

Wide Field Visual Information Encoding in the Blow Fly

Evren Tumer

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1 Introduction

The H1 neuron in the blow fly is known to encode information about horizontal motions across the full visual field. This neuron has been well characterized [1] and is often used to test theories on information encoding in sensory neurons [2, 3]. In this lab, students will prepare a fly by opening the back of the head and exposing the H1 neuron. Then the fly will be mounted in front of an Light Emitting Diode (LED) display and the H1 neuron will be monitored with an electrode as the pattern on the LED display moves. The main stimulus variable is the angular velocity of the pattern projected by the LEDs at the fly's visual field; which will be recorded simultaneously with the spike train coming from the H1 neuron. This data can be used to analyze how the spike train is encoding information about the stimulus.

The H1 is a directionally sensitive motion detection neuron, that spikes at changing rate for motion in one direction (preferred direction) across the visual field and stops spiking when motion is in the opposite direction (non-preferred). So the cell has a much smaller dynamic range in response to motions in the non-preferred direction. In order to encode information about motion in both directions accurately the fly has two H1 cells, one in behind each eye, with opposite preferred directions. The spiking rates of the H1 has been studied under many changing conditions, including changing the average intensity, the contrast, and angular velocities of the stimulus [1] also adaptation in the H1 cell has been studied [4].

2 Experimental Setup

2.1 Flight Arena and Stimulus

The main setup of this experiment involves a flight arena in which the fly is held fix in front of an LED screen. The screen consists of 96 columns of green LEDs; each column has 10 LEDs all of which can be at one of two voltage levels. A digital I/O board, controlled by a computer, can independently switch each column between the two levels. Thereby creating a stimulus of bright and dark vertical bars which can be moved in front of the fly at different angular velocities. Figure 1 illustrates the basic flight arena set up, notice that there are 24 columns excluded from the back of the arena to allow for the electrode rig that will be used for the electrophysiological measurements. So the 96 columns of LEDs cover a total angle of 288 degrees around the fly with each column subtending 3 degrees.

2.2 Preparation and Electrophysiology

The neural activity of the H1 neuron is recorded extracellularly by a tungsten electrode. These signal have magnitudes of tens of microvolts and require amplification. Figure ?? illustrates the basic experimental setup. The signal from the electrode is first passed through a preamplification stage which uses Field Effect Transistor amplifiers to minimize the amount of current drawn through the electrode. From the preamp the signal is passed to a differential amplifier that removes common-mode signals between the reference wire and the electrode and amplifies the signal with a gain of 1000. Now the signal is large enough to be recorded on the computer.

Blowflies, *Sarcophaga bullata*, are used in this experiment. Flies are purchased as pupae and allowed to mature in a plastic insect cage. A good cage will come with a door on top that is large enough to fit one's hand through. In order to get the flies out of the cage without allowing them to escape, this door is removed and the waist end of a pair of pantyhose is attached over the hole. By cutting away the foot part of the pantyhose one can reach into the cage to catch the flies while blocking their escape. Place the pupae into the cage along with a small bowl of water and a small bowl of sugar. Also a heat lamp can speed up the eclosion (hatching) of the flies, which takes about a week. It is also suggested to keep the cage somewhat humid which can be done by periodically (every day or so) spraying water into the cage.

Most papers on H1 measurements use 2-3 day old female flies. It's good to let the flies mature for 2-3 days after hatching, this allows the cuticle behind the head to harden and the visual system to develop, but usually the flies can be used up to 1.5 weeks after hatching. Though I have not yet understood why, I have had the best success with the female flies. The two sexes can be easily distinguished while the flies are copulating. The females are usually smaller, with a rounder abdomen, and a smaller orange spot on their butt. After 1.5 weeks the flies can be disposed by placing the cage in the freezer for 10-15 minutes. This anesthetizes the flies after which they can be killed and thrown out. There's nothing like the smell of a week old fly cage in the morning. Have fun kids.

After catching a fly in a plastic test tube, place the test tube into the freezer for 15-20 minutes, anesthetizing the fly. Remove it from the test tube and cut off the wings and legs with a scalpel. Glue down the wing stubs as well as the legs, this will help to minimize signals from muscle motion. Next glue the fly to a small piece of metal and cut off the proboscis (tongue) and glue the stub of the proboscis down. Glue the head of the fly down to the thorax, making sure that the head is far enough down that the back of the eyes is fully exposed and then cut away the cuticle behind one of the eyes. A good tool for this is made by breaking off a small piece from a carbonized steel razor blade. These razors break cleanly and can be used to make small sharp edges, which can be glued down to a popsicle stick and used as a dissection tool. Clear away the air sacs in the back of the head, which look like spider webs, and place some fly saline in the opening (see appendix for formula). Now the visual system should dramatically appear before you. The visual neurons start to branch out from the midline of the head to the eye. Figure 2 illustrates the layout of the visual system and points to the approximate location of the H1 neuron. Finally, using a scalpel, make a small incision between two of the plates on the top part of the abdomen, this will help when inserting the reference wire into the fly. Let the fly get used to her new "situation" by placing her in the dark for about an hour. Add more saline if the head cavity starts to dry out.

Now the search for the H1 begins : don't worry, the force is strong in you, this should present no particular problem. For the electrophysiology 1-5 M Ω , 0.005 inch diameter tungsten electrodes were used. Place the electrode as close as possible to the opening in the back of the fly's head and insert the reference wire into the fly's abdomen. Use the operating microscope and the micromanipulator to position the electrode over the region shown in figure 2.

Start the LED pattern moving with a sinusoidal angular velocity (see next section) as you slowly move the electrode toward the tissue. The directional sensitivity of the H1 will make it easy to find, since the neuron will only spike when the pattern is moving in the preferred direction. There seems to be some neural activity that can be picked up from the photoreceptors, or something closer to the eye than the H1, which occurs every time the pattern moves. This can be distinguished from the H1 since it occurs in both directions of motion.

2.3 Stimulus Control Software

Figure 3 shows the front panel of the lab view program that controls the stimulus and the data acquisition and processing. The program can alter the average intensity, contrast, spatial wavelength, and angular velocity of the pattern. The program also controls the digitization of the measured electrophysiological voltages, low pass filters them to remove noise, then high pass filters them to accurately pull out the spikes. The raw signal can be sampled at rates up to 100 kHz, but 20-30 kHz is usually more than enough to get accurate spike timing. Each part of the program is described below with the numbers corresponding to numbers in figure 3. Note that boldface type refers to the controls on the front panel of the program.

1. Controls that handle the intensity and contrast of the pattern. When the **Update C/I** button is turned off these values will remain fixed. With the button in the "On" position the pattern intensity and contrast will be updated once every **C/I Update Rate** milliseconds. The two different voltage levels that the LEDs can have are determined by

$$V_{low} = 10I(1 - m)$$

$$V_{high} = 10I(1 + m)$$

where I is **Average Intensity** and m is **Contrast**. Both I and m range between 0 and 1, which leads to output voltages ranging between 0 and 20 Volts.

2. This section of the panel controls the analog input and output through a National Instruments AT-MIO-16-E10 board. The analog output is used to control pattern intensity and contrast by setting the two

voltage levels that the LEDs can switch between. The analog input is for sampling the voltage measurements from the electrode. The voltage range of the Analog-to-Digital Converter (ADC) is set by **AI Voltage Limits**. **AI Scan Rate** sets the sampling rate of the buffered input and **AI Buffer Size** sets the size of the internal buffer. The length of data that is to be pulled out of the buffer and processed at a time is set by **Data to pull out** (in milliseconds). This value must be set to a value less than the buffer size divided by the scan rate.

3. This control sets the pattern that will be moved in front of the fly by initializing a 15 element array, each element of which consists of an 8 bit (1 byte) integer that controls 8 of the LED columns. A bit that is set to 1 will set it's corresponding LED column to V_{low} 0 valued bit will set that LED column to V_{high} . The first element of the array sets the eight LEDs on the boards numbered P12 and P13, the next element sets P14/P15, and so on around the arena, with the 15th element setting P2/P1. For example, an element of the pattern is set to 9, this corresponds to a binary value of 00010001, and sets the first and the fourth column of LEDs to the low voltage and the other 6 to the high voltage. With all of the elements of the array set to 1, the pattern will have dark bars spaced at 24° in front of a bright background. The pattern does not change in structure during motion, it only changes in position.
4. This control determines how the pattern will move. Many different functions can be placed here, but the current version of the program only has the capability for constant angular velocity, the value of which is set by control **Ang Freq**, and a sinusoidal angular velocity for which the frequency (in Hz) and the amplitude (in $^\circ/\text{sec}$) are set by **Sin Ang Freq** and **Sin Amp**, respectively.
5. This controls the low pass filtering and spike sorting routines. The low pass filter is implemented by an average over a number of samples set by **Filter Order**. Then a two point numerical derivative of that signal is used to high pass filter the signal and take out any slow variations (non-spikes). The threshold control sets the level at which the fully filtered (both low and high pass) signal triggers a spike. Spikes are located by finding a peak in the signal after a trigger occurs. The fully filtered signal is output to the plot window **Filt Data** to help

determine the threshold in real time. The signal from the electrode after pre-amplification and amplification is plotted above that in the window **Vm Chart** and the angular velocity of the pattern is plotted in **AF Chart**. Then the time intervals between spikes and the spike amplitudes are plotted to **ISI Plot** and **Spk Amp**, respectively. To search for positive going spikes **Pk/Val** is set to 0 and to find negative going spikes it is set to 1.

6. The controls **W DF** and **Spike Time DF** determine the files to which the angular velocity and the spike information will be written. The former file will contain two columns of numbers, the first indicating time (in seconds) and the second the value of the angular velocity of the pattern at that time. The latter file has three columns, the first is the absolute time of the spike since the beginning of the experiment (in seconds), the second column is the amplitude of the spike (in Volts) and the last is an estimate of the width of the spike (in samples). The raw signal can also be saved to the file indicated in **AI DF**, but the data is only written out while **Wrt TMS** is set "On" since this can quickly become a rather large file.

3 Results

A section of the raw voltage measurements taken during constant angular velocity stimulus of $100^\circ/s$ is shown in figure 4. Figure 5 zooms in on one of those spikes. Sometimes the fly will try to move its body or other appendages, during which the electrode picks up a very large signal. An example of this is shown in figure 6. These episodes must be filtered out of the data analysis which can be done by vetoing any spikes occurring within some time window of the onset of the muscle movement. Large spike amplitude records should be able to indicate when muscle movement occurred.

A simple initial experiment is to measure the response of the H1 to constant angular velocity pattern motions of different magnitudes. From this data the average firing rate can be calculated for different angular velocities of the pattern. Some typical results from this experiment are shown in figure 7. Each data point was computed by collecting approximately 1000 spikes while stimulating the neuron with a low contrast ($m = 0.2$), low intensity ($I = 0.2$) pattern composed of dark bars on a bright background with a 24°

spatial wavelength. To figure out the spiking rates, the spike train was broken into segments of 200 ms in length and the number of spikes occurring in each segment were counted to form what is called the spike density function. Averaging the spike density function over the whole recording gives the average spiking rate. The H1 neuron can adapt to static stimuli and change its response over time, but inspection of some of the spike density functions showed no indication of significant adaptation. Note that these responses will change with the pattern's spatial wavelength, intensity and contrast.

In the above data the spike rate seems to saturate as moderate angular velocities and then degrade at large angular velocities. Further work of this type could involve increasing the contrast and intensity of the pattern and studying the affects on the spike rate response. It has been shown the higher intensities can increase the angular velocity at which the spiking rate begins to degrade [5]. Experiments can also be done on the affects of changing the spatial wavelength of the pattern. A good reference for this type of a study can be found in [1].

To further illustrate the directional sensitivity of the H1 neuron, figure 8 shows the response of the cell to a sinusoidal angular velocity profile. When the pattern is moving in the unpreferred direction, which is negative angular frequencies here, the spike rate quickly drops to zero. But the spike density function seems to quickly saturate during motion in the preferred direction so it does not seem to have a wide dynamic range for encoding the stimulus. We may be driving the system out of its linear response regime with such large angular velocities ($\approx 150^\circ/s$) or it may be necessary to use functions other than spike density to decode the angular velocity from the spike train [2]. An example where the spike rate does seem to encode the amplitude of the stimulus is shown in figures 9 and 10. Here the stimulus only moves in the preferred direction with a much smaller maximum amplitude than before. Though somewhat noisy, the spike density function is correlated with the amplitude of the angular velocity as seen in figure 10.

Besides spike rate, it has been proposed that the precise timing of spikes can carry much more information than spike rates, which average out spike time variability. One way to use estimate how individual spike times encode information about the stimulus involves taking a spike train and breaking up into small time bins (approximately 1-2 ms wide). Each time bin takes on a binary values, being assigned a 1 if a spike happened during that bin and a 0 if not. The stimulus that led to this spike train is also binned in time windows of the same size. Now the problem becomes one of finding a linear

kernel that takes the discretized spike train and estimates the input stimulus (angular velocity) :

$$\hat{y}_i = \sum_j h_j x_{i-j}$$

where \hat{y}_i is the estimated angular velocity of the pattern at bin i , x is the discretized spike train, and h_j is the kernel we want to find. The most straight forward way to solve for the kernel is to minimize the mean squared error between the prediction (\hat{y}) and the measured stimulus.

$$E = \frac{1}{2} \sum_i (y_i - \hat{y}_i)^2 = \frac{1}{2} \sum_i (y_i - \sum_j h_j x_{i-j})^2$$

where y_i is the measured angular velocity. The kernel that minimizes this error function is obtained by solving the equation

$$\frac{\partial E}{\partial h_j} = 0$$

which is obtained by solving the Wiener-Hopf equations

$$C_J^{yx} = \sum_j h_j C_{J-j}^{xx}$$

with the auto and cross correlations defined by

$$C_k^{yx} = \sum_i y_i x_{i-k}$$

A good stimulus for determining the linear kernel is a white noise distribution of angular velocities poses a problem for this setup. Since each LED column is 3 degrees wide, the pattern must move at least that much within one update for any change to occur in the pattern. So if the pattern is being updated at 200 Hz, the pattern must be moving at $600^\circ/s$ for any pattern motion to take place. At such high angular velocities the response of the neuron may be degraded and may not be linear. Usually these experiments have been done with a monitor, instead of an LED screen, which allows for higher spatial resolution but also stimulates less of the visual field. One way to get around this may be to use low pass filtered white noise as the stimulus. If the stimulus has a correlation time long enough to overcome the filtering effects of the low spatial resolution but short enough to still create a wide bandwidth signal it may be possible to estimate the kernel. At the time of

this writing I had yet gotten this to work. If these problems are overcome, once a kernel is estimated, other angular velocity profiles can be presented to the fly and the estimation errors using the kernel can be compared with an estimate of the stimulus made using the spike density function. Students can also try to see what parameters can be changed to drive the system out of the linear regime and into one where the linear kernel can not accurately estimate the stimulus.

4 Appendix

4.1 Fly Saline

As used in [6] :

110 mM NaCl
5.4 mM KCl
1.9 mM CaCl₂
20 mM NaHCO₃
15 mM TRIS(base)
13.9 mM glucose
73.7 mM sucrose
23 mM fructose

Adjust the pH to 7.2 at 25°C with 1 N HCl.

References

- [1] Hendrik Eckert. Functional properties of the H1-Neurone in the third optic ganglion of the blowfly, *Phaenicia*. *J. Comp. Physiol. A*, 135:29–39, 1980.
- [2] Fred Rieke, David Warland, Rob de Ruyter van Steveninck, and William Bialek. *Spikes : Exploring the Neural Code*. MIT Press, 1997.
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- [4] R. R. de Ruyter van Steveninck, W. H. Zaagman, and H. A. K. Mastebroek. Adaptation of transient responses of a movement-sensitive neuron in the visual system of the blowfly *Calliphora erythrocephala*. *Biol. Cybern.*, 54:223–236, 1986.
- [5] G. D. Lewen, W. Bialek, and R. R. de Ruyter van Steveninck. Neural coding of naturalistic motion stimuli. <http://xxx.lanl.gov/abs/physics/0103088>.
- [6] T. M. Brotz, M. Egelhaaf, and A. Borst. A preparation of the blowfly (*Calliphora erythrocephala*) brain for in vitro electrophysiological and pharmacological studies. *J. Neurosci. Meth.*, 57:37–46, 1995.

5 Figures

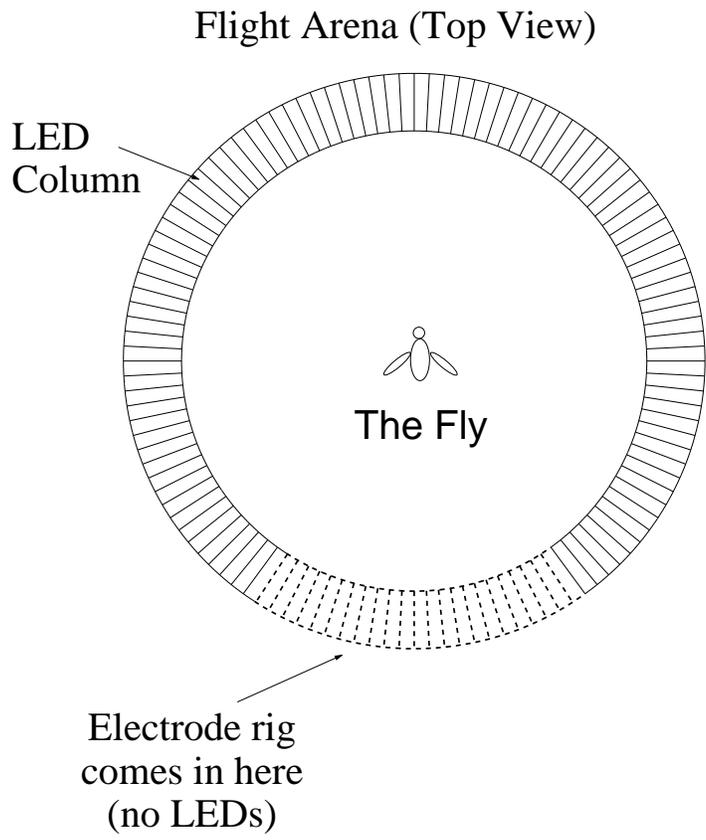


Figure 1: Diagram of the flight arena in which the fly sits. There are 24 LED columns missing behind the fly to allow the electrode rig access.

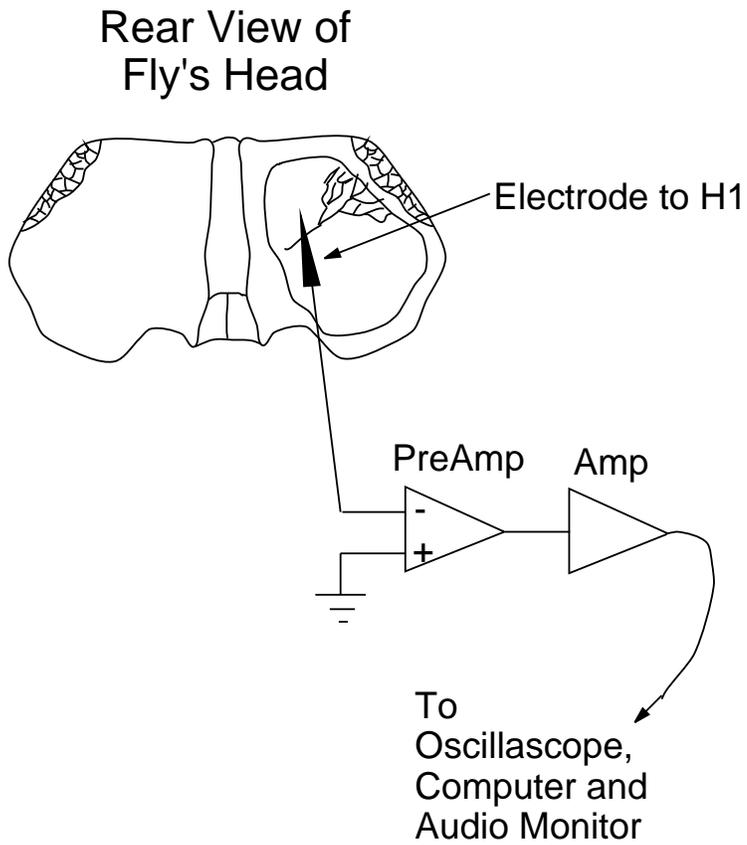


Figure 2: View of the back of the fly's head with an approximate place to begin searching for the H1.

Figure 3: Front panel of the Labview program that controls the LED screen and digitizes and processes the spike train data.

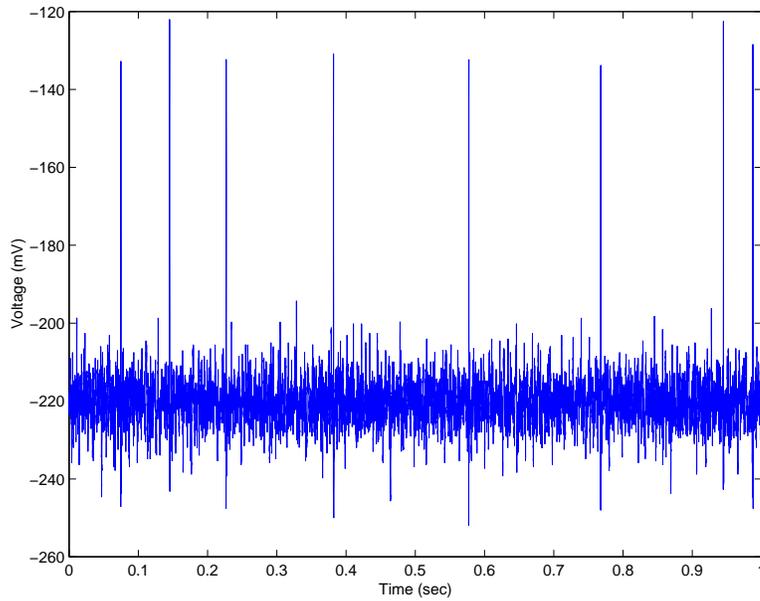


Figure 4: An example of the raw signal as sampled by the computer. This data was taken while the fly was exposed to a $100^\circ/s$ constant angular velocity pattern motion.

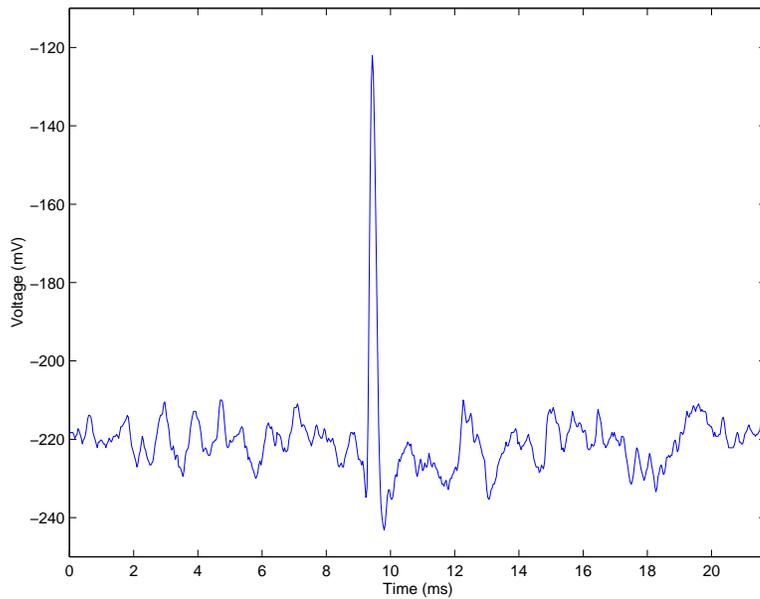


Figure 5: Zoom in on one of the spikes seen in figure 4.

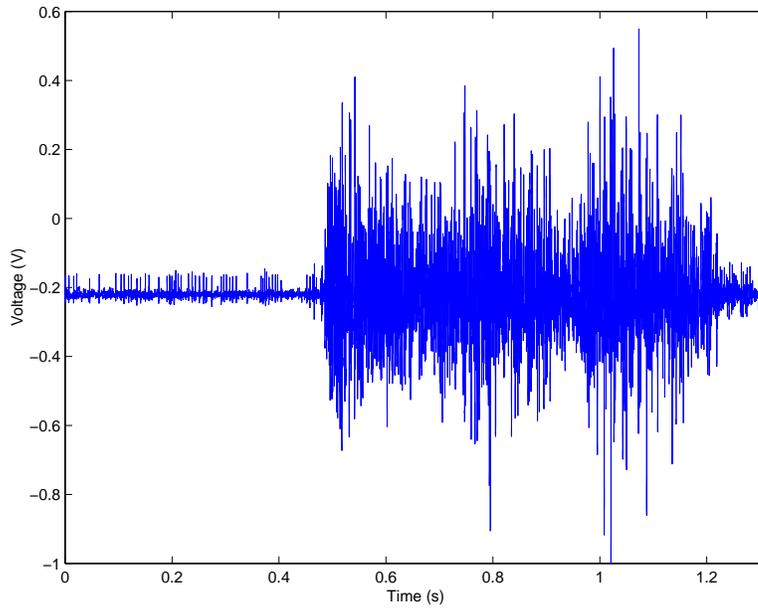


Figure 6: A common source of interference in the measurements is caused by muscle motions. Notice how small the H1 spikes ($\approx 50mV$) are in amplitude compared to the large muscle related activity (beginning at 0.5 sec).

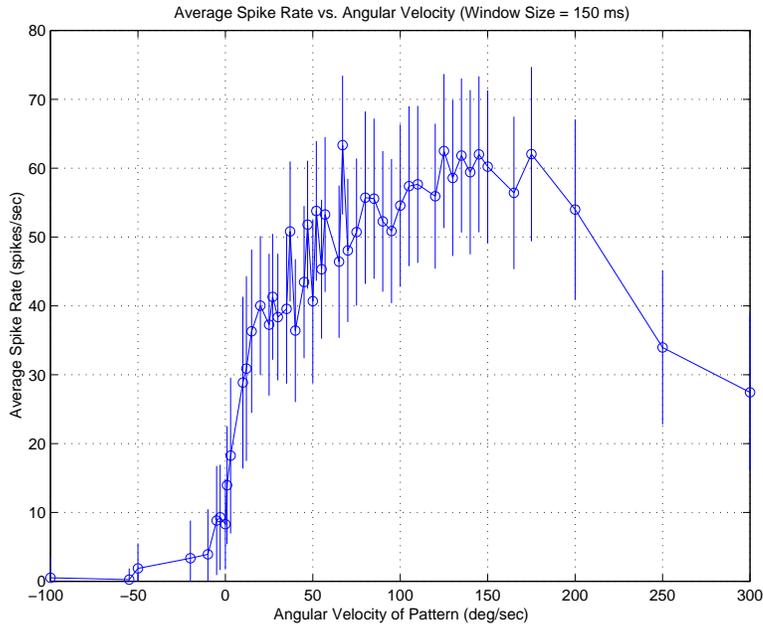


Figure 7: The average spiking rate, calculated using spike counts in 150 ms windows, for various constant angular velocity stimuli. This was done with a low contrast pattern ($m = 0.2$) with a spatial wavelength of $24^\circ/s$. The error bars denote one standard deviation of the spike density function.

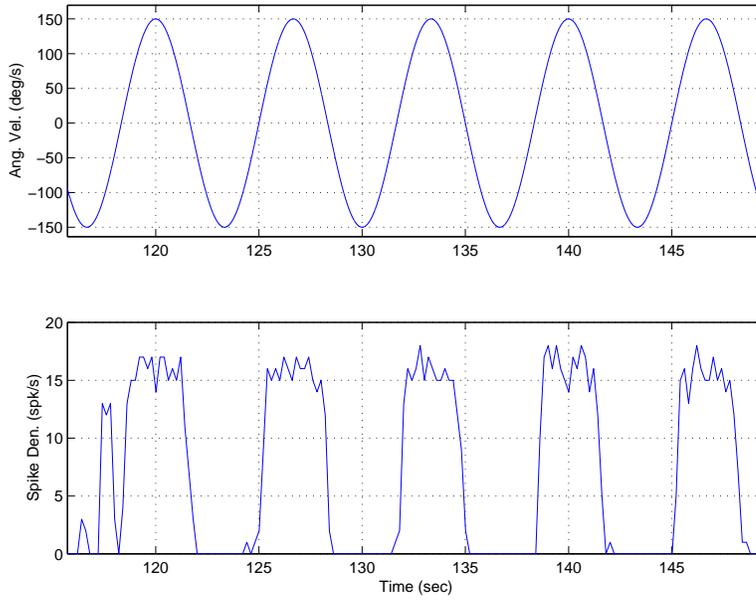


Figure 8: Spike density function response (bottom) of the H1 to a sinusoidal angular velocity stimulus (top) using 200 ms windows. The stimulus moves the pattern in the preferred (positive) and non-preferred directions (negative). Notice how the neuron stops spiking when the pattern is moving in the negative direction. The neuron does not seem to have a large dynamic range for encoding the amplitude of the stimulus which may be due to the large amplitude angular velocity fluctuations driving the neuron out of its linear regime.

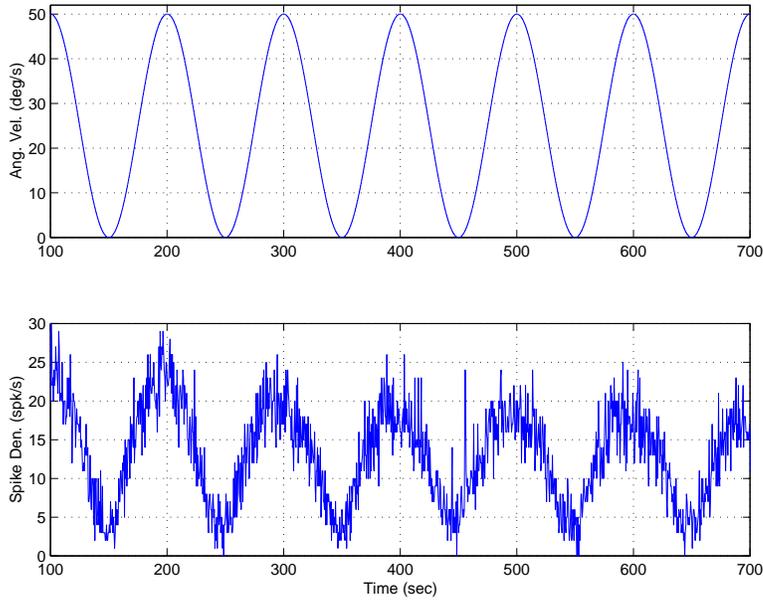


Figure 9: Spike density function response (bottom) of the H1 to a sinusoidal angular velocity stimulus (top) using 500 ms windows. The stimulus moves the pattern only in the preferred direction (positive). Here the spike density function codes the amplitude of the stimulus with higher dynamic range than in figure 8. Note that the maximum angular velocity here is only $50^\circ/s$.

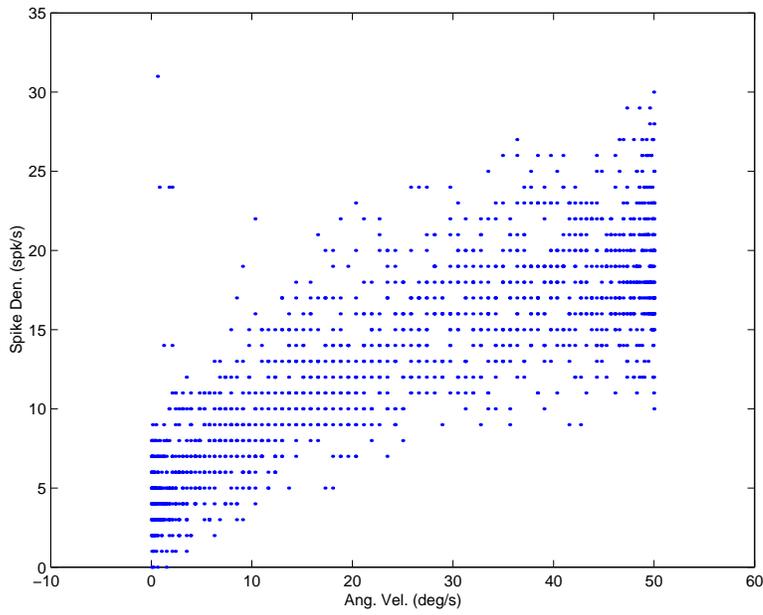


Figure 10: A plot of the spike density versus the angular velocity from figure 9 to illustrate the correlation between the two.