

6-2016

Information Encoded by Dragonfly Visual Neurons MDT3 and DIT3 in Response to Looming Stimuli

Cara Slugaski

Union College - Schenectady, NY

Follow this and additional works at: <https://digitalworks.union.edu/theses>



Part of the [Animal Sciences Commons](#)

Recommended Citation

Slugaski, Cara, "Information Encoded by Dragonfly Visual Neurons MDT3 and DIT3 in Response to Looming Stimuli" (2016).
Honors Theses. 213.
<https://digitalworks.union.edu/theses/213>

This Open Access is brought to you for free and open access by the Student Work at Union | Digital Works. It has been accepted for inclusion in Honors Theses by an authorized administrator of Union | Digital Works. For more information, please contact digitalworks@union.edu.

Information Encoded by Dragonfly Visual Neurons
MDT3 and DIT3 in Response to Looming Stimuli

By

Cara Slugaski

* * * * *

Submitted in partial fulfillment
of the requirements for
Honors in the Department of Biological Sciences

UNION COLLEGE
June, 2016

Abstract

SLUGASKI, CARA Information Encoded by Dragonfly Visual Neurons DIT3 and MDT3 in Response to Looming Stimuli. Department of Biological Sciences, June 2016.

ADVISOR: Robert Olberg

The Target Selective Descending Neurons (TSDNs) DIT3 and MDT3 in the Aeshnid dragonfly have been shown to respond best to expanding visual stimuli that appear to be approaching, also called looming stimuli. The ratio between the length of the object (L) and the absolute approach velocity (V) completely determines the time course of angular expansion of the object's image. This study investigated how these neurons responded to the change in expansion of the stimuli, along with which properties of the looming stimuli (target angle, angular expansion rate, or angular acceleration) excite DIT3 and MDT3, respectively.

In this experiment, we dissected the animal to expose the nerve cord and then drove a microelectrode into individual axons. We distinguished between DIT3 and MDT3 by displaying a raster stimulus; the response to different areas of the visual field allowed us to determine which neuron we had penetrated. DIT3 has been shown to prefer visual stimuli in the contralateral portion of the visual field, while MDT3 prefers stimuli in the ipsilateral portion of the visual field. We then exposed the animal to stimuli with different looming properties to test for variations in response.

We found that these neurons are most sensitive to looming stimuli with large L/V values. The spikes for DIT3 and MDT3 occurred earlier as the L/V values of the stimuli successively increased. We also found that both DIT3 and MDT3 respond to a consistent angular expansion rate, while the other parameters resulted in inconsistent spike times relative to changing L/V values. This implies that these TSDNs encode information regarding angular velocity down the nerve cord, information that may be important for the animal to intercept its prey.

Introduction

Aeshnid dragonflies are among the largest North American insects and also among the fastest and most accurate predators. During prey pursuit, they quickly respond to deviations in their flying-prey's trajectory and alter their own in order to achieve interception. Rotations of the dragonfly's head constantly track the movement of its prey, allowing interception at its exact angular position (Mischiati et al., 2014). In the dragonfly nerve cord, there are eight pairs of large visual neurons, called Target Selective Descending Neurons (TSDNs). These neurons respond selectively to small moving stimuli, which mimic the movements of dragonfly prey. All eight of these neurons demonstrate preference to direction and some even to target size (Frye and Olberg, 1995).

TSDNs provide information to the wing motor circuitry, which allows the animal to control its trajectory. These neurons prefer distinct direction and sizes of objects in the dragonfly's field of vision. They receive information about the properties of these stimuli and convey it to the prothoracic mesothoracic, and metathoracic ganglia. Stimulation of TSDNs results in an adjustment of the wing positions (Gonzalez-Bellido et al., 2012). They transmit information down the nerve cord to the wing-motor circuitry, which controls flight, presumably allowing for a flight path correction. The information encoded by these neurons is hypothesized to guide the steering movement of the dragonfly's wings, even allowing the dragonfly to predict the location of their prey for accurate interception. It is believed that the steering that guides interception is driven by the body dynamics of these animals and prey motion. The dragonfly head-body angles guide the animal to align it with the flight path of its prey (Mischiati et al., 2015).

A subset of the TSDNs shows large and precisely timed responses to the expanding image of an approaching object. These images are often referred to as “looming stimuli”. The pattern of visual responses, i.e., their receptive fields and directional preferences, suggests that the looming-sensitive neurons are the specific TSDNs called DIT3 and MDT3. DIT3 responds significantly to an expanding object, and MDT3 responds to a looming stimulus with an accelerated expansion (Gonzalez-Bellido et al., 2012).

A receptive field is an area of the dragonfly’s field of view that excites (or inhibits) a certain neuron. Evidence suggests that MDT3 and DIT3 have two distinct receptive fields. In addition to its response to looming, MDT3 is excited by translating stimuli presented in the left, or ipsilateral, portion of the visual field. DIT3 is excited by looming and translating stimuli presented in the right, or contralateral, portion of the visual field. (Gonzalez-Bellido et al., 2012).

In this study, we investigated the responses of these looming-sensitive neurons, DIT3 and MDT3, to systematic changes in the parameters of approaching objects. The ratio of target size (L) to approach speed (V) completely determines the time course of angular expansion of a looming object on the retina. A smaller L/V defines a smaller object or higher approach speed, and a larger L/V a larger object or lower approach speed. In our stimulus manipulations we held the approach speed constant and varied the size of the virtual object (a black circle on the white projection screen). A larger target has a larger initial size and accelerates more slowly, while a smaller target, despite starting out smaller, accelerates more quickly. This changing property of the objects allowed us to see how DIT3 and MDT3 responded differently according to the change in expansion of the looming stimuli. According to Sun and Frost, the responses in pigeon nucleus rotundus neurons occur earlier for bigger looming objects (Sun et al., 1998).

The central complex, or CC, is an area in the brain of insects that contributes to spatial orientation and locomotion. It has been found that the neurons in this area of the brain responded with high sensitivity to looming stimuli that mimicked objects approaching on a direct collision course. The central complex is thought to incorporate sensory information about the animal's surroundings, which direction the animal is moving, and produces motor commands determining future directions of the animal (Rosner and Homberg, 2012).

The Descending Contralateral Movement Detector (DCMD) seen in Orthopteran insects gives a stronger response to stimuli that are traveling towards the eye. Over a wide range of L/V values, this neuron shows preference for approaching objects over receding objects (Rind and Simmons, 1992). DCMD has been found to have greater sensitivity to expanding, as opposed to contracting targets. It is a movement detector neuron to black and expanding stimuli (Schlotterer, 1977). Further, there is a direct correlation between the firing rate of DCMD relative to collision and the L/V parameter. The correlation coefficient for L/V and the peak time of this neuron's response was 0.92, showing that DCMD fires sooner when presented with a stimulus with a larger L/V (Gabbiani et al., 1999). This neuron can detect looming objects and contribute to predator avoidance by inducing a steering mechanism (Frye and Olberg, 1995). We wanted to see if TSDNs' response to looming stimuli in Aeshnids evoked a similar steering mechanism that would direct them toward their prey.

We also aimed to show the different information encoded by DIT3 and MDT3 when exposed to these looming stimuli. We wanted to determine which parameters of these stimuli have the greatest effect on neuronal responses. These parameters included target angle, angular expansion rate, and angular acceleration. We hypothesized that each cell encodes a different

piece of information with regards to the properties of the stimuli. Specifically, the response rates of DIT3 and MDT3 are dependent on the expansion rate of these looming stimuli.

Methods

In these experiments, we used Aeshnid dragonflies to investigate the responses of two different looming sensitive neurons, DIT3 and MDT3, to changing parameters of simulated object approach.

Dissection

We began our procedure by anesthetizing the animal in ice, assuring its immobility during the dissection. We removed the legs and dissected through to the thorax, making fine cuts to expose the ventral nerve cord, prothoracic, and mesothoracic ganglia. We used a metal “spoon” to secure the nerve cord by placing it underneath the cord, between the prothoracic and mesothoracic ganglia. This was done to stabilize the nerve cord during recording. Using a piezo-driven micromanipulator, we penetrated individual axons in the nerve cord with a 3M KCl filled aluminosilicate glass microelectrode (Sutter Instruments) with a typical resistance of 25-40 MOhms. The animal was positioned ventral side up centered in front of the screen, with the head 13cm from the screen, 11cm above the table, and angled 50 degrees from the table surface. This put the dragonfly’s head (and thus its field of view) centered horizontally and vertically with respect to the screen’s dimensions (which was 15cm high and 20cm wide).

Neuron Penetration and Recording

We ensured that penetration of the axon had occurred by observing standard neuron resting potential via an oscilloscope. To identify TSDNs, we displayed a raster stimulus on the projection screen. The raster plot consists of a target object tracing across every possible spot on

the screen, originating from each direction (up, down, left, and right). By matching an action potential to the target's position and direction of origin when firing occurred, we were able to map out the receptive field of the cell we had penetrated. This information was vital in ascertaining the specific TSDN we had penetrated. Further, to confirm the cell was looming-sensitive, we displayed a standard looming stimulus, to which a marked response would serve as confirmation. Coupling a positive looming response and the cell's receptive field, we could reasonably deduce the identity of the cell (MDT3 or DIT3). Previous research has shown that MDT3 has an ipsilateral receptive field, while DIT3 has a contralateral field. As both cells are known to be looming sensitive, we would expect a response to a looming stimulus from both MDT3 and DIT3.

Experimental Stimuli

Once we determined which TSDN we penetrated, we exposed the dragonfly to expanding stimuli with different looming properties to test for a variation in response. These stimuli were projected on a screen located in front of the dragonfly, with the dragonfly oriented so that the dorsal/frontal region of the compound eye viewed the screen. The stimuli varied in both their placement on the screen and the properties of the looming objects. Targets were presented expanding from the center of the screen, simulating a direct hit, as well as originating from points off-center, analogous to a target the animal would narrowly miss in its wild habitat, as can be seen in Figure 1.

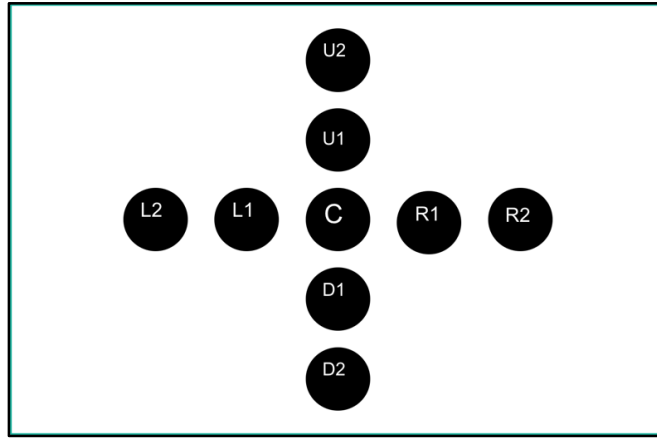


Figure 1. Diagram of Looming Stimuli Placements White box represents the projection screen displayed to the animal, and each black circle shows a spot where a looming target was displayed. Letters denote placements relative to center (C), the center of the animal's field of view, simulating a direct hit target approaching. L, R, U, and D represent targets left of, right of, above, and below center, respectively. 1 and 2 indicate distance from center, with 1 denoting a deviation of 5.8mm from center, and 2 a deviation of 11.7 mm from center.

L/V is a ratio of target size to expansion speed that represents the changing size of a looming object. There is a different time course expansion for different L/V values; the larger target approaches more gradually, while the smaller target stays small and accelerates quickly at the end. To test the changing property of L/V , we altered either the size of the target. The changing property of the stimuli allowed us to see how both DIT3 and MDT3 responded according to the change in expansion.

Electrical recordings were processed through an amplifier (Neuroprobe, WPI) and converted to digital signals using PowerLab (AD Instruments) hardware, and were saved for visualization and analysis with LabChart software (AD Instruments) and MatLab.

Data Processing

After sorting spikes in LabChart, we processed the spiketimes for analysis, using custom MATLAB scripts. Data from the “near-hit” trials were run through scripts which extracted the number of action potentials detected in response to each stimulus. This was then used to

determine the differential sensitivity of MDT3 and DIT3 to the directionality of near-hit looming stimuli.

Results

It is known that different TSDNs have different receptive fields. Specifically, MDT3 is more reactive to the ipsilateral side of the visual field, while DIT3 is more reactive to the contralateral side of the visual field. The looming properties that were tested to account for variation between DIT3 and MDT3 were time-to-contact, target angle, angular expansion rate, and angular acceleration. These properties were plotted against L/V to discern how they each affect spike timing. We tested for consistencies between the looming properties of the stimuli and the spike timing of DIT3 and MDT3.

For each respective cell, we compared the time of the first spike and the mean spike times to each of the looming parameters. If either of these neurons fired at a consistent value for one of these parameters, the same value for that property would be plotted for the varying L/V ratios. Instead of seeing fluctuation in the graph as the L/V values changed, we would see a horizontal line depicting the consistent spike times despite L/V changing.

The time-to-contact parameter of the looming stimuli did not result in consistent firing of DIT3. We did see that DIT3's response to looming objects with increasing L/V values showed a steady decrease in response time. For the time-to-contact property of the looming stimuli, we did not see consistency with timing of first spike nor the average spike time for DIT3 (Figure 2).

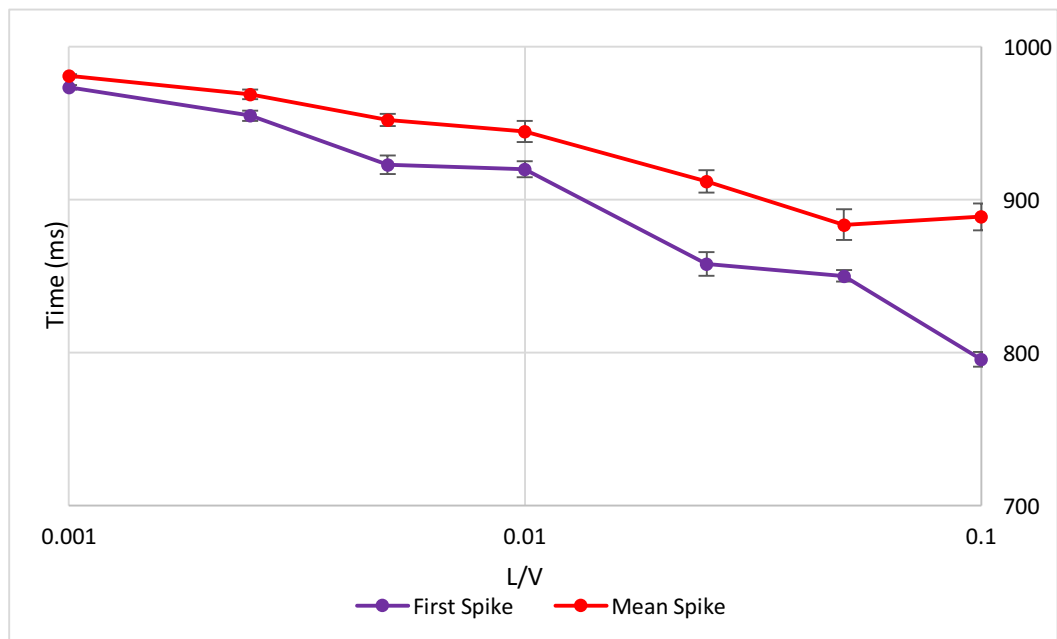


Figure 2. DIT3 first spike and mean spike timing relative to changing L/V values. Error bars represent standard error. N = 141. The contact time is 1000 milliseconds.

Similar to that of DIT3, MDT3 spike timing was not consistent with the time-to-contact looming parameter of these stimuli. Neither the time of the first spike nor the average spike time for MDT3 showed consistency as the L/V values changed (Figure 3).

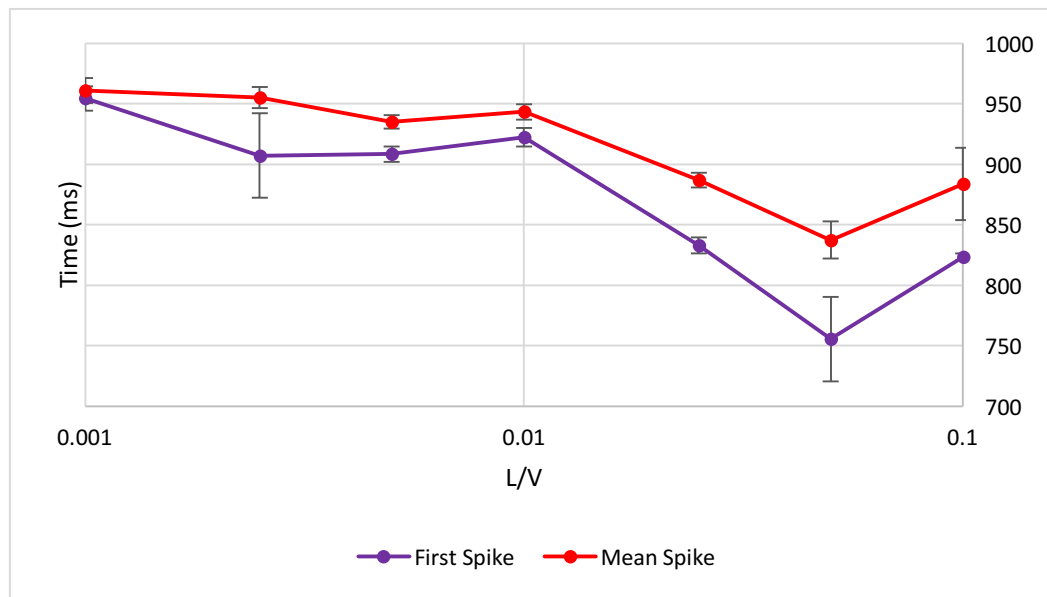


Figure 3. MDT3 first spike and mean spike timing relative to changing L/V values. Error bars represent standard error. N = 182. The contact time is 1000 milliseconds.

Neither the first spike time nor the mean spike time was consistent relative to time-to-contact, except for MDT3 as low L/V values (i.e. small looming targets). Therefore, we calculated the values three parameters, visual angle, angular velocity, and angular acceleration corresponding to the spike times. We see that for DIT3, the plot illustrates that the spike timing did not change drastically in response to the changing angular velocity, or expansion rate (Figure 4).

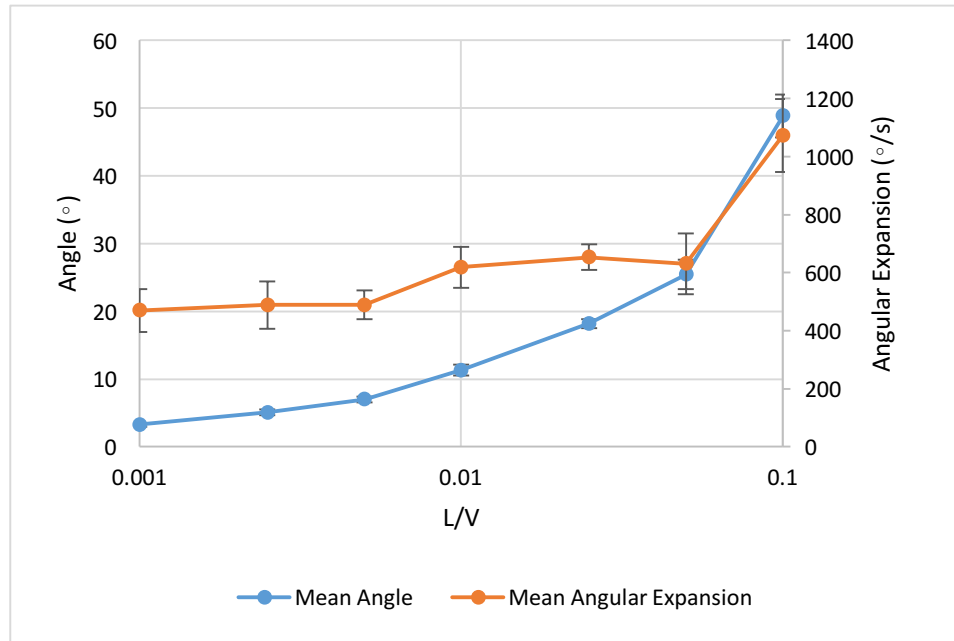


Figure 4. DIT3 angle and angular expansion rate relative to changing L/V values. The plot represents target angle and expansion rate at the time of the first spike. Error bars denote standard error. N = 141. The contact time is 1000 milliseconds.

The spikes for DIT3 were thus triggered at a consistent angular expansion rate. Since the timing of the spikes did not appear to vary much in response to this changing parameter, it suggests that the cell fires at the same expansion rate (Figure 4). When looking at DIT3's spike timing relative to target angle, we see that there is much less of a consistent firing relative to this parameter (Figure 4). DIT3 does not fire at a consistent target angle. DIT3 responded consistently to the expansion rate, aside from one value at an L/V of 0.1 that lies outside the range where spikes provide consistent information about angular expansion. The average spike

times for DIT3 in response to the changing expansion rate also remained constant. The values for the average spike times do not appear to change as the angular expansion rate changes, albeit there is a single value at an L/V of 0.1 that lies outside of the consistent range (Figure 5).

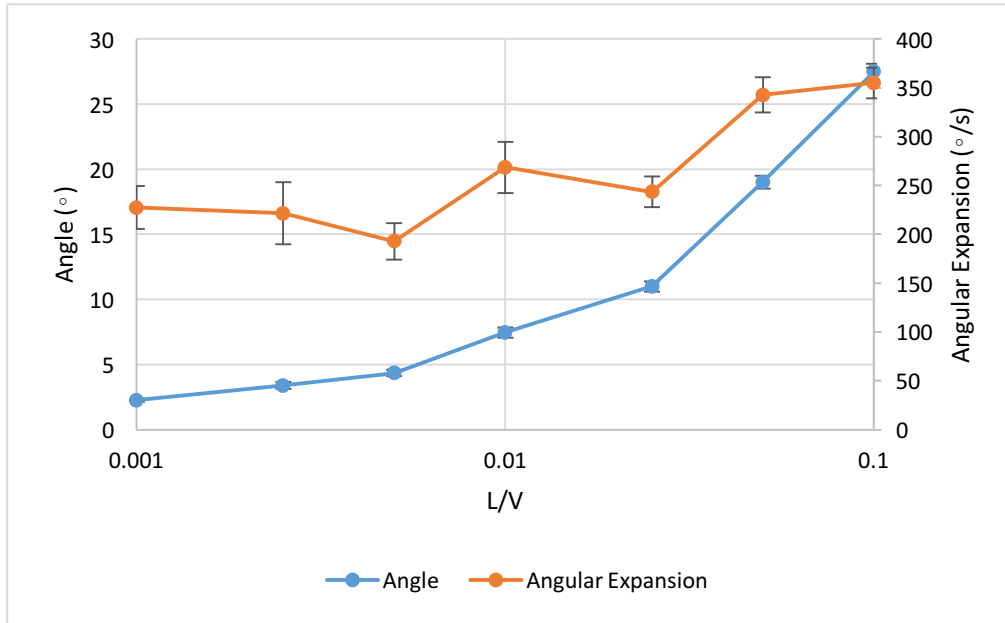


Figure 5. DIT3 mean angle and mean angular expansion rate relative to changing L/V. The plot represents the target angle and expansion rate at the time of the mean spike. Error bars denote standard error. N =141. The contact time is 1000 milliseconds.

We plotted the angular acceleration value at the time of the spikes to see whether the acceleration property of the looming stimuli excited DIT3. The timing in the spikes appeared to vary for different angular accelerations. As L/V increased, the value for angular acceleration that elicited the first spike decreased (Figure 6). This trend was also observable with the angular acceleration values for the mean spike times. The angular acceleration values that represent a mean spike time were not consistent (Figure 7). DIT3 spikes were not triggered at a constant angular acceleration.

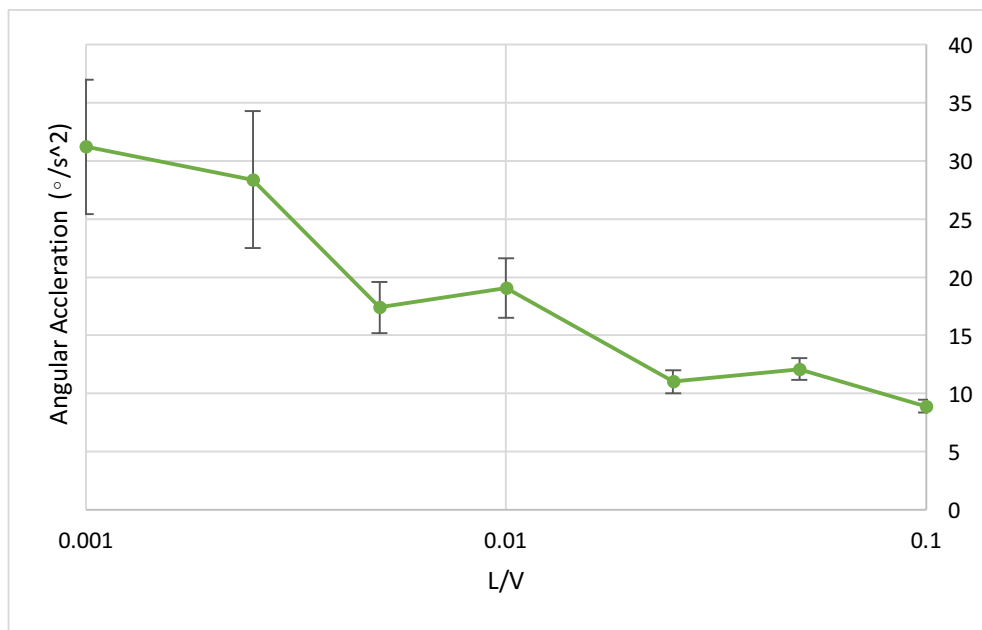


Figure 6. DIT3 angular acceleration relative to changing L/V values. The plot represents the angular acceleration at the time of the first spike. Error bars denote standard error. N = 141. The contact time is 1000 milliseconds.

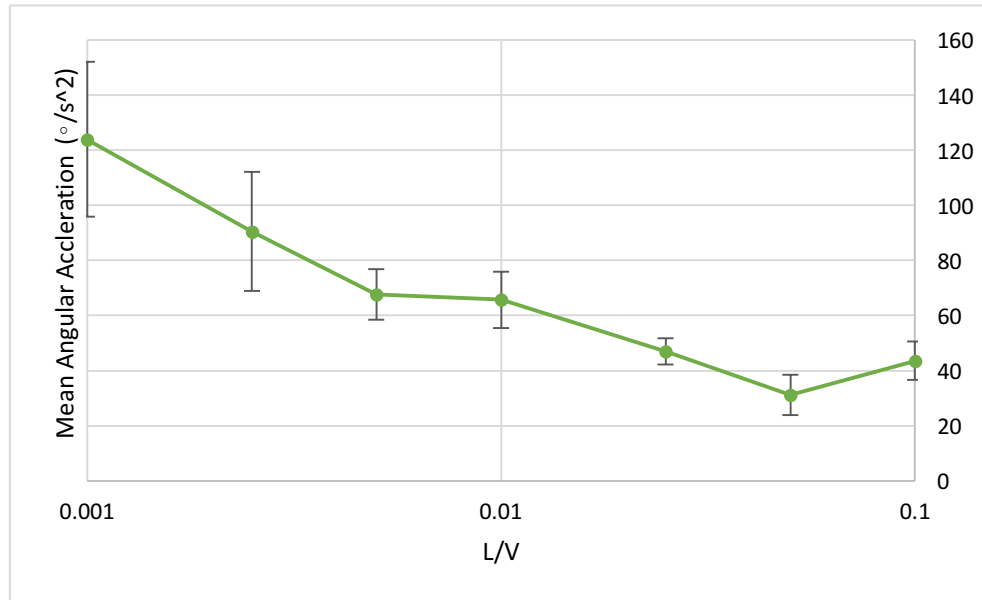


Figure 7. DIT3 mean angular acceleration relative to changing L/V values. The plot represents the angular acceleration at the average spike time. Error bars denote standard error. N = 141. The contact time is 1000 milliseconds.

DIT3 showed consistent firing for a certain angular expansion rate, so we wanted to see if this held true for MDT3. We compared the first spike timing of MDT3 relative to angular expansion rate, and we saw that the values did not change as the L/V value varied. There is a consistency in the first spike timing of MDT3 relative to changing angular expansion (Figure 8). Similar to the data for DIT3, there is a value for MDT3 at an L/V of 0.1 that lies outside the

range in which spikes provide consistent information about expansion rate. Looking at the first spike timing relative to target angle for MDT3, we see that the values vary as L/V changes. There is no consistency seen between first spike timing and the target angle for MDT3 (Figure 8). These trends are also perceptible for the average spike times of MDT3 relative to target angle and angular expansion rate. MDT3 average spike times are consistent with a specific angular expansion rate. The average spike times of this cell are not consistent with the changing target angle values (Figure 9).

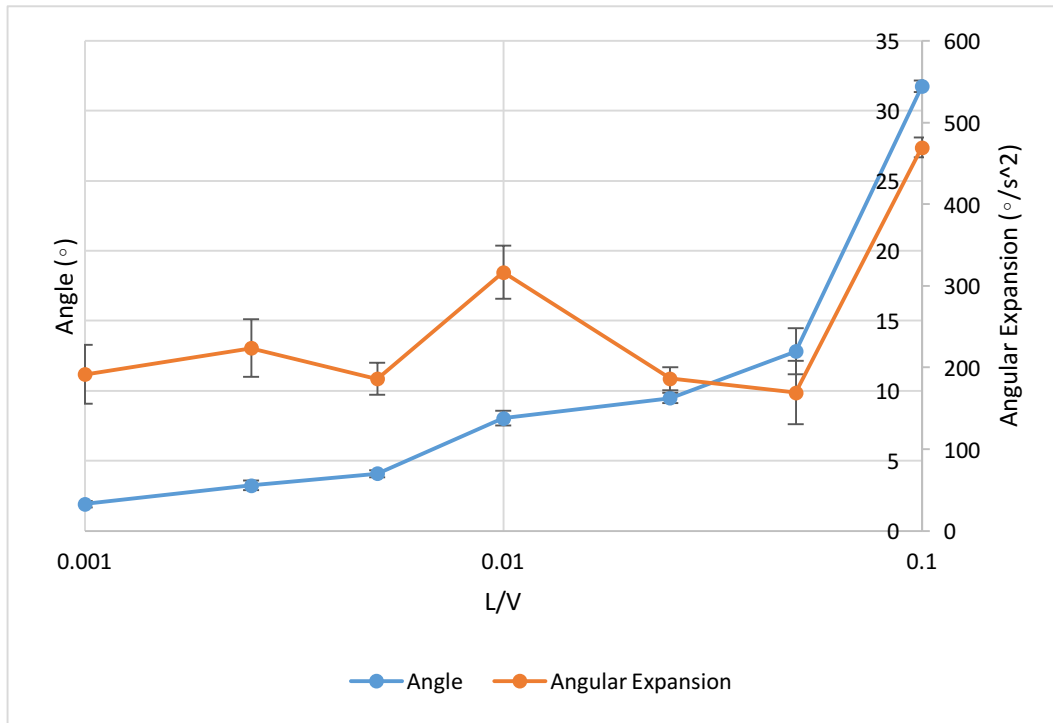


Figure 8. MDT3 angle and angular expansion rate relative to changing L/V values. The plot represents target angle and angular expansion at the time of the first spike. Error bars denote standard error. N = 182. The contact time is 1000 milliseconds.

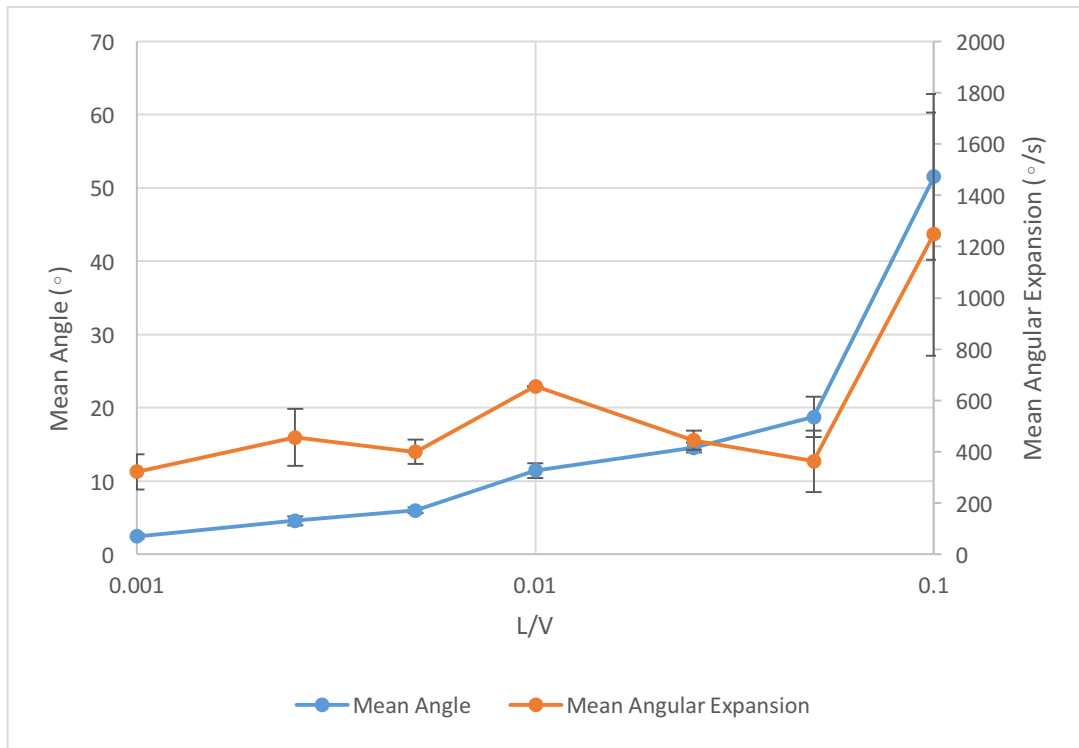


Figure 9. MDT3 mean angle and mean angular expansion rate relative to changing L/V values. The plot represents target angle and angular acceleration at the average spike time. Error bars denote standard error. N = 182. The contact time is 1000 milliseconds.

The last property we wanted to test for a consistency in MDT3 was angular acceleration. We plotted the value for angular acceleration at the time of the first spike and the time of the mean spike to varying values of L/V and looked for consistencies. Like DIT3, the first MDT3 spikes were not triggered at a constant angular acceleration. The spike timing varied for each respective L/V value, showing no consistent trend as the angular acceleration changed (Figure 10). Likewise, the the mean spike times for MDT3 were not consistent with a specific value for angular acceleration (Figure 11).

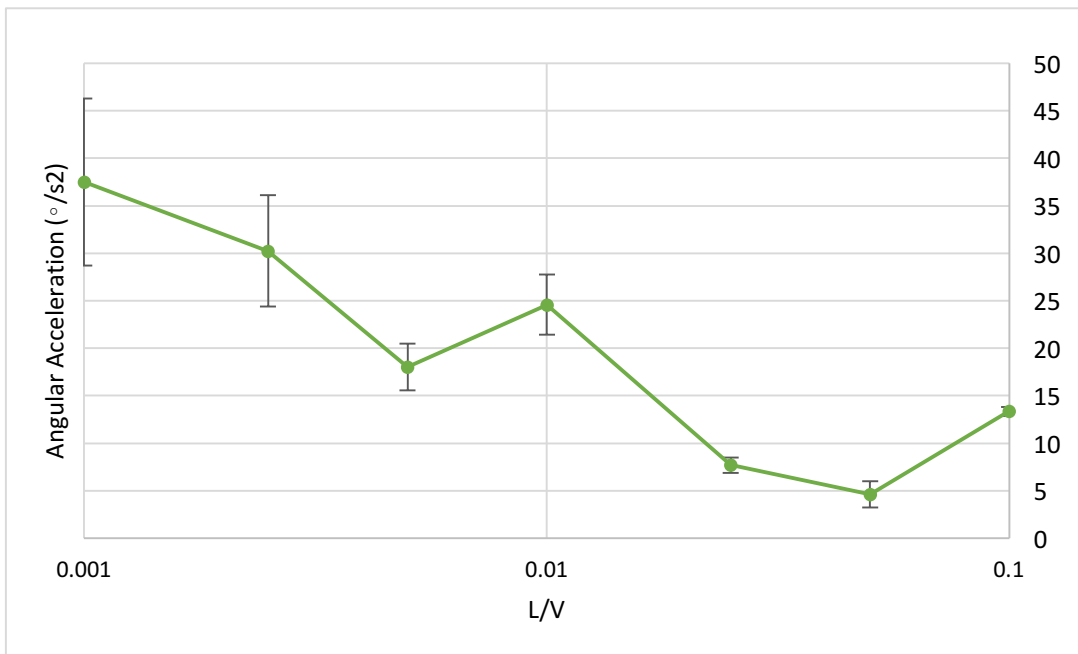


Figure 10. MDT3 angular acceleration relative to changing L/V values. The plot represents angular acceleration at the time of the first spike. Error bars denote standard error. N = 182. The contact time is 1000 milliseconds.

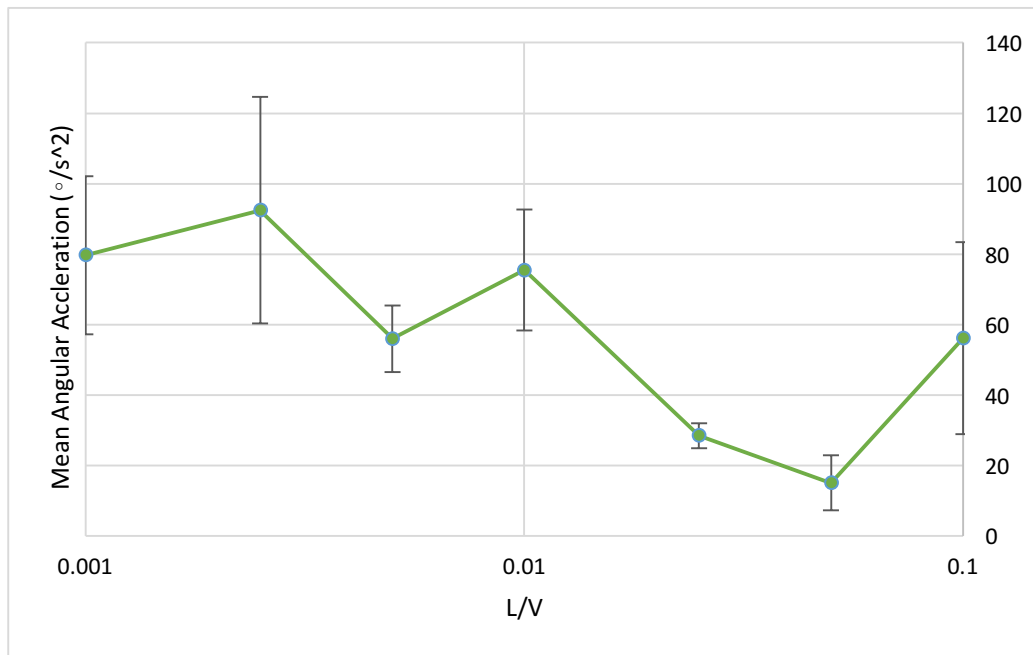


Figure 11. MDT3 mean angular acceleration relative to changing L/V values. The plot represents the angular acceleration at the average spike time. Error bars denote standard error. N = 182. The contact time is 1000 milliseconds.

Discussion

The Target Selective Descending Neurons DIT3 and MDT3 both transmit visual information down the nerve cord regarding the expansion rate of looming targets. They respond best to expanding objects that mimic the approaching movements of their prey. The wing-motor circuitry receives information from these TSDNs and allows for a flight path correction in order for the animal to accurately intercept its prey. We wanted to test exactly what properties of these looming stimuli excite both DIT3 and MDT3.

We exposed the dragonfly to stimuli with different looming properties to look for a disparity in the response of these neurons to the changing parameters. We changed the value of L/V to see how DIT3 and MDT3 responded according to the change in the looming properties. This allowed us to deduce which factors within these stimuli the neurons are most sensitive to. The property that these neurons respond to consistently would thus be the information they encode that allows the dragonfly to be so precise in its interception.

Like DCMD in Orthopteran insects, DIT3 and MDT3 in dragonflies are more sensitive to stimuli that are expanding, specifically to those stimuli that have a larger L/V value (Gabbiani et al., 1999). The timing of firing of DCMD is directly correlated to increasing values of L/V , as is that for DIT3 and MDT3. While it is predicted that this property of DCMD allows the animals to avoid being captured by predators through a steering mechanism (Rind and Simmons 1992), this property of DIT3 and MDT3 may provide the dragonfly with a different advantage. The sensitivity to a larger L/V value may give dragonflies the ability to respond best to prey that are larger or have a slower approach speed.

The central complex part of the insect brain provides information about spatial orientation and locomotion to the animal (Rosner and Homberg, 2012). Resembling the neurons

present in this area of the brain, DIT3 and MDT3 also respond best to looming objects approaching on a direct collision course. The central complex is believed to produce commands that determine future directions of the animal, while DIT3 and MDT3 could also potentially contribute to a dragonfly's change in flight path to intercept its prey.

The spike timing for both DIT3 and MDT3 was consistent with a certain expansion rate. This was the only parameter that these neurons fired consistently at. Neither DIT3 nor MDT3 was triggered at a constant target angle or angular acceleration, which suggests that both of these neurons encode information about expansion rate. The two looming-sensitive TSDNs, DIT3 and MDT3, have receptive fields on opposite sides of the visual field (contralateral and ipsilateral, respectively). This allows the dragonfly to receive information concerning expansion rate across the entire visual field.

Spike timing for DIT3 and MDT3 does not predict time-to-contact for varying values of L/V . For the foraging dragonfly, this could imply that the animal prefers stimuli of a certain absolute size. Dragonflies are selective for object size, which suggests that they are also able to determine the distance of their prey (Olberg, 2005). Although spike timing does not predict the time-to-contact information for different L/V s, what we know regarding the dragonfly's preference for stimuli of a certain size indicates that the dragonfly is able to get around this problem. If the dragonfly consistently approaches its prey at a given speed, the values for L (target size) and V (approach speed) are both known values. In that case the L/V value would thus be constant, and the spike timing would provide accurate information regarding time-to-contact of the dragonfly to its prey.

Target angle and angular acceleration are also properties that DIT3 and MDT3 do not encode for; yet the dragonfly remains able to predict the exact location of its prey and precisely

intercept it. Although MDT3 and DIT3 do not encode information regarding target angle and angular acceleration, the information encoded about angular velocity combined with the dragonfly's ability to obtain time-to-contact information suggests that the animal relies most heavily on this information during prey pursuit.

References

- Frye, MA and Olberg, RM (1995) Receptive field properties of feature detecting neurons in the dragonfly. *J.Comp.Physiol. A* 177:569-576.
- Gabbiani, F, Krapp, HG, Laurent, G (1999). Computation of object approach by a wide-field, motion sensitive-neuron. *J. Neuro.* 19(3):1122-1141.
- Gonzalez-Bellido PT, Penga H, Yanga J, Georgopoulos A, and Olberg RM (2012). Eight pairs of descending visual neurons in the dragonfly give wing motor centers accurate population vector of prey direction. *PNAS* 110(2): 696-701.
- Mischiati M, Lin H-T, Herold P, Imler E, Olberg R, Leonardo A (2015) Internal models direct dragonfly interception steering. *Nature* 517:333-338.
- Olberg, RM (1986) Identified target-selective visual interneurons descending from the dragonfly brain. *J. Comp. Physiol.* 159:827-840.
- Olberg, RM, Worthington AH, Fox JL, Bessette, CE, Loosemore, MP (2005). Prey size selection and distance estimation in foraging adult dragonflies. *J Comp Physiol A* 191:791-797.
- Rind, FC, Simmons, PJ (1992). Orthopteron DCMD neuron: a reevaluation of responses to moving objects. *J Neurophys.* 68(5):1654-1666.
- Schlotterer, GR (1977). Response of the locust descending movement detector neuron to rapidly approaching and withdrawing visual stimuli. *Canadian J Zoology.* 55(8):1372-1376.
- Sun, H, Frost, BJ (1998). Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. *Nat Neurosci.* 1(4):296-303.