

Horizontal Motion Detection by the Blowfly H1 Neuron

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Introduction

Electrophysiological recordings are often performed in animals which require significant preparation and training. The blowfly, on the other hand, is a readily accessible model for this technique because of its large size and characterized cells. One of these cells in particular, H1, is known to respond preferentially to horizontal visual movement (Eckert, 1980). The goal of this project was to become proficient in basic electrophysiology by targeting H1 and driving its activity through a programmable arena of LEDs.

Methods

Fly Preparation

Three cups of *Calliphora vomitoria*, commonly known as blue bottle flies, were ordered from Forked Tree Ranch, Idaho. This constituted approximately six thousand flies in the pupa stage, which were able to be utilized throughout the course of the project by temperature-controlling hatch time. Two containers were used, one for storing flies for future use and one for storing flies for immediate use. The future use container was simply the box in which the flies arrived, in addition to a petri dish which was kept full with 0.5M sucrose solution. This box was covered with pantyhose to control fly transportation, such that a cut leg of the pantyhose could act as an entry point and could be tied off when not in use. This box was then kept in a refrigerator to prevent rapid hatching of pupae. The second container, for immediate use, consisted of a milk carton with the top cut off, a petri dish of 0.5M sucrose solution, and a pantyhose wrapping. This container was kept in the open to store healthy flies. Flies were transported from the future use to the immediate use container as necessary, however towards the end flies were used straight out of the future use container stored in the refrigerator.

Fly surgeries began by anesthetizing the flies in the freezer for approximately 3.5 minutes and then cutting off the legs and wings. Flies were then fixed to a short, thin piece of metal by melting wax onto the end with a soldering iron and holding the fly gently in place with tweezers until the wax hardened. The metal strip was then flipped over to show the ventral surface of the fly's head and held with an alligator clip. A second drop of wax was placed on this alternate side of the strip, and the proboscis of the fly was pulled down into the wax with tweezers and held until the wax hardened. This served to pull the head of the fly forward and down, exposing the back of the head. The strip was then flipped over, and an incision was made in the back of the head with a scalpel to reveal as much of the brain on a given side as possible. This last step proved unnecessary once recordings were done with quartz electrodes, as mentioned below, and in fact recordings were of much higher quality and fly mortality went away completely when no exoskeleton was removed at all. In these cases, electrodes were simply driven through the exoskeleton in the back of the head.

Stimulus

Two arenas were utilized during this project. The first, donated by Dr. Ralph Greenspan and built by Drs. Michael Reiser and Michael Dickinson (Reiser and Dickinson, 2008), consisted of 44 panels of 8x8 individually controllable LEDs. Unfortunately, though much time was spent troubleshooting the system,

the lack of documentation eventually forced us to move to a different rig. The second rig consisted of 96 columns and 90 rows (about 270 visual degrees) of LEDs which were controllable by column. Matlab scripts written by Jeff Moore and Adam Calhoun were used to deliver stimuli by communicating with the arena through NI-DAQ cards with a 1 MHz baud rate. All stimuli used consisted of intensity-varying horizontal sine waves with a wavelength of 30 degrees of visual angle. The main stimulus used (findH1.mat) moved the sine wave left and right with a period of 2 seconds, and slower and faster stimuli were generated by playing a reduced subset of frames or multiple repetitions of some frames of the main stimulus. One LED was recorded to time-lock recordings to stimulus; however, because of the imprecise method of manipulating stimulus velocity, this time-locking only occurred for the original stimulus speed.

Recording

After preparing the fly, the metal strip containing the fly was fixed on to a holder and placed in the arena. A metal grounding wire was then inserted through the abdomen of the fly. Three types of electrodes were tested for recording: pulled glass pipettes, tungsten electrodes, and pulled quartz pipettes. Of these, quartz pipettes with a tip opening of approximately 2-5 microns proved the easiest to use. These did not require a craniotomy, and instead pipettes were pushed directly through the exoskeleton in the back of the head.

Finding H1 was accomplished by playing the intensity sine wave stimulus and lowering electrode until a unit was found on an audio monitor and oscilloscope. Typically, the best results came from inserting the electrode slightly lateral to the medial segment of the back of the head, and slightly dorsal of the horizontal midline. This is possibly because the fibers from H1 funnel through this region, and as shown it is even possible to record from both H1 neurons in this location. Significant grounding measures were necessary to reduce line noise, the most useful being a grounded lid of aluminum foil over the arena. Additionally, two in-laboratory preamps proved problematic in retrospect after trying a separate preamp supplied by Jeff Moore and the Kleinfeld Lab. Finally, the audio monitor used was slightly faulty and would sometimes cause the recording to become completely flat, likely due to a short. This was solved simply by turning the audio monitor off and then on again.

Analysis

Spike sorting was done through the Matlab program wave_clus (Quiroga et al., 2004). Because the recorded units had a high signal-to-noise ratio and were very distinct, this semi-automated method proved to be quick and reliable. Additional analyses were done through custom Matlab scripts.

Results

Although we managed to find H1 a number of times, the following data was taken from a particular session where both H1 neurons were simultaneously present and well isolated in the recording. This is made clear by the directional selectivity of the cells, where each cell had a clear preferred direction which induced increased firing, and an anti-preferred direction which induced inhibition. The excitation and inhibition produced by horizontal movement were both shown to be distinct from baseline firing (fig 1).

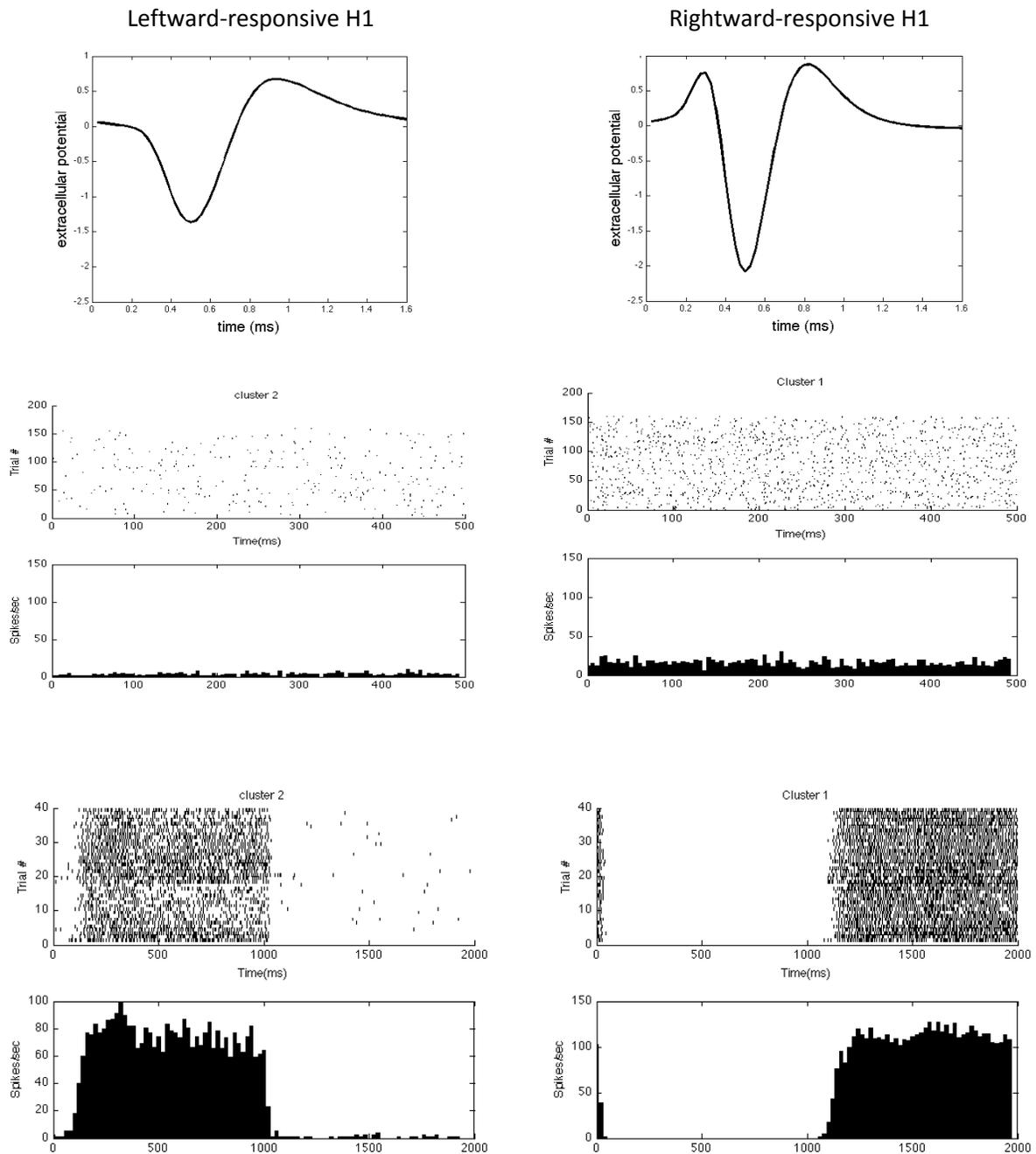


Figure 1: Left column) leftward-responding H1. Right column) rightward-responding H1. Top row) average waveform from isolated unit. Middle row) raster plot and peristimulus time histogram of baseline responses, 500 ms blocks of no visual activity. Bottom row) raster plot and peristimulus time histogram of 30° sine wave of light intensity reversing direction at 0.5 Hz. Each trial consists of one 2 s period.

The tuning of these cells with respect to frequency of moving the stimulus left and right was analyzed through calculating the maximum firing rate in response to stimuli of differing velocities. After fitting with a quadratic to obtain loose approximations, this yielded a maximum response of the rightward H1 to 0.27 Hz and the leftward H1 to 1.5 Hz (fig 2).

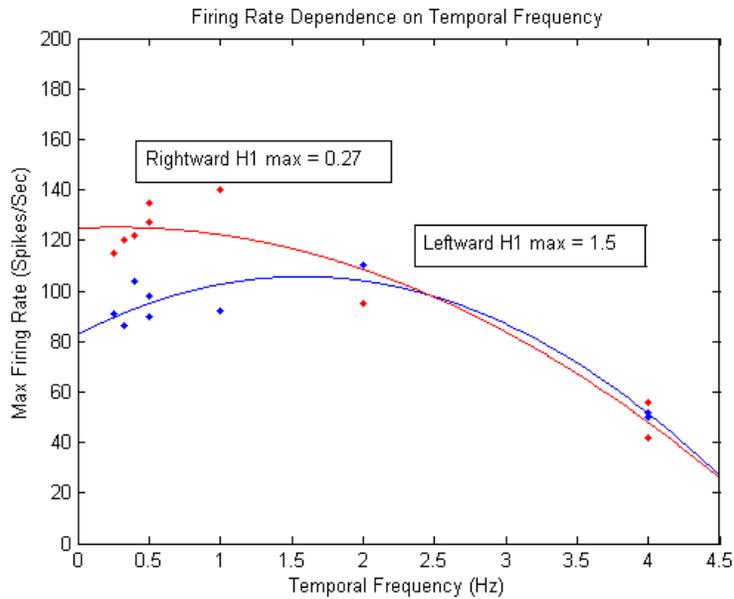


Figure 2: Maximum firing rate as a function of temporal frequency. All stimuli consisted of intensity varying as a sine wave with a wavelength of 30° and direction-reversal frequency as given on the x-axis. Each cell showed roughly similar patterns of response.

The variance of the inter-spike intervals was also analyzed at different speeds. The rightward responding H1 (black in figure 3) decreased average and variance of inter-spike intervals from 0.25 Hz to 0.5 Hz, and became highly inconsistent with response to 2 Hz stimuli. The leftward responding H1 (red in figure 3) showed inconsistency starting with 0.5 Hz, and with 2 Hz became very variable and inconsistent (figure 3).

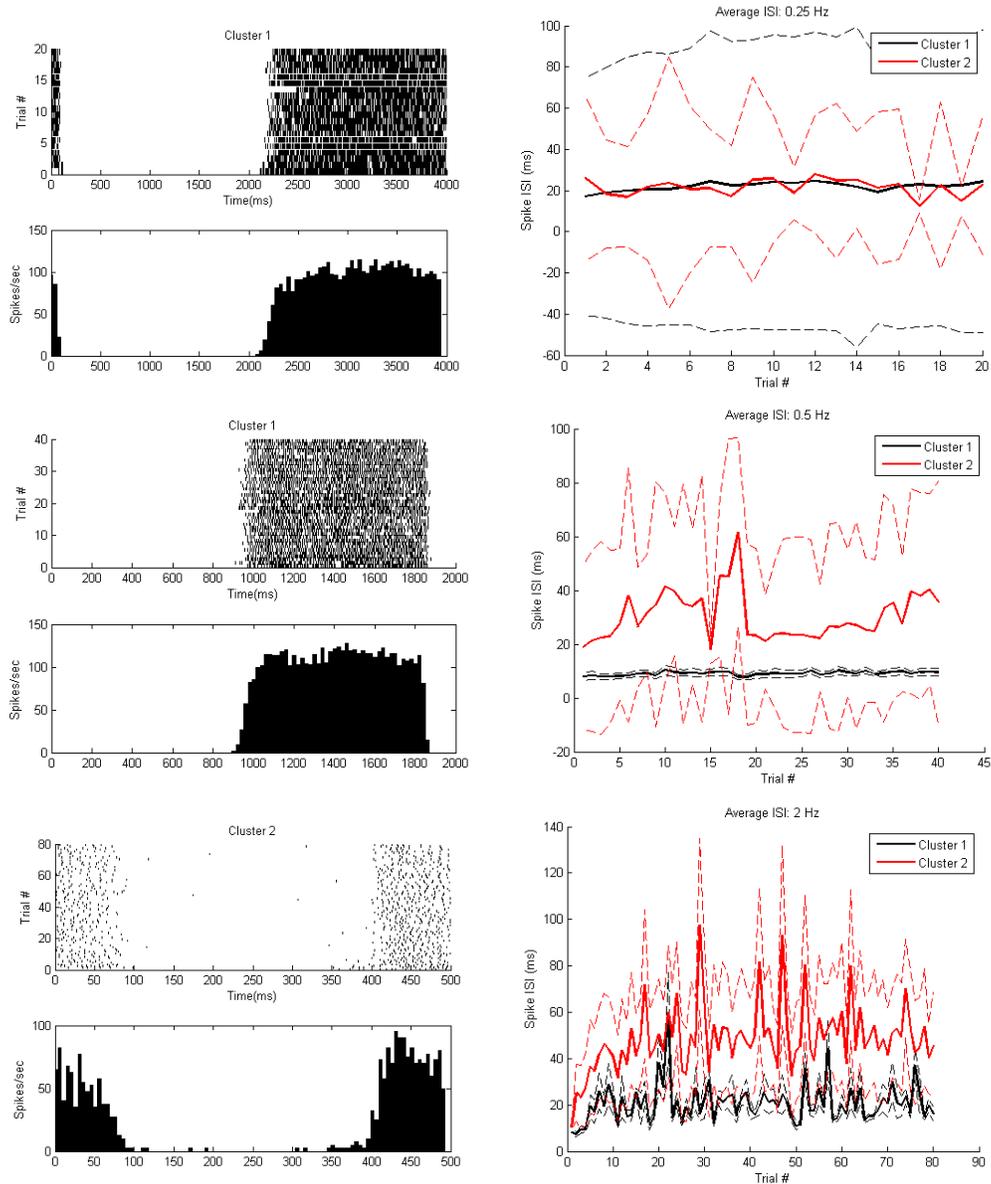


Figure 3: Inter-spike interval analysis. All stimuli consisted of intensity varying as a sine wave with a wavelength of 30° and direction-reversal frequency as given in the title. Left column) peristimulus time histogram examples of the rightward-responsive H1 corresponding to speeds given on the right column. Right column) In each panel, cluster 2 (red) represents the leftward-responsive H1, while cluster 1 (black) represents the rightward-responsive H1. Solid lines show averages, dashed lines give +/- 95% confidence intervals. Top) 0.25 Hz, Middle) 0.5 Hz, Bottom) 2 Hz.

We also looked at a stimulus which faded in or out of maximum intensity over time, to look at the firing rate response to intensity. Because of the limited stimuli we were using during the recording, this was done by looking at a stimulus which faded out going right to look at the rightward-responsive H1, and a stimulus which faded in going left to look at the leftward-responsive H1. On fading out, the firing rate of the cell dipped, rose again, and then decreased in a generally linear manner to zero about halfway through the intensity decrease. The burst of activity after the initial dip here could represent the

contrast sensitivity of the cell, where the gain was lowered to account for decreasing intensity. With fading in, the cell had a rapid rise in activity about halfway through the intensity increase, followed by a generally linear increase, and then slightly more slowly until a steady-state firing rate was reached. Both of these effects show that each cell was minimally responsive to about half the possible intensity of the arena. Also in the case of both cells, a short burst of action potentials was fired when the stimulus suddenly turned off or on, regardless of motion direction (figure 4).

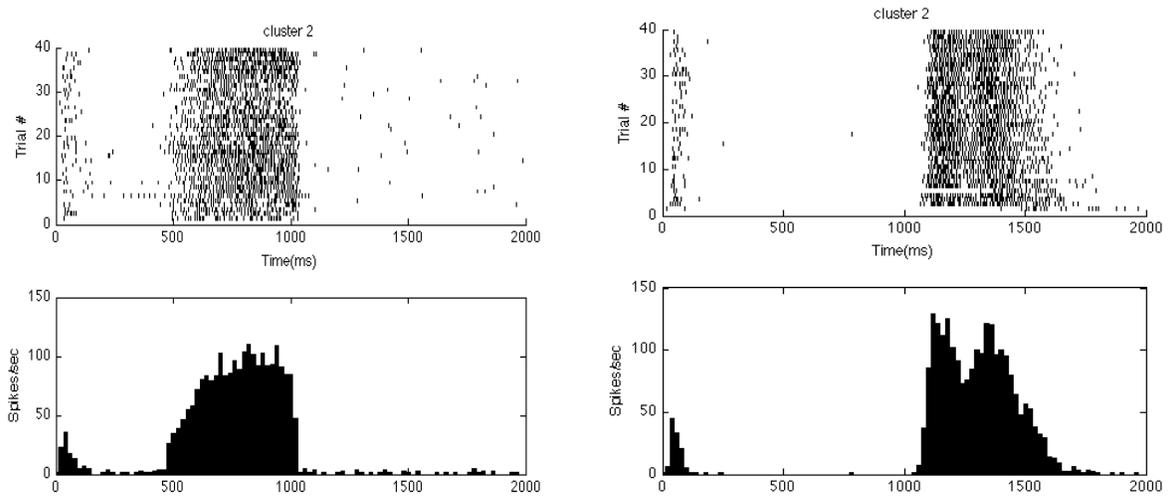


Figure 4: Response to graded intensity. Stimuli consisted of intensity varying as a sine wave with a wavelength of 30° , where maximum intensity was raised or lowered linearly during one direction of movement. Left column) leftward-responding H1 with stimulus which faded in during leftward movement. Right column) rightward-responding H1 with stimulus which faded out during rightward movement.

On a side note, other visual cells were occasionally encountered which did not preferentially respond to horizontal direction of motion (figure 5).

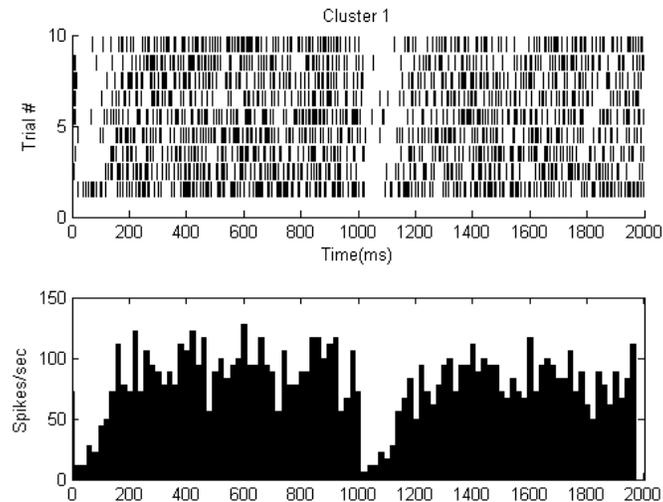


Figure 5: Visually responsive non-H1 cell. Raster plot and peristimulus time histogram of 30° sine wave of light intensity reversing direction at 0.5 Hz. Each trial consists of one 2 s period.

Discussion

Through troubleshooting stimulus arenas, fly surgeries, and recording techniques, we were eventually able to obtain and record reliably from H1. By recording simultaneously from both H1 neurons in the fly, we were able to compare the firing rate, frequency tuning, inter-spike interval variance, and graded response between each cell in a single animal. While each H1 showed generally similar properties, there were certain differences observed. Compared to the leftward-responsive H1, the rightward-responsive H1 showed higher baseline activity and lower velocity tuning. The rightward-responsive H1 also showed reduced inter-spike interval variability with increasing temporal frequency from 0.25 Hz to 0.5 Hz, while the leftward-responsive H1 both retained a high variability and had a higher variability of trial-by-trial average. The rightward-responsive H1 also showed a bump in activity to a decreasing intensity stimulus while the leftward-responsive H1 showed a rapid onset at a time slightly different from the increased activity of the opposite neuron, both possibly due to contrast detection thresholds and gain changes in activity. Despite these differences, each cell had a strong excitation towards preferred movement and inhibition towards anti-preferred motion, each cell had comparable response latencies to direction change, and each cell demonstrated a generally linear response of firing response to light intensity.

References

- Eckert H (1980) Functional properties of the H1-neurone in the third optic ganglion of the blowfly, *Phaenicia*. *Journal of Comparative Physiology A: Neuroethology*
- Quiroga RQ, Nadasdy Z, Ben-Shaul Y (2004) Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering. *Neural Comput* 16:1661–1687
- Reiser MB, Dickinson MH (2008) A modular display system for insect behavioral neuroscience. *Journal of Neuroscience Methods* 167:127–139