZE) + i) = norm(xyzData(i, :));
        end

        xData([(((n-1)*PACKAGE_SIZE) + 1):((n)*PACKAGE_SIZE)]) =
xyzData(:,1);
        n

    catch
        warning('Something happened.');
```

```

        fclose(s);
    end % End Try-Catch
end

```

```
end % End for loop
```

```
fclose(s); % Close serial communication with Arduino.
```

```
magFFT = fft(mag);
```

```
xDataFFT = fft(xData);
```

PlotsPlotsPlots.m

```
% The number of samples in a single package from the Arduino.
```

```
PACKAGE_SIZE = 128;
```

```
% The number of packages recieved from the Arduino.
```

```
PACKAGE_NUM = 1;
```

```
% The number of values in each sample from the Arduino.
```

```
SAMP_DIM = 3;
```

```
% x1 = abs(fftshift(csvread("cyl_dir_mag_102.csv")));
```

```
% x2 = abs(fftshift(csvread("cyl_dir_mag_127.csv")));
```

```
x3 = abs(fftshift(csvread("cyl_dir_mag_152.csv")));
```

```
x4 = abs(fftshift(csvread("cyl_dir_mag_177.csv")));
```

```
x5 = abs(fftshift(csvread("cyl_dir_mag_202.csv")));
```

```
x6 = abs(fftshift(csvread("cyl_dir_mag_227.csv")));
```

```
x7 = abs(fftshift(csvread("cyl_dir_mag_252.csv")));
```

```
freq = ((0:(length(x7)-1))-(PACKAGE_SIZE/2)) ./ (0.001*PACKAGE_SIZE);
```

```
figure
```

```
hold on
```

```
% plot(freq, x1, 'color', [1 63/255 85/255])
```

```
% plot(freq, x2, 'color', [1 178/255 53/255])
```

```
plot(freq, x3, 'color', [1 74/255 186/255])
```

```
plot(freq, x4, 'color', [215/255 106/255 1])
```

```
plot(freq, x5, 'color', [116/255 116/255 1])
```

```
plot(freq, x6, 'color', [6/255 233/255 0])
```

```
plot(freq, x7, 'color', [0.0000 115/255 14/255])
```

```
hold off
```

```
legend('3.0V', '3.5V', '4.0V', '4.5V', '5.0V')
```

```
axis([0 250 0 50])
```

```
xlabel('Frequency (Hz)')
```

```
ylabel('Amplitude')
```

Multiplots.m


```

% The number of samples in a single package from the Arduino.
PACKAGE_SIZE = 128;
% The number of packages recieved from the Arduino.
PACKAGE_NUM = 1;
% The number of values in each sample from the Arduino.
SAMP_DIM = 3;

% x1 = abs(fftshift(csvread("mag_102.csv")));
% x2 = abs(fftshift(csvread("mag_127.csv")));
x3 = abs(fftshift(csvread("cyl_dir_mag_152.csv")));
x4 = abs(fftshift(csvread("cyl_dir_mag_177.csv")));
x5 = abs(fftshift(csvread("cyl_dir_mag_202.csv")));
x6 = abs(fftshift(csvread("cyl_dir_mag_227.csv")));
x7 = abs(fftshift(csvread("cyl_dir_mag_252.csv")));

% y1 = abs(fftshift(csvread("coin_direct_mag_102.csv")));
% y2 = abs(fftshift(csvread("coin_direct_mag_127.csv")));
y3 = abs(fftshift(csvread("noise1_mag_153.csv")));
y4 = abs(fftshift(csvread("noise1_mag_178.csv")));
y5 = abs(fftshift(csvread("noise1_mag_203.csv")));
y6 = abs(fftshift(csvread("noise1_mag_228.csv")));
y7 = abs(fftshift(csvread("noise1_mag_253.csv")));

% z1 = abs(fftshift(csvread("coin_noise_mag_102.csv")));
% z2 = abs(fftshift(csvread("coin_noise_mag_127.csv")));
z3 = abs(fftshift(csvread("noise_mag_153.csv")));
z4 = abs(fftshift(csvread("noise_mag_178.csv")));
z5 = abs(fftshift(csvread("noise_mag_203.csv")));
z6 = abs(fftshift(csvread("noise_mag_228.csv")));
z7 = abs(fftshift(csvread("noise_mag_253.csv")));

freq = ((0:(length(x7)-1))-(PACKAGE_SIZE/2)) ./ (0.001*PACKAGE_SIZE);

figure
subplot(3,1,1);
hold on
% plot(freq, x1, 'color', [1 63/255 85/255])
% plot(freq, x2, 'color', [1 178/255 53/255])
plot(freq, x3, 'color', [1 74/255 186/255])
plot(freq, x4, 'color', [215/255 106/255 1])
plot(freq, x5, 'color', [116/255 116/255 1])
plot(freq, x6, 'color', [6/255 233/255 0])
plot(freq, x7, 'color', [0.0000 115/255 14/255])

```

```

hold off

title('Direct Placement')
legend('3.0V', '3.5V', '4.0V', '4.5V', '5.0V')
axis([0 250 0 50])

subplot(3,1,2);
hold on
% plot(freq, y1, 'color', [1 63/255 85/255])
% plot(freq, y2, 'color', [1 178/255 53/255])
plot(freq, y3, 'color', [1 74/255 186/255])
plot(freq, y4, 'color', [215/255 106/255 1])
plot(freq, y5, 'color', [116/255 116/255 1])
plot(freq, y6, 'color', [6/255 233/255 0])
plot(freq, y7, 'color', [0.0000 115/255 14/255])
hold off

title('Dampened')
ylabel('Amplitude')
axis([0 250 0 1000])

subplot(3,1,3);
hold on
% plot(freq, z1, 'color', [1 63/255 85/255])
% plot(freq, z2, 'color', [1 178/255 53/255])
plot(freq, z3, 'color', [1 74/255 186/255])
plot(freq, z4, 'color', [215/255 106/255 1])
plot(freq, z5, 'color', [116/255 116/255 1])
plot(freq, z6, 'color', [6/255 233/255 0])
plot(freq, z7, 'color', [0.0000 115/255 14/255])
hold off

title('Off-target Stimulation')
axis([0 250 0 1000])
xlabel('Frequency (Hz)')

```