# Functional properties of the blowfly H1 neuron

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**Abstract** It is known that all species of fly possess two H1 neurons, originating in each lobula plate and terminating in the contralateral plate. These neurons exist within a class of directionally-selective neurons in the fly visual system, and have been studied quite extensively due to their recording accessibility and the simplicity of their receptive fields. Here we sought to characterize H1's response properties through a simple setup which allowed us to parametrically vary aspects of a fly's visual space while we recorded extracellularly from an H1 neuron. Consistent with the literature, we find that H1 responds strongly to movement in the posterior-to-anterior direction. Additionally, we find that it is sensitive to different movement speeds, and suggest that it is more specifically tuned to strong boundaries moving in visual space, rather than simply movement.

## Introduction

It is known that all species of fly possess two H1 neurons, each originating in the lobula plate ipsilateral to the eye it receives input from and terminating in the contralateral plate. These cells were functionally and anatomically characterized as early as the 1970s (Hausen 1976), and have been extensively studied since due to their accessibility and receptive field simplicity. They exist among a larger class of motion-sensitive cells in the fly visual system, and their function has primarily been identified as coding for horizontal forward motion (i.e., in the posterior to anterior direction) (Borst & Helmstaedter 2015). In this report we sought to characterize H1's response properties through a simple experimental setup that allowed us to parametrically vary aspects of a fly's visual space while we recorded extracellularly from an H1 neuron.

We demonstrate that H1 is maximally responsive to stimuli moving in the posterior-to-anterior direction. Additionally, we quantify H1 response as a function of stimulus movement speed, and test different stimulus patterns in order to better assess its spatiotemporal tuning.

## Materials and Methods

### Animal Preparation

Blowflies (members of the *Calliphoridae* family) were anesthetized using $CO_2$. During anesthesia, their wings were clipped and they were secured lengthwise within the tip of a clipped transfer pipette, such that the head came out of the tip and the body was immobilized. Hot wax was used to secure the front of the head to the chest, such that the back of the head was easily accessible. Here we cut a window using an opthalmic scalpel and removed fat deposits within using a KimWipe, exposing the lobula plate containing the telodendron of the contralateral H1. To ensure longevity of the

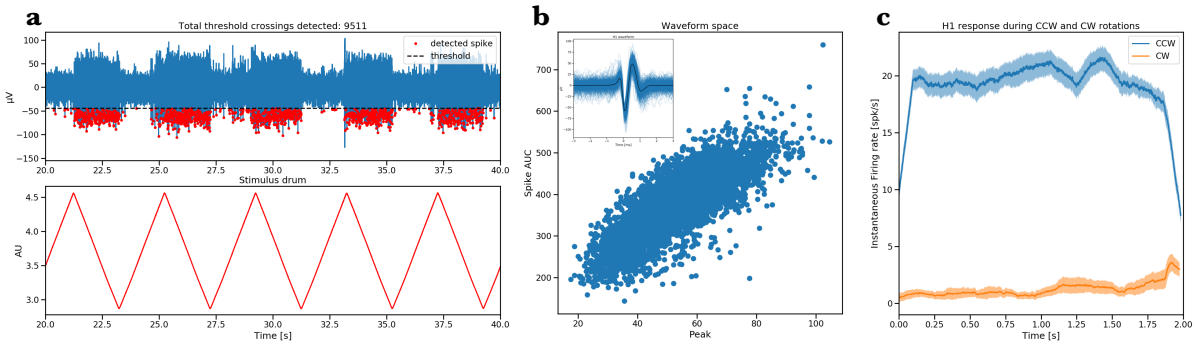


**Figure 1. Identification of the H1 neuron.** An oscillating drum with a constant spatial frequency was used to identify the H1 neuron. **a.** *(Top panel)* Extracellular potential recorded while the fly viewed a an oscillating constant spatial frequency *(bottom panel)*. **b.** Waveforms in peak-spike AUC space, showing H1 is easily identifiable as a single cluster. *(Inset)* Example and averaged waveform of H1. **c.** Averaged firing rate response of H1 to epochs of counter-clockwise stimulus rotation (blue) and clockwise stimulus rotation (orange). Error bars are 95% confidence intervals.

preparation, the exposed chamber was filled with Brotz saline (Brotz et al., 1995).

## Experimental setup

The prepared fly was positioned centrally on our recording bench, such that a rotating drum display could be positioned immediately in front of the eye contralateral to our recording window. This drum was controlled by a servo motor connected to a computer, such that we could precisely control its rotational speed and direction. A reference tungsten electrode was advanced into the edge of the recording window, such that it came into contact with the saline. Using a Sutter micromanipulator, a tungsten recording electrode was advanced into recording chamber, roughly centered in the cuticle window.

## Recording

Analog extracellular potential signal was amplified using an AM-3000 by a factor of 1000 and filtered online with a high pass filter of 300 Hz and a low pass filter of 3 kHz.

Drum position and extracellular potential signals were digitized at 10 kHz and sent to a computer. Extracellular signal was also routed to a speaker such that we could listen to signs of H1 once we began to advance the recording electrode into the brain. For finding H1, we set the stimulus drum to display a constant spatial frequency strip (see **Figure 3a**, bottom panel) and to oscillate at a period of 3.5 seconds. We then advanced the recording probe into various parts of the exposed lobula plate until we found H1.

## Analysis

Extracellular signal was demeaned and thresholded at -3 times its standard deviation for spike detection. 6 ms windows were taken around each threshold crossing in order to capture the waveform. Duplicate spikes were eliminated and several features (e.g. AUC, peak and trough, voltage potential rebound AUC, etc) were computed for every waveform. For analyses characterizing the time-varying instantaneous firing rate (IFR), binary spike trains were convolved with a boxcar function 200 ms in length to produce a continuous firing rate signal. Time-collapsed firing rates were computed by simply dividing the number of spikes observed in an epoch by the number of seconds in that epoch. All analysis was performed with custom scripts written in the Julia language, version 1.1.1.

## Results

### Identification of the H1 neuron

H1 was clearly identifiable by a robust response to the oscillating constant spatial frequency strip stimulus (**Figure 1a**). As expected, counter-clockwise (CCW) rotation of the drum (resulting in the appearance of posterior-to-anterior movement) caused large increases in spiking activity, whereas clockwise (CW) rotation drastically reduced H1 spiking. Our SNR and thresholding was good enough to clearly characterize the extracellular waveform of H1 (**Figure 1b**).

In order to better understand the temporal dynamics of H1, we computed its instantaneous firing rate across time by convolving its binarized spiking data with a 200 ms boxcar function. This continuous signal was split into CW and CCW epochs and averaged to find the trial-averaged H1 response (**Figure 1c**). CCW stimulus presentation clearly produces a robust response.

### H1 firing rate decreases with stimulus presentation speed

With H1 located, we sought to characterize its tuning as a function of stimulus presentation speed. Using the same constant spatial frequency strip as before, we progressively increased the speed of rotation from a period of 20 seconds to a period of 3 seconds in 8 evenly spaced intervals. Plotting the instantaneous firing rate of each speed suggested an inverse relationship between speed and firing rate (**Figure 2a**). Ignoring temporal dynamics, we computed the time-collapsed firing rate for each speed by simply dividing the number of spikes observed by the length of the epoch. While this method does not allow one to estimate time-varying dynamics, it is unbiased by choice of kernel function and thus well suited for simply assessing firing rate as a function of speed. As with the previous analysis, we find that H1

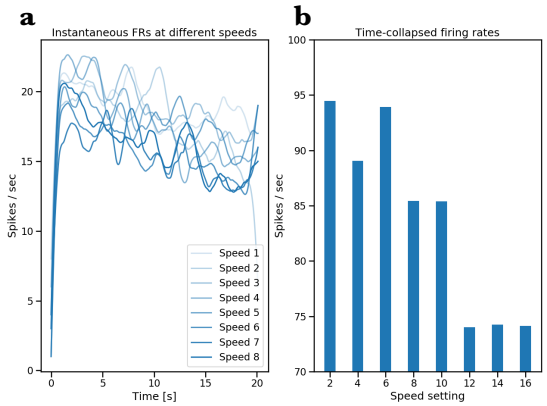


**Figure 2. H1 responses as a function of presentation speed.** The constant spatial frequency strip was presented at varying rotational speeds and spikes were recorded. **a.** Instantaneous firing rates for each rotational speed. **b.** Time-collapsed firing rates (by simply counting the number of spikes and dividing by the trial length) of the same trials.

firing rate decreases with stimulus presentation speed (**Figure 2b**). More specifically, we find that there is a sharp drop-off once the movement speed hits a period smaller than about 5 seconds. Further experiments should extend this analysis to faster and slower speeds.

### H1 is strongly tuned to hard boundaries

Having characterized some of the response properties of H1 to a stimulus with a constant spatial frequency, we sought to more specifically understand what aspects of the visual stimulus besides speed affect its firing. We experimented with three other kinds of stimuli, two of which parametrically varied the contrast and the size of the stimulus, respectively, and a third which displayed a random pattern (**Figure 3**). As we'd previously observed a robust response to CCW rotation with a period of 3.5 seconds, we set each strip to continuously rotate at that period for 7 rotations in order to estimate trial-averaged time-varying firing rates for

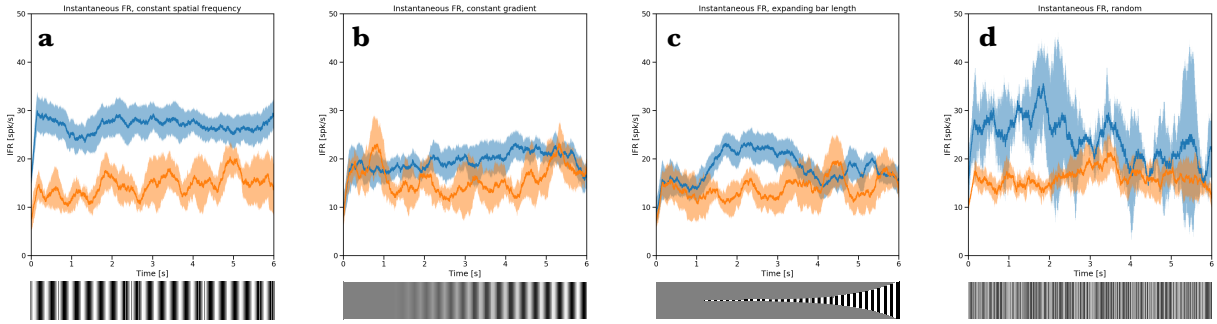


**Figure 3. H1 responses to varied stimuli.** H1 was continuously presented with each of the four stimuli, in order to assess its tuning. Trial-averaged firing rates were computed for counter-clockwise (blue) and clockwise (orange) presentations. **a.** Responses to the constant spatial frequency strip, used in figures 1 and 2. **b.** Responses to the contrast gradient strip. **c.** Responses to the expanding bar length strip. Note the increase in firing rate locked to the appearance of the bar. **d.** Responses to the random stimulus strip.

each stimulus. For comparison, we performed the same experiments using the CW rotation. As with previous figures, H1 responds significantly more strongly to CCW presentation of the constant spatial frequency strip (**Figure 3a**) than to CW rotation.

The contrast gradient strip (**Figure 3b**) shows weak ramping in response to the increasing contrast in CCW rotation, but more data is needed to demonstrate that this is significantly more than the CW presentation.

The expanding bar length strip (**Figure 3c**) shows significant modulation in firing rate for CCW rotation as soon as the stimulus comes into view, but it decays back a couple seconds after.

The random stimulus (**Figure 3d**) produces a strong but varied response for CCW rotation. Qualitatively, peaks in response seem to appear whenever there is a lower-frequency change in the statistics of the strip (such as when we go from a bulk darker region to a lighter region, or vice versa).

CW rotation for all stimuli strips is comparable.

## Discussion

To summarize, we sought to functionally characterize the tuning of the identified H1 neuron in *Calliphoridae* through a setup which allowed us to parametrically vary different aspects of visual space. We know that H1 is tuned to horizontal posterior-to-anterior movement, but how does this scale with movement speed? With the size of the visual object? We hope that we've been able to answer some of these questions, in part.

Together, our results suggest that H1 is optimally tuned to movement at a certain speed (perhaps matching object speeds experienced naturally during flight), and that it is strongly tuned to strong boundaries moving in visual space, rather than simply any kind of horizontal forward movement.

## Literature Cited

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