

Fly Vision – Virtual Reality and Electrophysiology

Orientation Selectivity of H1 Neuron for Horizontal Linear Motion

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Introduction:

Blowflies (scientific name *Calliphora erythrocephala*) possess multiple classes of visually responsive neurons. Of particular interest to this study is the H1 neuron, which responds to large-field horizontal motion by integrating responses from multiple horizontally selective (HS) cells near the retina [1] and generating action potentials at larger frequencies for movement in the preferred direction [2]. In this study, we aimed to test for the response characteristics of the H1 neuron, in particular its direction selectivity.

Past reports have focused largely on H1 responses to rotational motion, presenting flies with moving vertical bars and gratings that rotated horizontally in its visual field around a cylindrical LED array. In this study, we aimed to apply a more natural visual stimulus: linear motion. Because freely moving flies experience more translational than rotational motion during their flight paths, we hypothesized that responses of H1 neurons should have a large component that responds to linear motion of a particular direction. Therefore, we devised a stimulus that could simulate linear motion in arbitrary directions with the same LED array setup, which would permit us to investigate H1 direction selectivity, something not possible with simple rotating stimuli.

Methods:

Stimulus:

The blowflies were immersed in a virtual reality arena, which simulated moving patterns of light by successively flashing on and off adjacent LED columns arranged in a cylinder, with the fly at the center of the arena. Each LED column could be turned on or off independently, and the resulting image could be updated at a maximum frequency of 1 MHz. In order to create graded light intensities, we randomly flickered each LED column on and off with the probability of being on proportional to the desired intensity of light. Thus, each “frame” was composed of 1000 time steps of flickering, allowing the virtual motion stimulus to be presented to the fly at 1000 fps, well above the flicker fusion rate of 250 Hz for *Calliphora* [6].

We presented two main types of visual stimuli to the flies: the standard rotating sinusoidal grating and “linear” moving sinusoidal gratings on two virtual parallel walls moving past the fly at a particular orientation. The standard rotating sinusoid varied its light intensity relative to visual angle, whereas the linear stimulus varied light intensity relative both to position along the wall and to virtual linear distance from the center of the arena, according to the following equations:

$$x = R \cot(\theta)$$

$$d = R \csc(\theta)$$

$$I = \frac{\sin(2\pi(x/\lambda + t/T)) + 1}{2d^2}$$

where x is the position along the virtual wall relative to its closest pass to the fly at the center of the arena, d is the Euclidean distance from the fly at the center of the arena to a point on the virtual wall, θ is the deviation in visual angle from the “forward” heading, R is the radius of the cylindrical arena (set to 1 so that distances are measured in radians), t is time, λ is the spatial period of the virtual grating (set to $\pi/3$), T is the temporal period (set to 0.5 seconds), and $I \in [0,1]$ is the relative intensity at a given visual angle θ (see Figure 1b for summary).

The 96 vertical LED units comprised 288° of total visual space ($8\pi/5$ radians), with each LED bar taking up 3° of visual angle. Because of this, and because the angular frequency of the grating increases asymptotically with virtual distance to the wall, we took the mean integrated intensity for all points within each 3° -increment of visual angle as the intensity of the respective LED columns, thus avoiding artifacts.

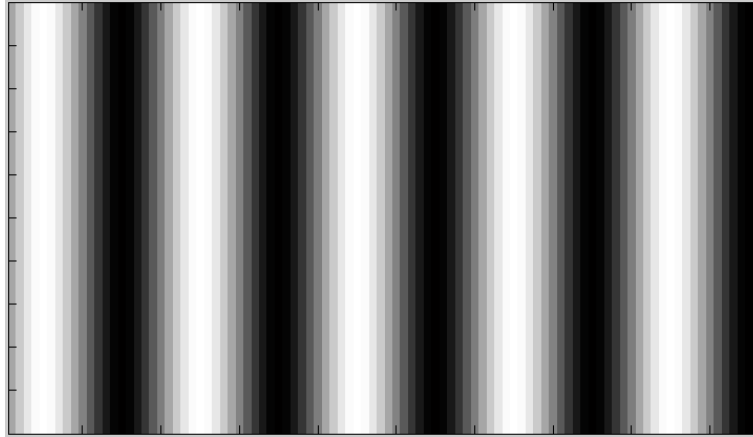
Electrophysiology:

While presenting these visual stimuli to the fly, we made electrophysiological recordings from its H1 neuron. We used quartz microelectrodes with 3-5 μm tips to probe the fly’s brain from the back of the head. The electrodes contained standard fly saline (110.0 mM NaCl, 20.0 mM NaHCO, 15.0 mM TRIS, 13.9 mM glucose, 73.7 mM sucrose, 23.0 mM fructose, adjusted to pH 7.2 at 25°C with 1N HCl [3]), which together with the sharp electrode provided a high-impedance electrical interface between the extracellular space in the fly’s brain and the electrode wire. The ground wire was placed in the fly’s back, and both fed into an amplifier and from there into a digital oscilloscope. Output was also sent to speakers so that action potentials could be easily heard during the probing procedure.

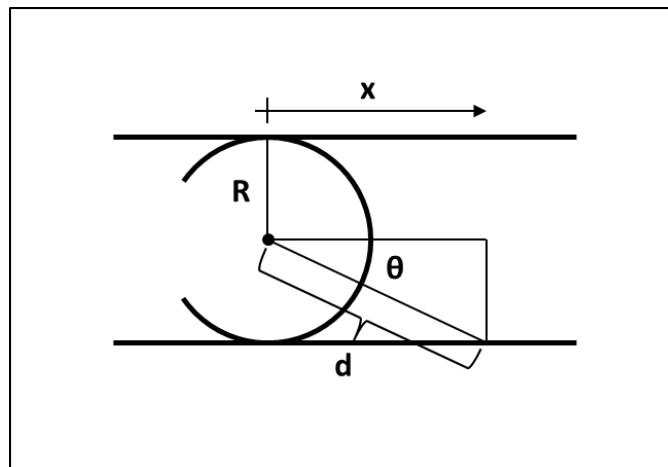
Finding H1 Cell:

Because H1 neurons are typically relatively easy targets in terms of the action potential signal they generate, we were able to use trial-and-error probing with the quartz electrode tip to find the H1 cell. This had to be done with the rotating stimulus playing (from Figure 1a), which produces a signature electrophysiological response in the H1 neuron (see Figure 2). We simply poked the electrode tip in and out of the back of the fly’s head with the stimulus on until we heard action potentials on the speakers. The H1 cell would fire strongly with the grating rotating in the preferred direction and weakly with it rotating in the opposite direction, so the rotating stimulus would shift between directions on each trial, and the changing responses could be heard within each trial.

a)



b)



c)

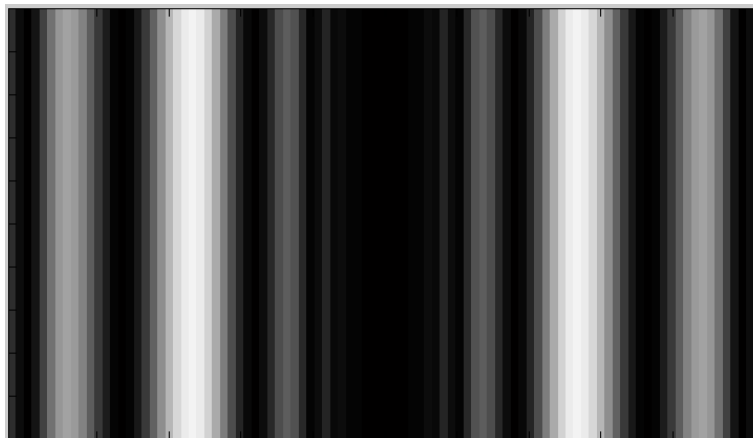


Figure 1. (a) sinusoidal grating used for rotating visual motion; (b) parameters used for relating visual angle in arena to positions of virtual LED walls; (c) sinusoidal gratings on parallel walls projected onto cylindrical LED array, appearing linear from center of arena

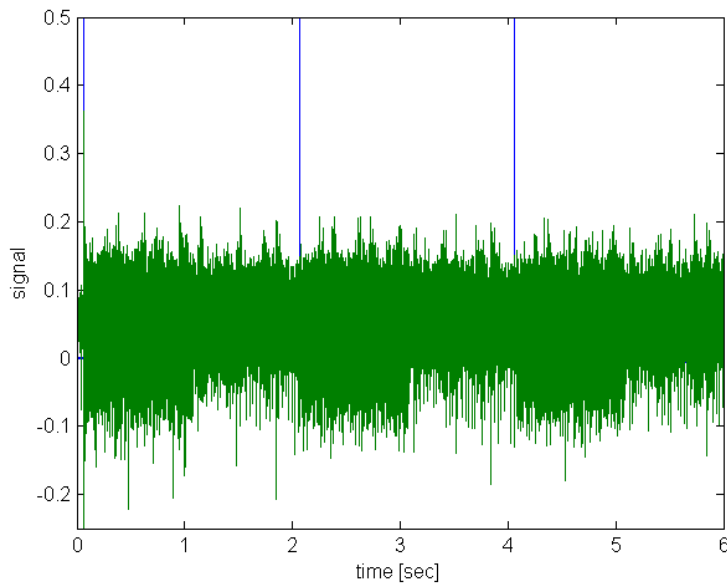


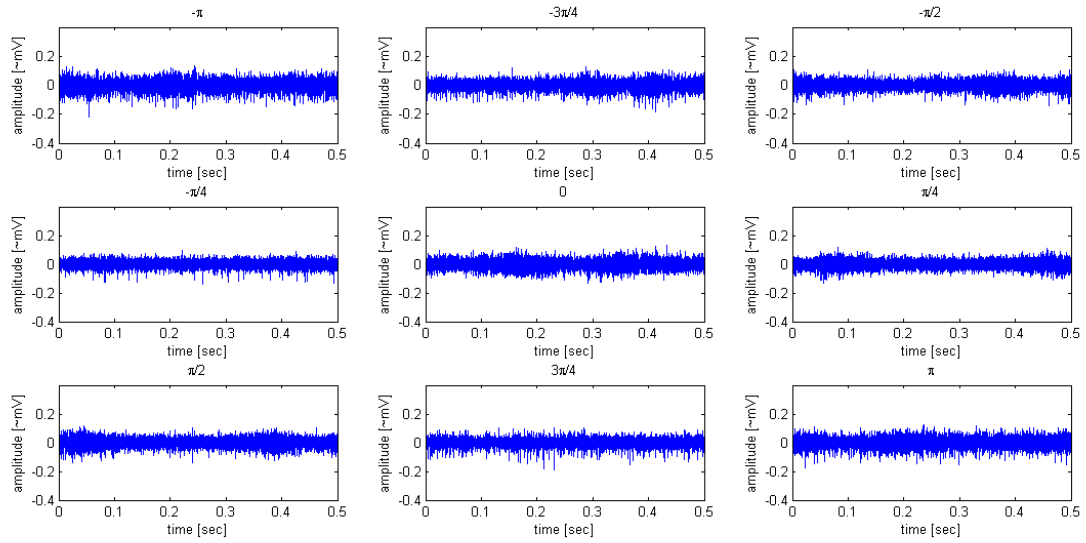
Figure 2. Response of H1 neuron to alternating rotating sinusoid: blue lines mark new stimulus cycles, green signal is proportional to measured voltage. Increased spiking apparent in first half of each cycle due to sinusoidal grating moving in preferred direction of the recorded H1 cell.

Trials:

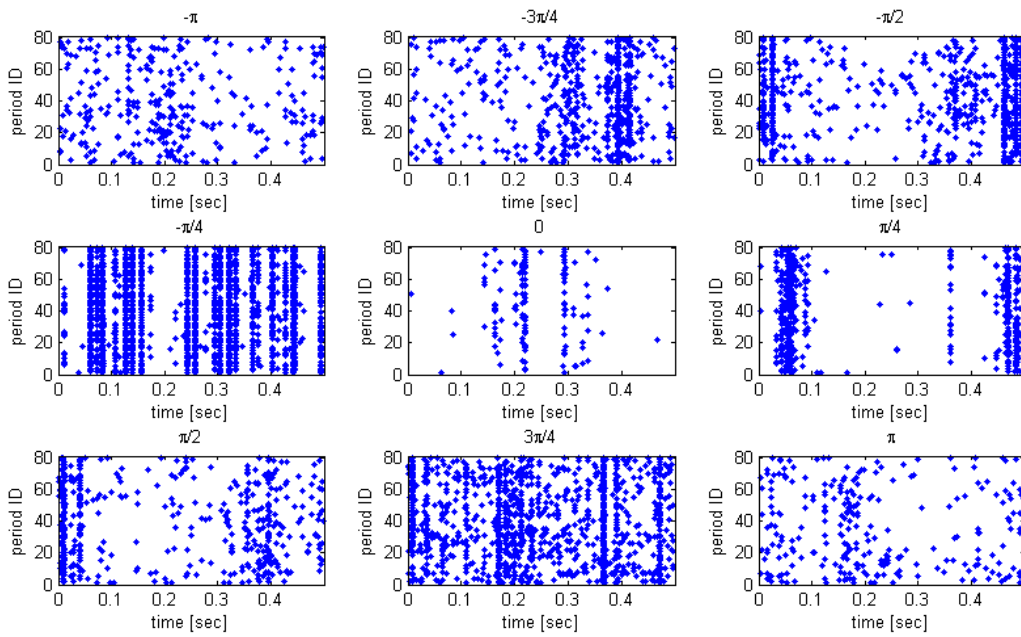
After the fly's H1 neuron was found, we prepared to switch to the virtual wall stimulus. We ran the stimulus for nine forward heading directions (-180° , -135° , -90° , -45° , 0° , 45° , 90° , 135° , and 180° , representing the direction the fly would see that it is moving in from its position and orientation), for multiple periods ($n = 80$) at each orientation. We measured the changes to voltage and interpreted them as changes in the spiking activity of the H1 neuron.

Results:

Figure 3 below shows sample trials recorded from the same cell for all nine directions, including both the voltage waveforms from single trials and the spike rasters for all 80 trials in each direction. Voltage waveforms were passed through a 3rd-order Butterworth filter between 600 and 6000 Hz to get rid of noise. Spikes were counted at all the time points when voltage dipped below four standard deviations from the mean.



a)



b)

Figure 3. (a) sample filtered waveforms at each of the nine stimulus directions; direction of stimulus given in radians above each plot; (b) spike rasters for all 80 trials of each stimulus direction

Note how some “spikes” occur at extremely consistent times within each period. This is likely some sort of artifact, the cause of which is unknown. Figure 4 below shows a histogram of mean firing rate in each of the nine stimulus configurations.

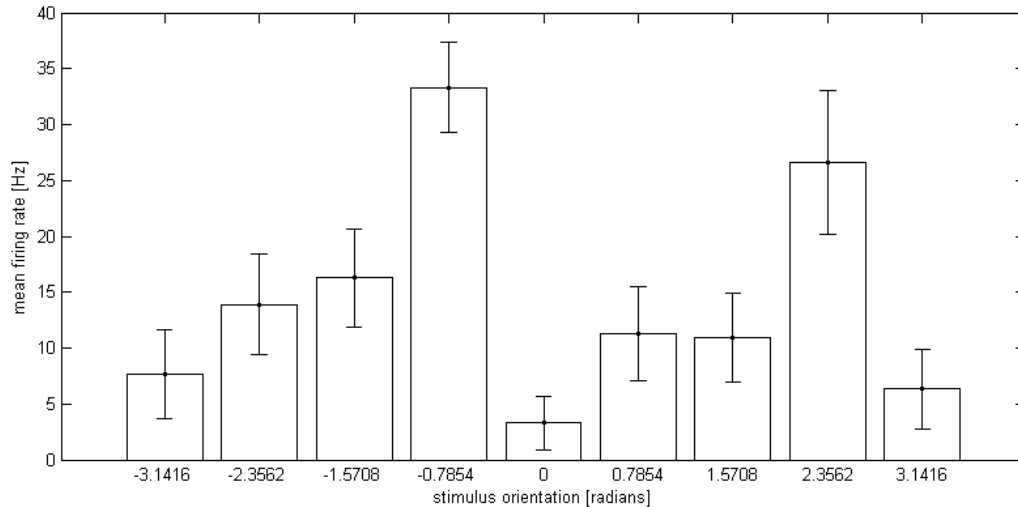


Figure 4. Mean spike rate histogram for all nine configurations; error bars show standard deviation from mean ($n = 80$).

Discussion:

Of note from Figure 4 is that the recorded H1 neuron seemed to respond very strongly to linear motion at both -45° and 135° , which are anti-parallel to each other, and relatively weakly to all other stimulus directions. If these recordings accurately reflect activity of the H1 neuron, it would indicate that it is more orientation-selective than direction-selective. However, there were numerous difficulties encountered in the experiment that would bring this conclusion into question.

Setbacks:

First of all, a number of setbacks over the weeks that recordings were attempted prevented quality recordings from H1 neurons. For instance, for several weeks, the flies we used seemed to be of poor quality, behaviorally speaking, and the length of their refrigerated hibernation and poor lighting conditions during maturation may have contributed to developmental defects. Additionally, we encountered a number of problems with both speakers and amplifiers, which prevented accurate electrophysiological recordings. Another problem early on may have been that we used standard leech saline (SLS) (85 mM NaCl, 4 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 10 mM Hepes, pH 7.4 adjusted with NaOH [4]) rather than fly saline (described above). It was not until all of these issues were addressed (new flies, proper fly saline, new amplifier and speaker) that we got any recordings, and those only from a single fly.

Potential Sources of Error:

There were also several sources of noise in the recordings that might have been indicative of severe problems. Figure 5 shows the power spectral densities (PSDs) for all trials and stimulus directions, with red arrows pointing out noisy artifacts encountered, including low-frequency buzzing (maroon/horizontal arrows) and regular interval noises (red/vertical arrows).

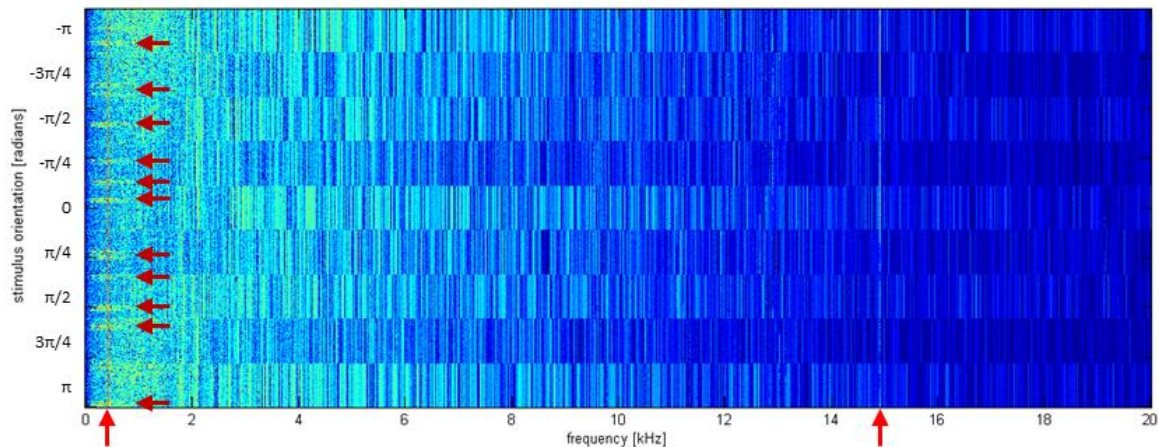
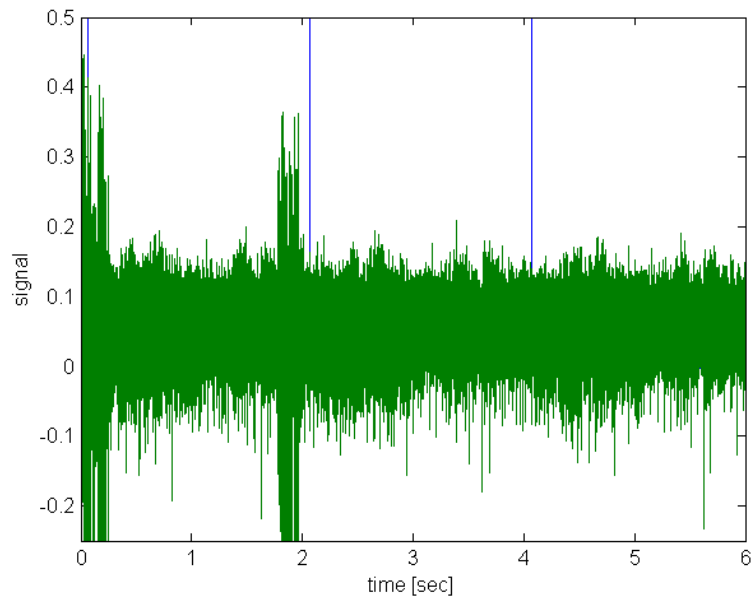


Figure 5. PSDs for all trials and all virtual wall stimulus directions; maroon/horizontal arrows show low-frequency buzzing artifacts; red/vertical arrows show regular-interval artifacts.

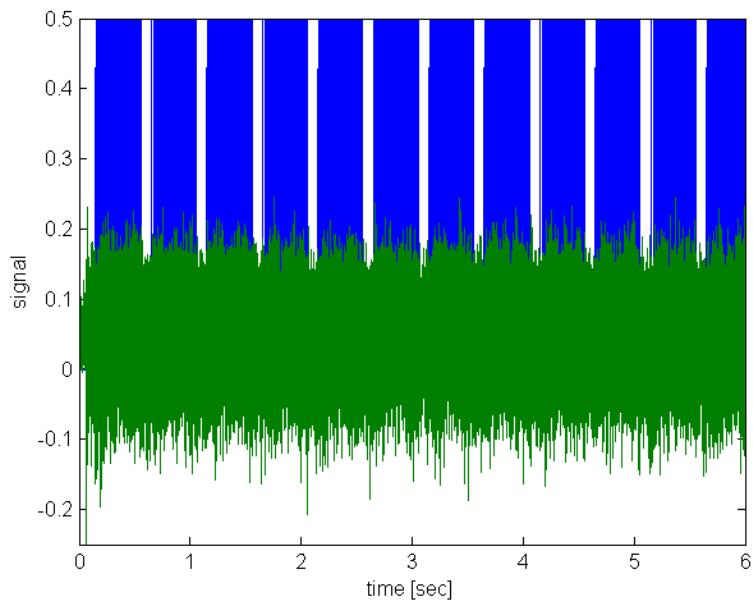
The vertical red arrows point out anomalous frequencies encountered in all trials and all stimulus orientations. These could help to account for the regular “spike” times that can be seen across trials in Figure 3b.

Finally, it is highly likely that the quality of the cell being probed degraded with time. Compare the pattern of response from the recorded cell in Figure 2 with that in Figure 6a, recorded at a later time. The difference in response between preferred and non-preferred direction of the rotating stimulus has significantly diminished with time. It may be possible that the later recordings used for the virtual wall stimuli could have detected spikes largely from neurons other than the original H1 cell that dominated the waveform in Figure 2. This clearly would affect the interpretation of results.

Finally, Figure 6b reveals a highly anomalous source of noise exclusive to the virtual wall stimulus. The blue vertical lines are supposed to indicate trial onset, but these become very noisy in the virtual wall case, especially when compared to the very clean trial onset signal from the rotating stimulus recordings. The cause of this discrepancy is unknown, but comparing the MatLab code that generated each of the types of patterns might elucidate the source of the error, even though the trial onset signals were not explicitly coded for. This may be related to the high-frequency noise visible in the PSDs in the previous figure.



a)



b)

Figure 6. (a) response to rotating stimulus, showing corruption of cells over time from those in Figure 2; (b) trial onset (blue) and voltage (green) recordings during virtual wall stimulus; trial onsets become noisy and occur at the onset of each period rather than each trial

Future work can use the lessons learned here to obtain cleaner recordings. What may also be of interest would be to modify the arena to test H1 responses to concentric stimuli coming in from arbitrary directions in 3D space.

References:

- [1] Borst, Egelhaaf (1990). Direction selectivity of blowfly motion-sensitive neurons is computed in a two-stage process. *Proceedings of the National Academy of Sciences USA*, 87: 9363-9367.
- [2] Borst, Haag (2002). Neural networks in the cockpit of the fly. *Journal of Comparative Physiology A*, 188: 419-437.
- [3] Brotz, Egelhaaf, Borst (1995). A preparation of the blowfly brain for in vitro electrophysiological and pharmacological studies. *Journal of Neuroscience Methods*, 57: 37-46.
- [4] Günzel, Schlue (1996). Sodium-magnesium antiport in Retzius neurones of the leech *Hirudo medicinalis*. *Journal of Physiology*, 491(3): 595-608.
- [5] Hausen (1982). Motion Sensitive Interneurons in the Optomotor System of the Fly. *Biological Cybernetics*, 46: 67-79.
- [6] Sherman, Dickinson (2003). A comparison of visual and haltere-mediated equilibrium reflexes in the fruit fly *Drosophila melanogaster*. *The Journal of Experimental Biology*, 206: 295-302