THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF PHYSICS

DEVELOPMENT OF AN OPTICAL BEHAVIORAL MONITORING DEVICE AND CLOSED LOOP REWARD DISPENSER FOR USE DURING IN VIVO ELECTROPHYSIOLOGY EXPERIMENTS ALLOWS FOR EXPLORATION OF THE ROLE OF THE ALM IN GENERATING MOTOR MOVEMENTS

OLIVIA VILELLA
SPRING 2018

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree in Physics
with honors in Physics

Reviewed and approved* by the following:

Dr. Patrick Drew
Professor of Biomedical Engineering
Thesis Supervisor

Dr. Richard Robinett
Professor of Physics
Honors Adviser

*Signatures are on file in the Schreyer Honors College.

Abstract

In mice, whisking and locomotion are motor behaviors used to explore an environment through acquisition sensory information. In addition to volitional exploratory behaviors, whisking and locomotion are in goal directed behaviors such as foraging for food. In order to quantify changes in neural activity related to whisking, I have developed an optical whisker-tracking device using near infrared LEDs and a high frame rate camera to monitor whisker movement in awake head fixed mice. We paired the whisker tracker with a spherical treadmill to monitor locomotion allowing for a quantifiable readout of two motor behaviors. Using these behavior monitoring devices we sought to determine if Anterior Lateral Motor (ALM) cortex, an area associated with motor movement planning, is necessary for the generation of volitional motor movements. We performed electrophysiology recordings using stereotrodes implanted in ALM in mice expressing transgenic receptors on all neurons allowing pharmacological control of neural activity. Recordings of Local Field Potentials (LFP) and Multi-Unit Activity (MUA) coupled with behavioral pre- and post-modulation of neural activity suggest that the ALM is either not necessary for the generation of volitional motor movements, or both the left and the right hemisphere require analysis. However, it is possible, given our small sample size, that a larger sample of experimental data would yield more conclusive results. Next, we seek to understand how activity in ALM is modulated during different types of motor behaviors, volitional vs. goal directed. I have built a reward dispensing system to allow for closed loop, randomized administration of sucrose or water as a reward for locomotion. With this device, future electrophysiology experiments can be performed to understand the role of ALM in goal directed behaviors contrasted against that of motor behaviors. My results suggest that ALM is not necessary for generation of contralateral motor movements and has established a method for testing whether ALM is necessary for the generation of reward seeking behavior.

Table of Contents

List of Figures

Acknowledgements

I would like to acknowledge Dr. Robinett and Dr. Drew for being my honors and thesis advisors, respectively. I'd also like to acknowledge Kyle Gheres for all of his assistance with my project.

Chapter 1

Introduction

Different animals have different ways of moving and exploring the world around them. Mice do this almost entirely with their whiskers. This behavior can be either exploratory (volitional) or goal directed (to receive a reward). Therefore, from whisking in conjunction with neurological data, experimental neurologists can determine a lot about the mouse brain, including motor planning areas. Since all mammalian brains have the same basic blueprint, these findings can relate to humans. For instance, the line between volitional and nonvolitional behavior is often blurred. By tracking whisking and neurological behavior, it is possible to learn more about the difference in these behaviors, and the cortices that they are involved in. Previous research indicates that the anterior lateral motor cortex is involved in motor planning, and the difference between the cortical activity in mice that expect a reward and do not receive it and mice that do not expect said reward could sharpen the line between volitional and nonvolitional behavior.

To address this issue, I completed several individual tasks with the end goal of differentiating between the neural activity and whisking of volitional behavior and trained behavior, with the hypotheses that trained behavior to expect a reward is essentially nonvolitional. First, I had to assist in the construction of a whisker tracking device in order to view whisking on a camera. There are two methods of tracking whiskers: optically, or with a contact device. The contact devices are not as accurate as optical images, so I decided to create a device in which the shadow of the mouses whiskers images against a light background in order to measure the angle of whisking. In order to do this, I needed a homogenous light source of appropriate intensity to be aimed at the mouses whiskers and reflected off of a mirror so the resulting shadows could be used to measure whisking.

In order to better understand the planning and generation of volitional motor movements, we made use of our optical whisker tracker coupled with in vivo electrophysiology of ALM. We then manipulated ALM with DREAADs to see if unilaterally altering neural activity would alter spontaneous whisking. These experiments involve looking observing the mice on the spherical treadmill and observing their ALM activity normally, and then using designer receptors exclusively activated by designer drugs (DREAADs) to examine is preventing the normal cortical pathways will prevent the same behavior. I will describe DREAADs and their role in neurology experiments. Here, clozapine N-oxide (CNO) was utilized to prevent the normal neuronal firing and determine if the ALM is the part of the brain that is necessary for the next step of this experiment.

Finally I constructed a closed-loop, behavior rewarding system, called a lickometer. Future experiments making use of the lickometer are centered around testing the neurological differences of goal-directed, volitional behavior. The experiments will involve mice being trained to expect a reward in the form of sugar water after a certain amount of running. After this training, the mouses neural activity will be monitored while running but there will be a randomization between reward and no reward. (in this case, in the form of sugar water versus water). This final step addresses the issue at hand, namely, whether the mice being trained to expect a reward is volitional or nonvolitional behavior.

In chapter 4, I detail how I coded the lickometer using LabView. I wrote a script to randomize output after a certain amount of input (the duration of the mouse running) and then inserted it into the larger code utilized in the lab for whisking and CBV camera data acquisition. Once the code worked as intended, I built the apparatus that will be used for the experiments.

Chapter 2

Development of Optical Device for Whisker Tracking

In order to determine if the ALM cortex is necessary for the generation of volitional motor movements, a reliable method of tracking the whisking and locomotion of the mice was needed. Previous lab members developed a method for measuring locomotion of head-fixed mice by placing them on a spherical treadmill attached to an encoder that generates a voltage when the mouse runs. In order to pair locomotion tracking with whisking, I needed to create a method of tracking whisker movement that did not interfere with natural whisking behavior. Using an optical method would allow us to observe whisker movement via a camera without interfering with the mouses ability to move its whiskers. In order to track whisking, a homogeneous light was aimed at the mices whiskers, and below the mouse, a mirror was set up to reflect light towards a camera. The whiskers, being backlit, appear as dark lines on a bright background allowing me to track the movement of the shadows of the whiskers. In order to obtain accurate data on whisking, the light would have to be homogeneous such that the shadows of the whisker contrasted with the background. In addition, the light would need to be of an intensity to prevent oversaturation on the camera. The initial method used was to use a near infrared LED and treating as a point source, expand the light using a Galilean telescope; however, the light was expanded unequally and was very bright at the center of the camera creating over saturation, which prevented visible whisker shadows. A Galilean telescope is a fairly simple optical device designed to expand a point source of light; indeed, all telescopes are designed to expand a point source of light as astrological bodies are distant enough that they can be approximated as a point source (Figure 2.1).

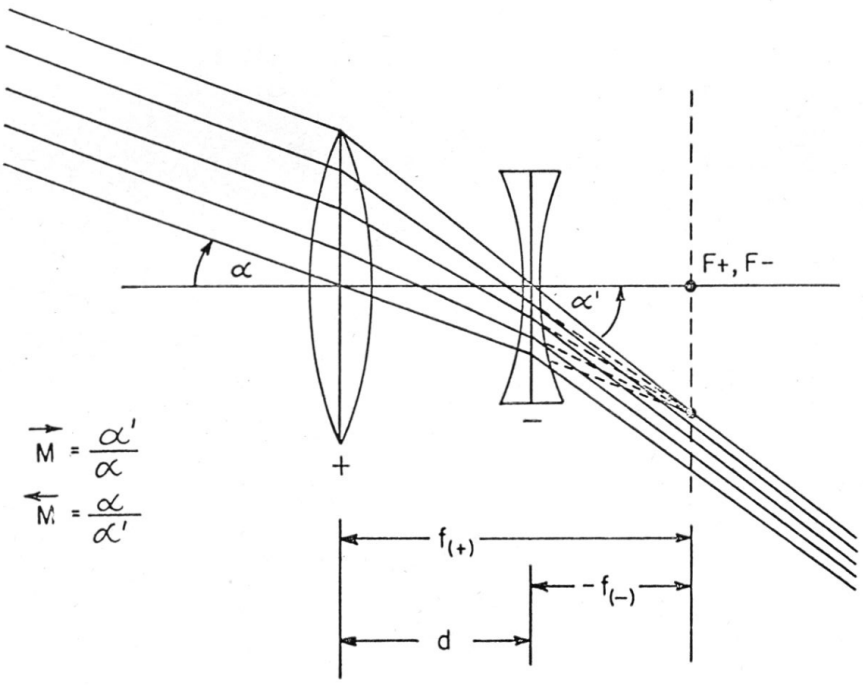

Figure 2.1: Galilean Telescope (Enoch, 1968)

The ray diagram of a Galilean telescope is quite simple and demonstrates how a point source is expanded. Light is focused through a convex lens, which acts as objective lens. The objective lens gathers the light and focuses them to create a real image. The concave lens is the ocular lens. The ocular lens is placed at the focal length of the objective lens to magnify the image. This set-up

works excellently for examining distant bodies; however, when applied to the LED light source it simple expanded the light to such a degree that it was saturated on the camera. From this point, I determined that collimation would be necessary to create a functional optical device.

Collimation of the light was the logical next step in creating a homogeneous light source. Mylar was used as a diffusion agent. The mylar helps diffuse the beam, expanding the concentrated power over a larger area solving two problems in one: it makes the imaging area larger and decreases the central hot spot from the Galilean method. The downside being this is accomplished by scattering light and then the beam had to be collimated so as to regain a non-diverging light source to maintain the light intensity at the mirror. This worked well because mylar is reflective. Some of the light was reflected back while some passed through, resulting in an overall decrease in brightness of the laser light. However, this effect results in diffusion of the light, and an overall inhomogeneous light source. Collimation was necessary and simple, as it only required a Fresnel lens. Fresnel lenses are used for when large apertures and short focal lengths are required, because a traditional lens would require a large amount of material to make. Fresnel lenses are created by dividing the lens into concentric annular sections, which then act as individual refracting surfaces (Hecht, 2002). A collimator narrows a beam of waves or particles into parallel rays, thus ensuring the uniform light source that was needed. A collimating lens is often very thick and convex in order properly collimate the light. But the size of such a lens would be untenable, hence the use of a Fresnel lens (Figure 2.2).

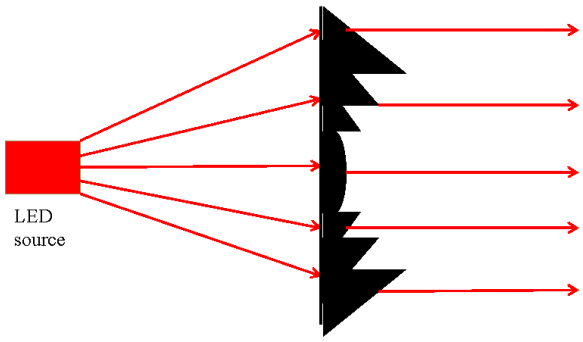

Figure 2.2: Fresnel Lens as a Collimator

A diagram of the finished optical device is given below, which includes the rays of light to show the resultant decrease and intensity and collimation (Figure 2.3).

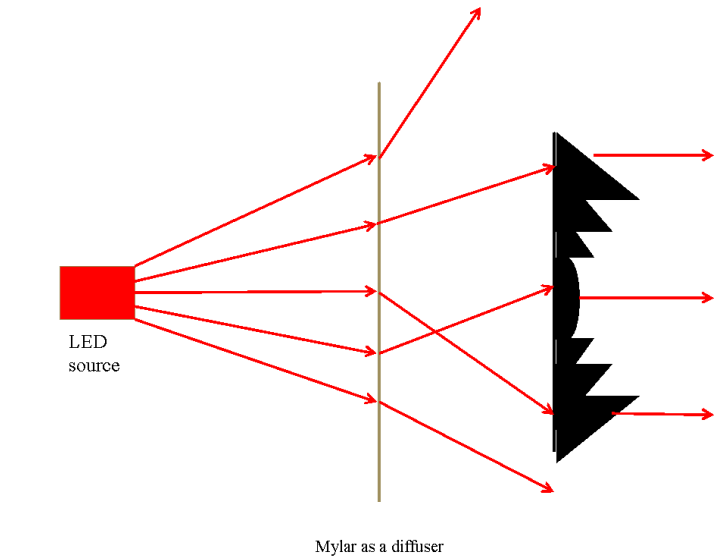

Figure 2.3: This set-up proved to work as intended. To quantify the homogeneity of the background light source, images were collected on a Basler camera and the difference from the average pixel intensity was calculated.

The data analysis involved radon transform. The radon function, as defined in MATLAB, computes projections of an image matrix along certain directions. The code was written such that the angle along which the image has the highest variance of pixel intensity was found, which corresponds to the light-dark pattern of the whiskers. As the mouse moves its whiskers the angle of greatest variance will change. We do this for 150 frames per second for 5 min and we can see whisker movement. After determining image homogeneity, an animal was placed with whiskers in the light path and whisker movement was tracked using the optical device (Figure 2.4).

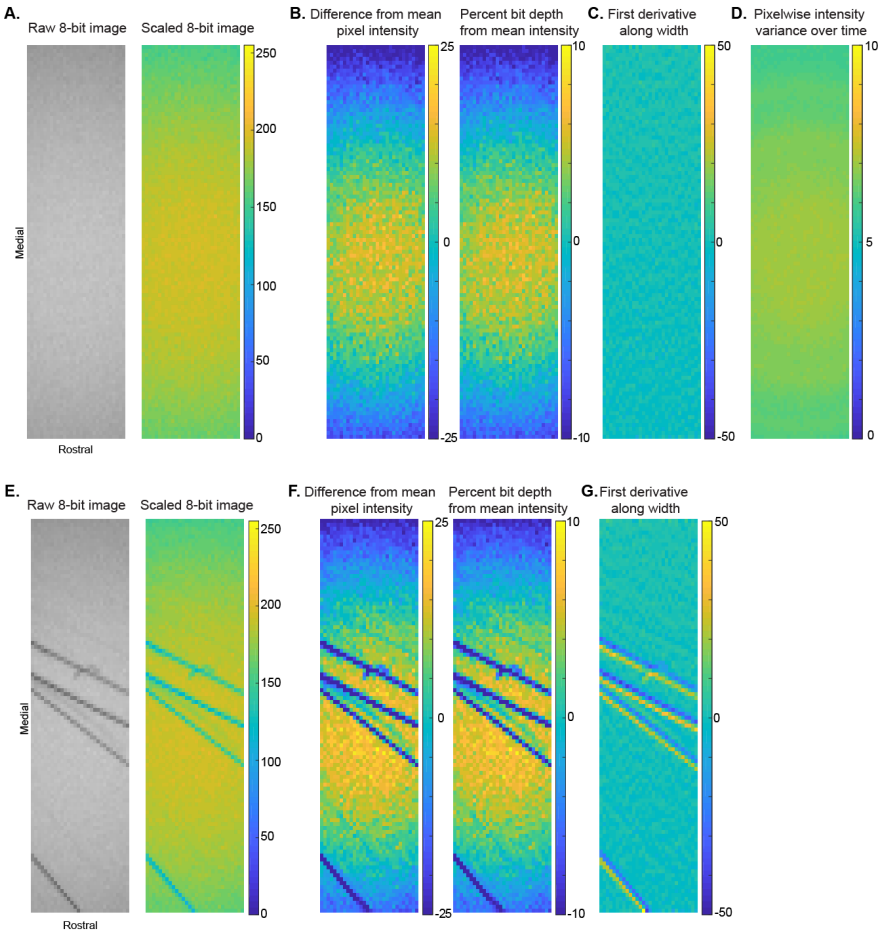

Figure 2.4: Raw Camera and Whisking Data

The optical device worked exceptionally well. Just from viewing the images of the camera without the mouse whisking, we can see that the intensity of the light is uniform, and the mean pixel intensities are very little changed (Figure 2.4-B). The data acquired from whisking is clear, with a sharp contrast between the shadows of the mouse's whiskers and the light (Figure 2.4-B). The whisker angles acquired by the Basler camera were also very distinct, and could easily be verified by images at the same points in time (Figure 2.5).

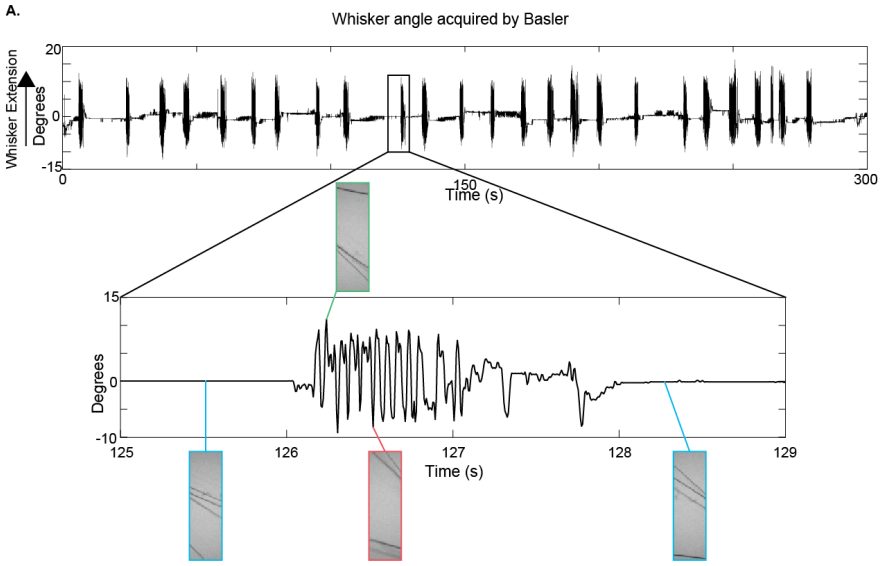

Figure 2.5: Whisking Data From Basler

Chapter 3

Pharmacological Modulation of ALM Activity Does Not Influence Execution of Volitional Motor Behaviors

Although neurology is a complicated science with many different facets, it can be neatly understood using the Hodgkin-Huxley Model. This is a mathematical rather than biological model, and it describes how action potentials are propagated. Hodgkin and Huxley performed experiments on the axons of squids and noticed multiple types of ion current, wherein there were specific sodium and potassium ion channels and chlorine was allowed to "leak" through the membrane. All of these ion-channels are now well-understood, but the implication of neuronal activity being controlled by voltage is fascinating, because it allows complex neural functions to be understood as fairly simple electrical circuits. In this view, the semi-permeable cell membrane is analogous to a capacitor, where the input current is defined as the flow of charges across the membrane due to the change in ion density. Here, the channels act as resistors (Figure 3.1).

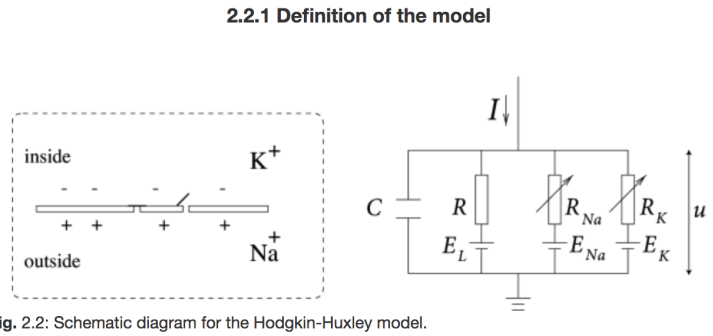

Figure 3.1: Schematic of Hodgkin-Huxley Model (Gerstner, 2018)

Using conservation laws, the total current over time can be written as a function of the capacitive current and the sum of the total currents over each ion channels (Equation 3.1). Using the definition of capacitance as charge over voltage and differentiating with respect to time, it becomes clear that in biological terms the voltage is the voltage across the membrane and the sum of the currents are those which pass through the cell membrane.

$$I(t) = I_c(t) + \sum_k I_k(t) \tag{3.1}$$

$$C\frac{\mathrm{d}u}{\mathrm{d}t} = -\sum_k I_k(t) + I(t) \tag{3.2}$$

The importance of the Hodgkin-Huxley model comes from the mathematical characterization of channels that are opened and closed. This means that the resistance of a channel can be measured with as a function of time and voltage. This required the introduction of gating variables that are the result of differential equations (Gerstner, 2018).

The amount of ion channels in a membrane is finite and will open and close stochastically. This results in a fluctuating pulse of current across the membrane. These pulses add to eventually create an action potential in a neuron, which creates the effect of multiple neurons firing in one cortical area. These cortical areas connect to each other and generate behaviors, such motor movements. In a mouse's brain, activity in the anterior lateral motor cortex informs directional movement (Chen, 2017). It is possible that this area is involved with volitional behaviors. In order

to test this, a mouse's activity in the ALM was first tested by running the mouse on a ball with a stereotrode. The response of the animal was then analyzed (Figures 3.2, 3.3, 3.4). These responses include measurement of the local field potential (LFP) and multi-unit activity (MUA). The LFP is an electrophysiological signal generated by the summed electric current flow, and MUA measures extracellular voltage.

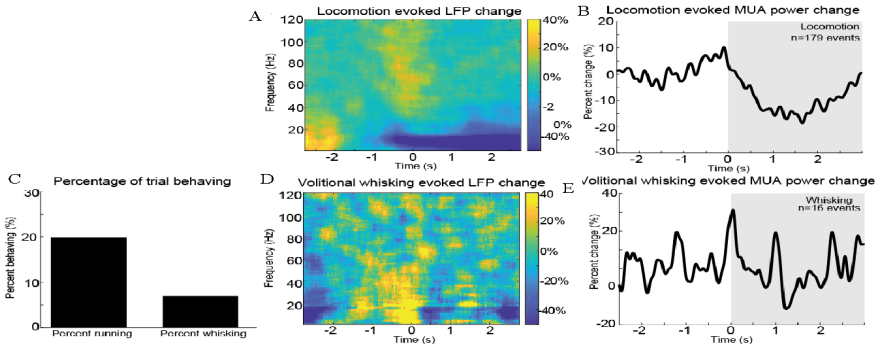

Figure 3.2: Normalized Spectrogram showing changes in LFP during Locomotion, Normalized changes in MUA, Fraction of Time spent Whisking/Running, Spectrogram of Whisking Events where Animal is not running, Normalized MUA for those events.

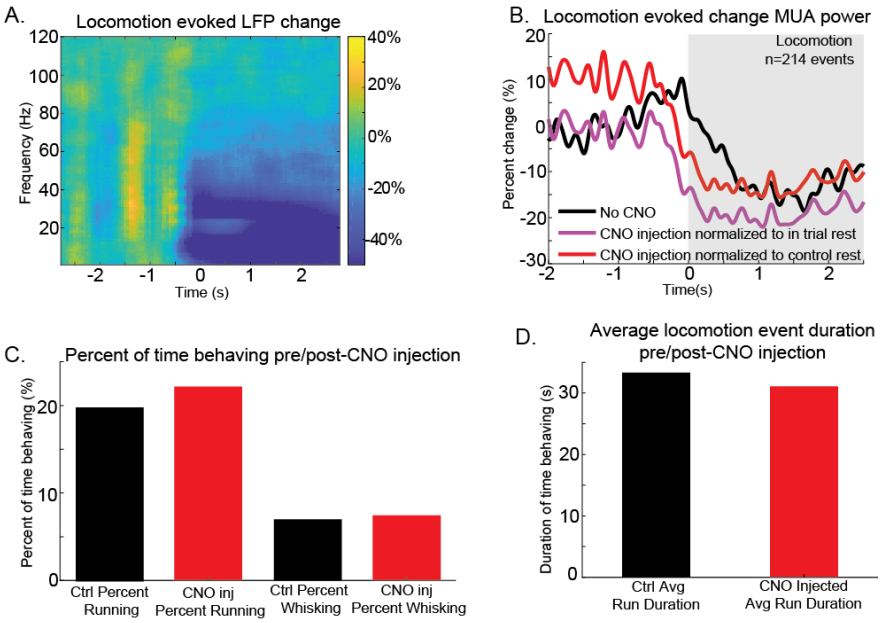

Figure 3.3: After CNO injection normalized LFP spectrogram, MUA power during local CNO injections, Percent time behaving before/after CNO injection, Duration of locomotion events pre/post CNO injection.

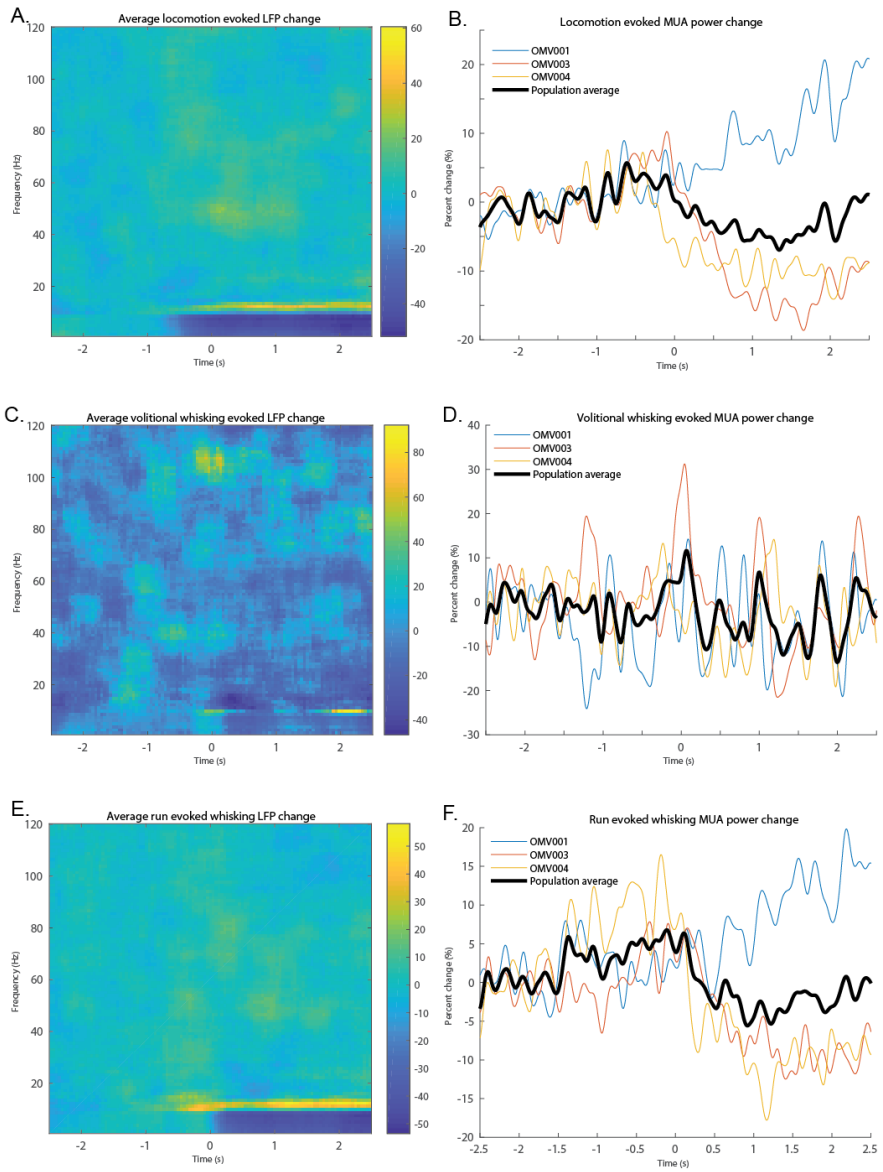

Figure 3.4: Top plots (A/B, E/F) display LFP/MUA when normalizing to period of onset; C/D show the same but normalized to average power prior to onset.

The figures of LFP, MUA, whisking, and locomotion are very clear. There is a noted change in both the LFP and MUA with changes in locomotion and whisking (Figure 3.2); however, there does not appear to be the expected response pre- versus post- CNO injection. For instance, there is no discernible change in the average locomotion event pre- versus post- CNO injection for a single animal (Figure 3.3-D). The population averages show little change in volitional locomotion and whisking as a function of MUA power change (Figure 3.4). This issues indicate that the ALM is not as intrinsic to volitional motor movement as previously thought, but it is possible the sample size was too small, or the right hemisphere also needed to be considered.

Chapter 4

Construction of Closed Loop Reward/Behavior Apparatus and Programming of Lickometer

Volitional behavior has been shown to be separate from reward seeking behavior. Volitional behaviors are the result of information that has been adjusted by the central nervous system (CNS), meaning, the animal thinks before performing them. The CNS is made up of the brain and the spinal cord, and in this context the cortex and the striatum are the most relevant sections. The striatum is a small group of subcortical structures, and it is one of the principal components of the basal ganglia. The basal ganglia are associated primarily with motor movement; they get information from the cortex and synthesize that information to achieve a desired goal by selecting the appropriate movement. Nonvolitional behaviors do not involve adjustment by the CNS, and are therefore more automatic and do not require as much synthesis of information as volitional behaviors does. Reward seeking behaviors have been shown be nonvolitional after training. An important facet of motor planning is the shifting between volitional and nonvolitional behaviors. The pre-supplementary motor area (SMA) in primates, which is analogous to the premotor cortex in mice, is related to motor planning and has been shown to be more related to habits than goal directed actions. Previous research indicates that harming this area of the brain will harm goal-directed movement but leave habitual movement in tact (Gremel, 2013). The future goal of the project is centered around testing the neurological differences of goal-directed, volitional behavior and habitual, nonvolitional behavior.

The previously described locomotion tracking apparatus was used with the mouse running on the ball to allow for monitoring of animal behavior. In addition, a lickometer was constructed and programmed to randomize output between sugar water and water reward for locomotion events meeting user-defined thresholds for duration. We hypothesize that dispensing reward for locomotion behaviors would alter the motor planning phase of movement generation resulting in differential ALM activation.

The theory behind this was that if a mouse was trained to expect sugar water after running on the ball for a certain amount of time, the mouse would keep running even after the output was binarized. The program was written in Labview, using a counter to keep track of how long the mouse runs and dispenses a neutral reward, water, or a positive reward, sugar water, to the mouse.

The section of the code that I wrote was fairly simple. Two solenoids were set up to respond to a randomized TTL pulse of 5 volts. I used a TTL pulse because the length of the pulse could be modulated to dictate how much volume was released from the reservoir of water or sugar water. The randomization itself was a simple loop in which the mouse running for a certain amount of time triggered the TTL pulse, releasing water or sugar water (Figures 4.1, 4.2).

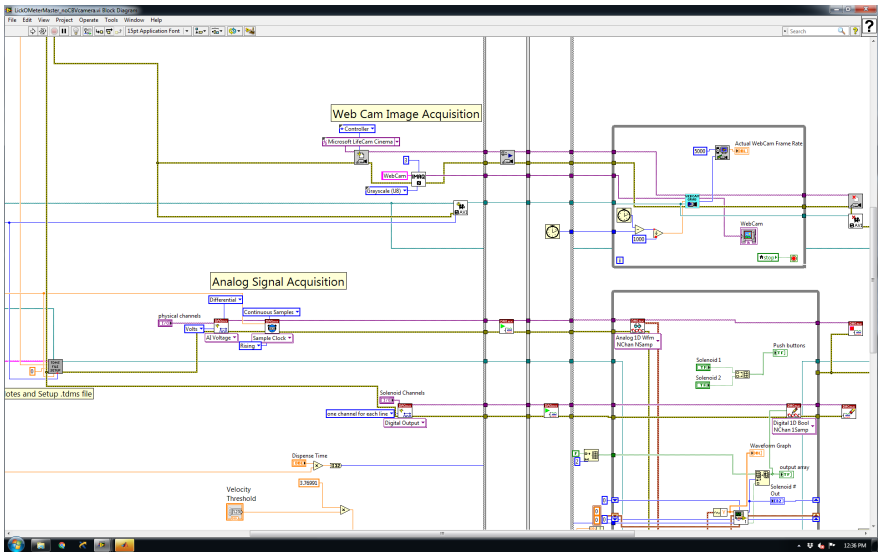

Figure 4.1: Block Diagram of Data Acquisition.

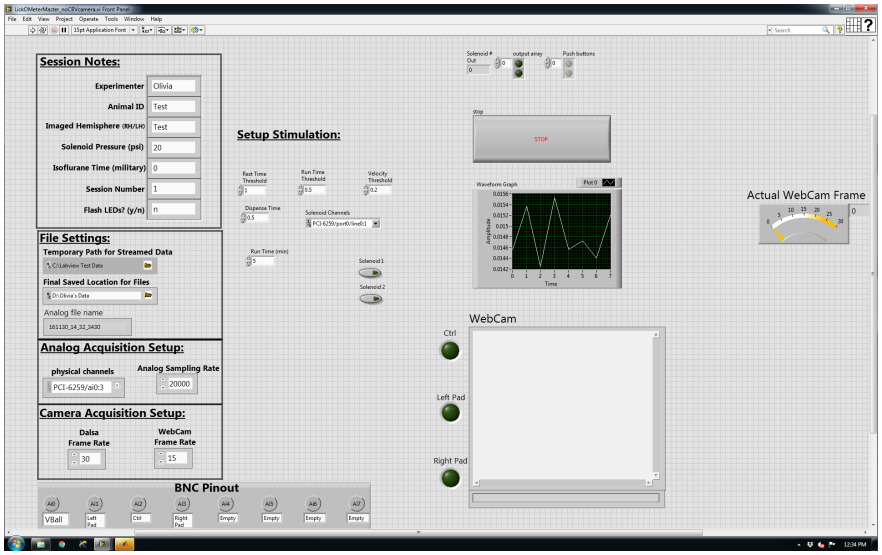

Figure 4.2: Front Panel of Data Acquisition.

An apparatus was then built to functionally distribute the water to the mouse in such a way that the mouse would be able to drink and would get it in a timely function. The construction was fairly simple, as two tubes were attached to the solenoid and held up on stands in the Faraday cage near the mouses mouth (Figure 4.3).

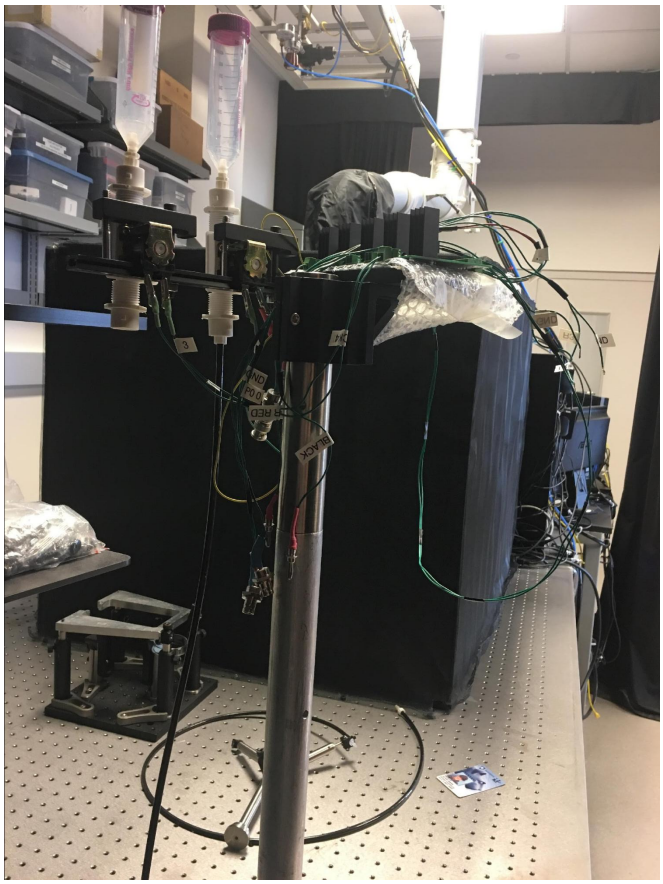

Figure 4.3: Completed Lickometer.

Although the apparatus itself has not been used experimentally, it has been thoroughly tested. All of the code works in the way it is supposed to, as does the physical construction designed to get the fluids to the mouse with the very easy step of holding the reservoirs far above the mice and allowing gravity to let the fluids drip towards the mouses mouth. Indeed, the code itself has been tested on live mice and data can be acquired using the lickometer code integrated into the larger code used for whisker tracking and CBV camera. This experiment will show fairly conclusively the relation between ALM and the goal-oriented versus habitual behavior.

Chapter 5

Discussion

Each section has its own set of results and conclusions to draw from them. We built and optical whisker tracking device using infrared light in order to avoid presenting a visual stimulus with a light and providing somatosensory stimulus by using a tracking device that relied on touch, and this well because I was able to create an optical device that diffused the LED and collimated the light, yielding a homogenous light source of appropriate intensity to whisker track. The pixel intensities had very little chance, and the Basler images demonstrated a clear connection between the measured whisking angle and whisking angle as captured by the pictures. This whisking device then was ideal to use for whisking data for determining the importance of the ALM in motor movement.

The results from the electrophysiology of the animals pre- and post- CNO injections are not as expected. For example, the LFP itself changed for the animals as a whole post-CNO injection, indicating that the electric current flowing across the neurons changed, as is clear from both the results for a single animal and those for the population. The MUA also showed change, indicating the extracellular voltage changed as well. However, there were no drastic changes in either the locomotion or whisking. This demonstrates that the ALM is not related to motor activity as we expected it to be. There are a couple of possibilities as to why this is, the most obvious being that the sample size is not large enough; however, these results are not just those averaged over the population, but also for each individual mouse. The next step in this process would be to examine the right as well as the left hemisphere, because although the literature indicates that one would be sufficient, it is possible that the bulk of the activity takes place in the right hemisphere and we are missing it. In addition, if neither of these hemispheres with a larger population shows change in locomotion or whisking, we could examine other cortical areas.

The purpose of the building of the optical device and CNO injection and resulting analysis are to demonstrate the change from volitional to nonvolitional behavior in the form of training. Although no data has been taken yet, the lickometer works as intended. Using a fairly simple code to randomize outcomes, a solenoid can open a channel between separate wells of water and sugar water as triggered by the mouses run time. The actual lickometer was simply constructed to utilize gravity, such that the liquid would flow down towards the mouses mouth. Although there was no data taken for testing, the lickometer itself worked with a mouse running on the spherical treadmill. Once the issue with the ALM has been addressed, more research can be done using the lickometer.

Bibliography

[1] W. Gerstner, "Hodgkin-huxley model," Apr 2018. [Online]. Available: http://neuronaldynamics.epfl.ch/online/Ch2.S2.html

[2] T. Chen, "A map of anticipatory activity in mouse motor cortex," *Neuron*, 2017.

[3] B. L. Roth, "Dreadds for neuroscientists," *Neuron*, 2016.

[4] C. M. Gremel, "Premotor cortex is critical for goal-directed actions," *Frontiers in Computational Neuroscience*, 2013.

[5] J. M. Enoch, "A spectacle contact lens combination used as a reverse galilean telescope in unilateral aphakia," *Optometry and Vision Science*, 1968.

[6] E. Hecht, "Optics," *Addison-Wesley*, 2002.

ACADEMIC VITA

Academic Vita of Olivia Vilella
olivia.vilella14@gmail.com

Education
Major and Minors: Major in Physics with Minors in Mathematics and Spanish
Honors: Graduating with Honors

Thesis Title: Development of and Optical Behavioral Monitoring Device and Closed Loop Reward Dispenser for use during In Vivo Electrophysiology Experiments allows for the Exploration of the Role of ALM in Generating Motor Movements
Thesis Supervisor: Dr. Patrick Drew

Work Experience
Summer 2017
Repperger Internship
Created a physical model to expand accuracy of modeling software depicting RF radiation affects in the human body
Fort Sam Houston Joint Air Force Base, San Antonio, Texas
Dr. Michael Jirjis

Awards: Millennium Scholar

Community Service Involvement: Trenton Area Soup Kitchen GED Tutor

Language Proficiency: English and Spanish