Visual Coding in the H1 Neuron of a Blowfly

Justin Chin

TA: Jeff Moore

UCSD Modern Physics Laboratory: Biological and Quantum Physics Course, Prof. Philibert Tsai

Introduction

The H1 neuron in flies has been a subject of interest in many electrophysiological studies of visual systems. The H1 neuron is an ideal target for research because of its accessibility, specificity, and predictability. Flies can be grown and breed with little maintenance and cost. Additionally, the H1 neuron is a fairly large neuron that spans across the majority of the fly's head, which makes locating the neuron easy with relatively little preparation. The H1 neuron is hard wired to respond primarily to horizontal motion, excited by regressive pattern motion and inhibited by progressive pattern motion. This response allows the fly to control its stability in flight as well as avoid objects.

The goal of this experiment was to become proficient in basic electrophysiology as well as coding in Matlab. I aimed to vary stimuli speed for each direction to find a correlation between stimuli speed and neural response.

Methods

Fly Preparation

A total of six cups of blue bottle flies (*Calliphora vomitoria*) were ordered from Forked Tree Ranch. These flies came in their pupae stage. They were placed in the refrigerator, where they could be stored for about two weeks until they were needed. The flies would hatch in one to two days in warmer temperatures and would live for about two weeks.

Two large plastic containers were prepared for the flies. A fist-sized hole was cut in the middle of each lid and a sleeve of a disposable lab coat was attached. This allowed the flies to breath as well as provide a way to remove individual flies when needed. The sleeve was tied off when not in use to prevent the escape of any flies.

Once pupae were added to the container, a tray of water and a tray of sugar were placed in the containers. The majority of my first batch died fairly quickly. It was determined that this was due to overcrowding, which was solved by placing pieces of cardboard and less pupae in the containers. Refilling the water and sugar also proved to be a problem. A small hole was punctured over the tray large enough to spray water, but small enough so that the flies could not escape. A large funnel was used to pour sugar into the tray either by opening a small portion on the side of the lid or through the

top.

When a fly was needed for the experiment, the sleeve was untied and a test tube was held in the sleeve until a fly crawled in. The fly was then placed in the freezer for 3-4 minutes. This would anesthetize the fly for about a minute allowing sufficient time to cut off the wings and legs. The fly was then mounted on a thin strip of metal with hot wax. The hot wax also covered their wounds from the amputations as well as inhibit any movement from the fly. The fly was positioned so that the head protruded from the edge. This was done so that the proboscis of the fly could be pulled down and attached to the bottom of the metal strip. This exposed the back of the fly's head. Additional wax was added if necessary to secure the head.

A scalpel was used to make three triangular incisions to remove the exoskeleton and reveal as much of one side of the fly's head as possible. I chose to make these incisions on the left side of the fly's head, although any side could be chosen. It is important to make the incisions as shallow as possible so as not to sever any neurons and kill the fly. Once the exoskeleton was removed, air sacs and the mucus substance were removed to expose the fly's brain. The fly's brain could be clearly seen as a denser white stringy fiber. A fly saline solution was put into the opening periodically to keep the brain moist and to keep the neurons from dying.

The prepared fly was then placed into the LED arena. A reference wire was inserted into the body of the fly. A 3MΩ tungsten electrode was inserted into the brain to record extracellular activity. The best results were produced when the probe was inserted toward the lower middle of the head.

Stimulus and Delivery

Stimulus was delivered by a 90 x 96 array of LEDs that covered approximately 270 degrees. The LED arena was controlled by three NI-DAQ cards with a 1 MHz baud rate. Each card controlled 32 columns of LEDS. Matlab scripts were used to code the stimuli through the NI-DAQmx C library. The first column was used as a time-locked counter, producing a spike that would indicate the start of each iteration of the stimuli. This is absent in one of my stimuli but was added in later trials.

Brightness could be controlled by setting a probability for each frame to be on or off. However, this was deemed unnecessary as the impact of photon noise was negligible.

The stimuli used were coded on Matlab. A previously programed sine grating stimuli was used to find the H1 neuron. This stimuli oscillated at 100 deg/sec for one second in each direction. Stimuli consisting of a single band rotating around the arena at set speeds, ranging from 67.5 - 1620 deg/sec, were written originally and purposefully for this experiment. These stimuli aimed to test the responsiveness of a H1 neuron to a rotating object in each direction at varying speeds.

Fly

LED Arena
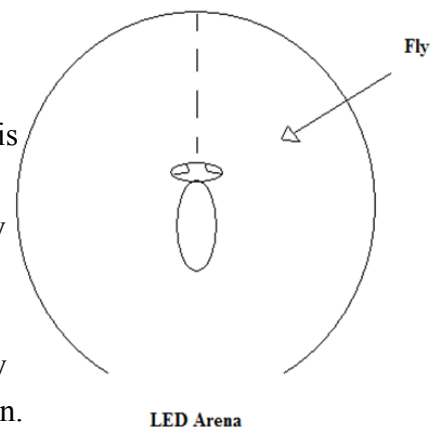
Recording

In order to acquire data, a MATLAB Data Acquisition Toolbox was used by using two analog inputs wired into an LED and an amplifier/filter. The analog input wired into the LED was non-functional. Instead, at the start of every trial, a signal was sent. Utilization of this LED ensured synchronization of the start of each trial with the input from the electrode. The other input came from an amplifier/filter, which amplified the voltage between the active and reference electrodes. The filters were set all the way open and the signal was amplified by 1000.

The electrode was inserted into the fly's head as sine grating stimuli was played continuously. An oscilloscope and a audio monitor was attached to the amplifier and was used to help locate the H1 neuron. The electrode was lowered until a patterned clicking noise much like a zipper sound could be heard and spikes could be seen on the oscilloscope.

Due to difficulties with the surgery and flies, only a limited number of useful trials were able to be recorded.

Analysis

Analysis was done on Matlab with custom Matlab scripts. Spikes were located by setting a threshold as the signal to noise ratio was high.

Results

The H1 neuron was located twice, though the second fly produced a weaker signal and was short lived. Recordings for the H1 neuron was successfully recorded and distinct neural response to horizontal movement was seen. Electrodes were inserted into the left hemisphere of the fly's brain and the sine grating stimuli was used to find and characterize the neuron (fig. 1a). An applied threshold was applied to obtain spike times. I used a threshold -45mV as this produced the most consistent results.

The sine grating stimuli was used not only to determine that the H1 neuron was located but to determine which eye the neuron was connected to. Previous reports have state that by inserting the probe near the middle of the brain, it was possible to obtain readings from both H1 neurons. The spiking rates showed that the left H1 neuron was obtained in this fly (fig. 1b-c).
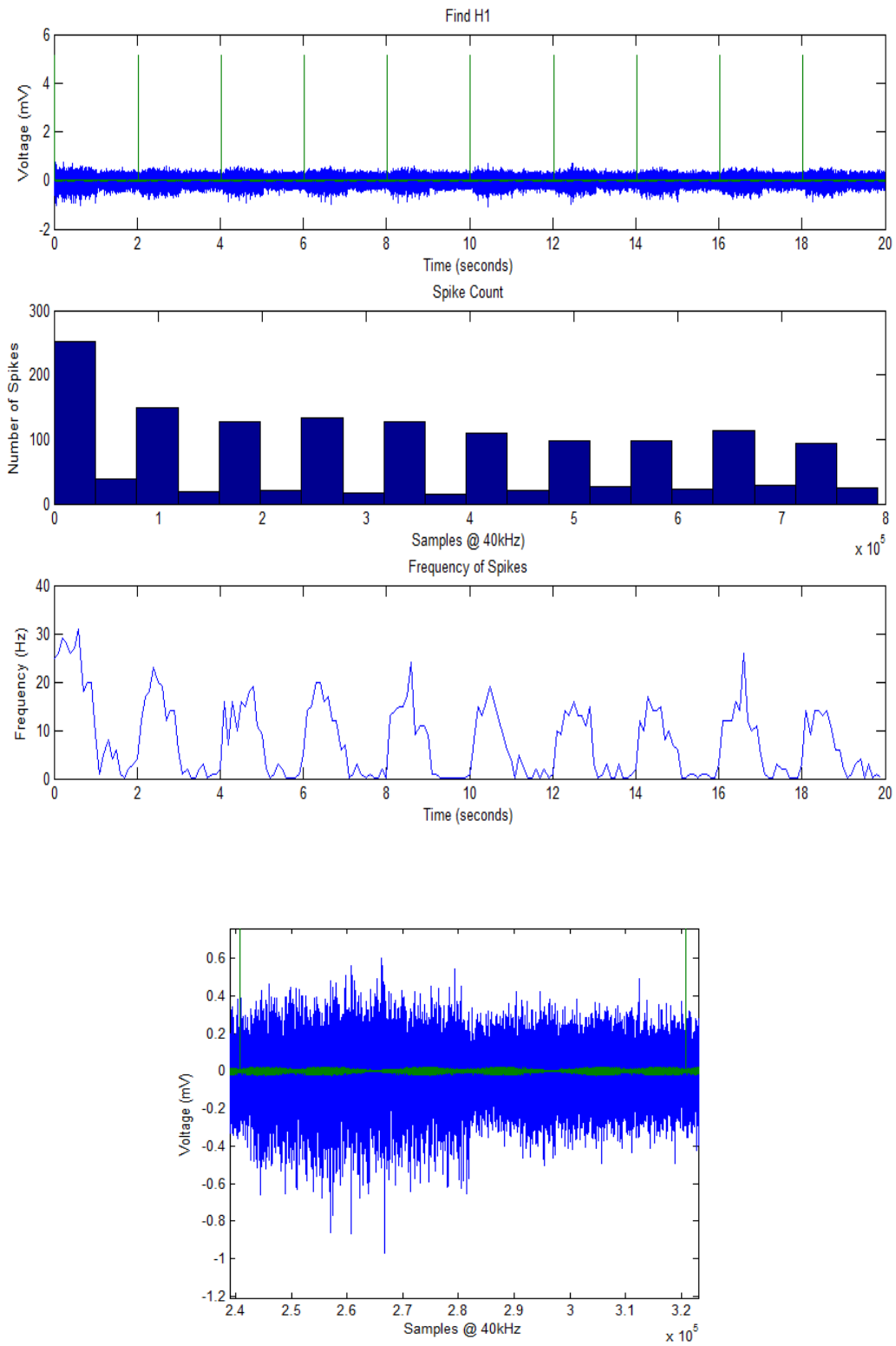
Figure 1a-d. a) Sample recording (sine grating @ 100 deg/sec). b) Spike count at a threshold of -0.45mV. c) Frequency of spiking over the recording. d) Close up of single trial. Direction shift occurs in the middle.

Upon finding and confirming the H1 neuron, I introduce my own stimuli randomly switching speeds (fig. 2). More trials may have been obtained if consecutive trials were performed, but I chose to randomize the trials to eliminate possible adaptation. Speed was inputted by revolutions per second from 0.25 rev/sec to 6 rev/sec. I was unable to perform any tests at a slower speed due to the limitations of the program. After every couple of trials, I reperformed the sine grating stimuli to ensure that the H1 neuron was still being recorded. The results of my experiment showed that the rate of spiking when the neuron was stimulated in the regressive direction was faster than when spiking occurred in the progressive direction. My analysis also showed that the neuron was most responsive to speeds of an object around 400 deg/sec.
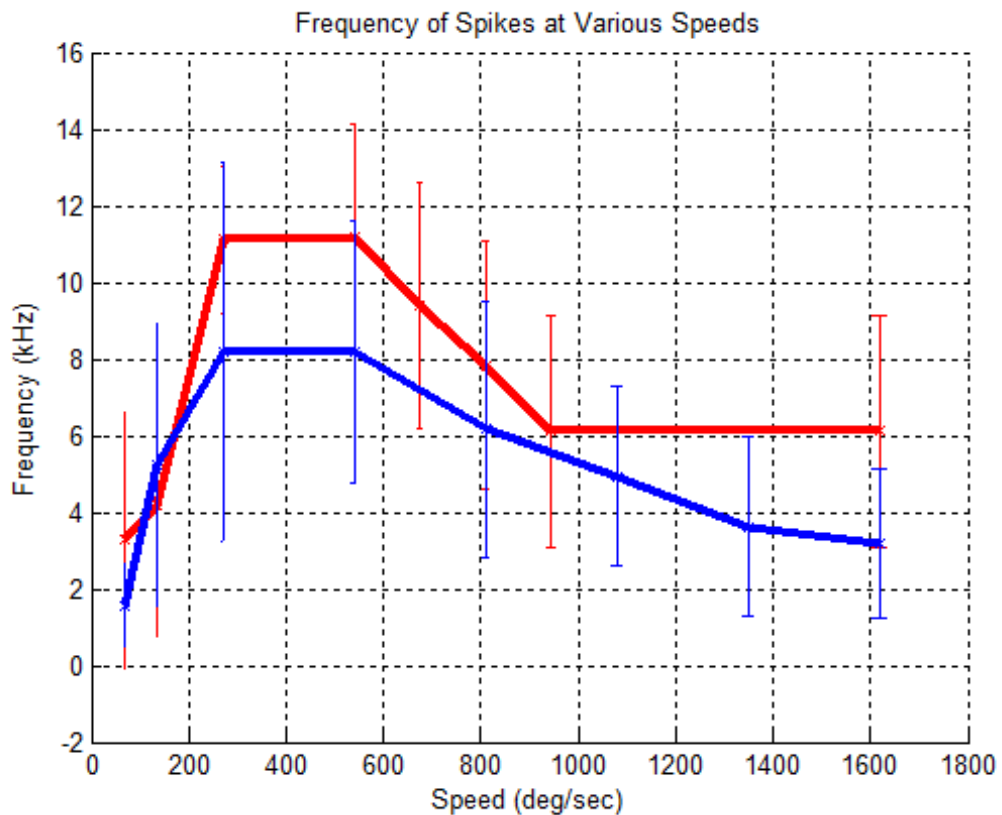

Figure 2. Neuron spike frequency from a single moving bar at various speeds. Blue indicates progressive motion (right to left) and red indicates regressive motion (left to right).

I finally introduced a third stimuli to the fly that consisted of two bands rotating in opposite directions (fig. 3). This simulated the fly moving forwards and backwards without rotation and my results showed continued spiking throughout the trials at all speeds tested. Speeds from 67.5 deg/sec to 270 deg/sec were measured with this stimuli.
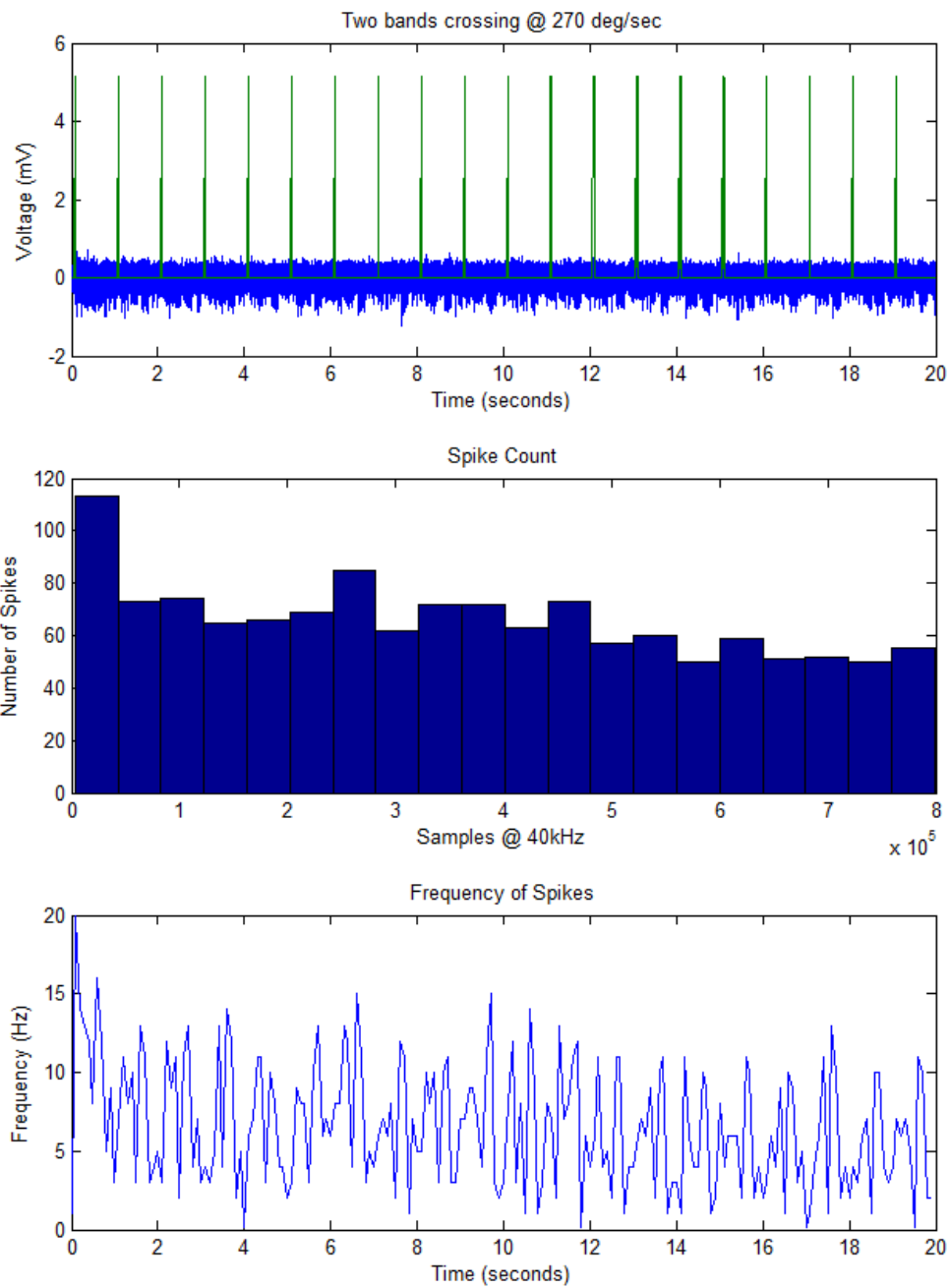
Figure 3. Example of two bands crossing stimuli.

Discussion

Despite the many roadblocks in the coding of the stimuli, preparing the flies, and finding the H1 neuron, data was successfully obtained with different forms of stimuli. I was only able to record the H1 neuron in three flies, however two of them were short lived. After only a few trials, all neural responses were gone. The final fly that most of the data was collected from also did not survive very long. Neurons could be audibly heard "dieing". The neural responses also became weaker throughout the experiment and this might have affected the data analysis. In spite of this, it was successfully shown that the H1 neuron responsiveness and activity is related to the speed of the visual stimuli. More trials would have been necessary to make any conclusive analysis on the optimal speed, however, I ran out of time to conduct any more trials.

The two crossing bands stimuli was created out of curiosity, but could be useful for future analysis to decipher how the neuron is responding to forward and reverse motions. An additional avenue for future study could be to analyze adaptation of the neuron.

References

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Jan G, Jutta K, Anne-Kathrin W., and Martin E. (2003) Impact of Photon Noise on the Reliability of a Motion-Sensitive Neuron in the Fly's Visual System. The Journal of Neuroscience