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Responses of Dragonfly Visual Neurons MDT3 and DIT3

to Near-Hit Looming Stimuli

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ABSTRACT

SHAPIRO, BENJAMIN GOODMAN   Responses of Dragonfly Visual Neurons MDT3 and DIT3 to Near-Hit Looming Stimuli. Department of Biological Sciences, June 2016

ADVISOR: Professor Robert Olberg

Dragonflies are known to have highly sophisticated visual processing systems, allowing precise flight control and incredibly accurate prey capture (Olberg et al., 2000). These processes are mediated by a group of neurons known as Target Selective Descending Neurons, or TSDNs. Of the TSDNs, MDT3 and DIT3 are known to respond to objects expanding into the animal's field of view, otherwise known as looming objects.

Through the use of intracellular electrical recording, we aimed to understand how these two neurons work together to scan the entire visual field, as well as how they respond to objects on a trajectory to miss the animal. We found that MDT3 and DIT3 share the workload roughly evenly, with each neuron responding best to objects in its receptive field. Further, each neuron responded more robustly in response to stimuli on course to miss the animal, rather than those on a collision trajectory. This leads us to the conclusion that MDT3 and DIT3 are tasked with confirming that the animal is on the correct path to intersect a prey object, and if it is not, to provide information about the last-second flight path corrections that must be made.

Introduction

Dragonflies have remarkable visual flight-control systems, allowing them to achieve an incredibly high 95% prey capture success rate. (Olberg et al., 2000) Their prey detection systems are mediated by a group of visual interneurons known as Target Selective Descending Neurons, or TSDNs. Eight pairs of these TSDNs originate in the brain, and have axons that project down the nerve cord. (Olberg, 1986) This allows information to be sent to the motor neurons controlling the wing muscles, altering the flight path to ensure prey capture. (Gonzalez-Bellido, et al., 2012)

TSDNs have a variety of receptive field locations, as well as target directional preferences, but they all respond to target motion somewhere in the dorso-frontal visual field. We were specifically interested in the TSDNs sensitive to looming objects, MDT3 and DIT3. Each of these neurons has a distinct receptive field, with MDT3 having a primarily ipsilateral receptive field, and DIT3 having a primarily contralateral field. Unlike most TSDNs, MDT3 and DIT3 have relatively uniform excitability across the entire dorso-frontal hemifield. In other TSDNs, excitability is largely concentrated around the visual midline. (Gonzalez-Bellido, Et. Al., 2012) This likely is due to the fact that sensitivity to expanding objects is best served by a large receptive field.

Sensitivity to looming objects has also been studied in the visual systems of other animals. In 2008 Yamawaki, et al. showed that descending neurons in the praying mantis respond to expanding images. Similar findings have also been shown in locusts by Rind and Judge in 1997, as well as in pigeons by Sun and Frost in 1998.

Our goal with this study was to determine the receptive fields of MDT3 and DIT3 with respect to looming objects. Additionally, we wanted to determine how these neurons respond to expanding stimuli which are approaching off center with respect to the animal, which we termed "near-hit stimuli". These targets approximate a prey object coming towards the dragonfly, but at an incorrect trajectory, such that interception would not succeed without course correction.

We hypothesized that MTD3 and DIT3's receptive fields in response to looming objects would align with their individual receptive fields. Further, we hypothesized that these TSDNs would respond to near-hit stimuli, as this would provide vital information for last-second flight trajectory correction, contributing to the high prey capture success rate seen in these animals.

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 19.3cm high and 25.7cm 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.
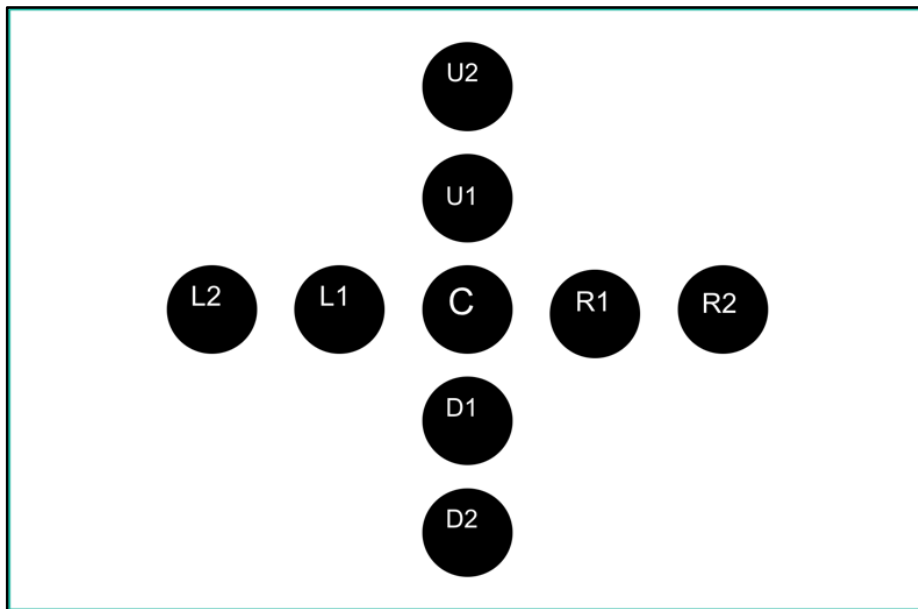

Figure 1. Diagram of Looming Stimuli Placements White box represents the projection screen displayed to the animal, and each black circle shows a spot where a looming target was displayed. Letters denote placements relative to center (C), the center of the animal's field of view, simulating a direct 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. The target used was a black circle with an absolute size of 0.5cm in diameter. The object originated 500cm away from the animal on the Z axis, and approached at a velocity of 20cm/sec.

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 spike times 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

The cells we recorded from were grouped by cell identity as determined by responses to raster stimuli (Figure 2) and to an on-center looming stimulus.
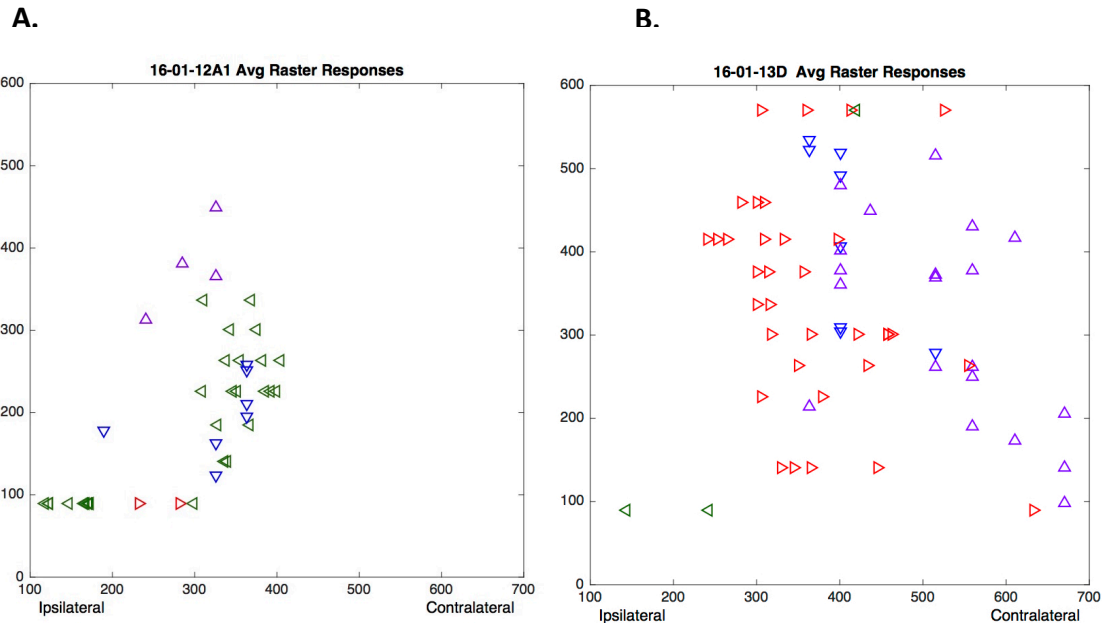
Figure 2. Responses to Raster Stimuli of Two Representative Cells. The boxes serve as a representation of the screen used to display stimuli to the animal. Each triangle indicates that an action potential was recorded when the stimulus was at that point on the screen, the direction of the triangle signifies the direction in which the stimulus was moving at the time of the action potential. **A** shows a cell with an ipsilateral receptive field, **B** shows a cell with a contralateral receptive field.

By combining the information gathered from the raster stimuli with a test for a response to a simple looming stimulus, we could determine the neuron we were recording from using the logic shown in Table 1. From this, we determine that in Figure 2, **A** was MDT3 and **B** was.

Table 1. Cell Identification Matrix

		Receptive Field in Response to Raster Stimuli	
		Ipsilateral	Contralateral
Response to Looming Stimulus?	Response	MDT3	DIT3
	No Response	Non-Looming-Sensitive Cell	

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DIT3. Determining the cell type was important as it allowed us to meaningfully analyze the rest

of our results. With the cell identified, we then displayed looming stimuli at different locations

on the screen. As seen below in Figure 3, we saw a marked difference in response patterns in

MDT3 as opposed to DIT3.
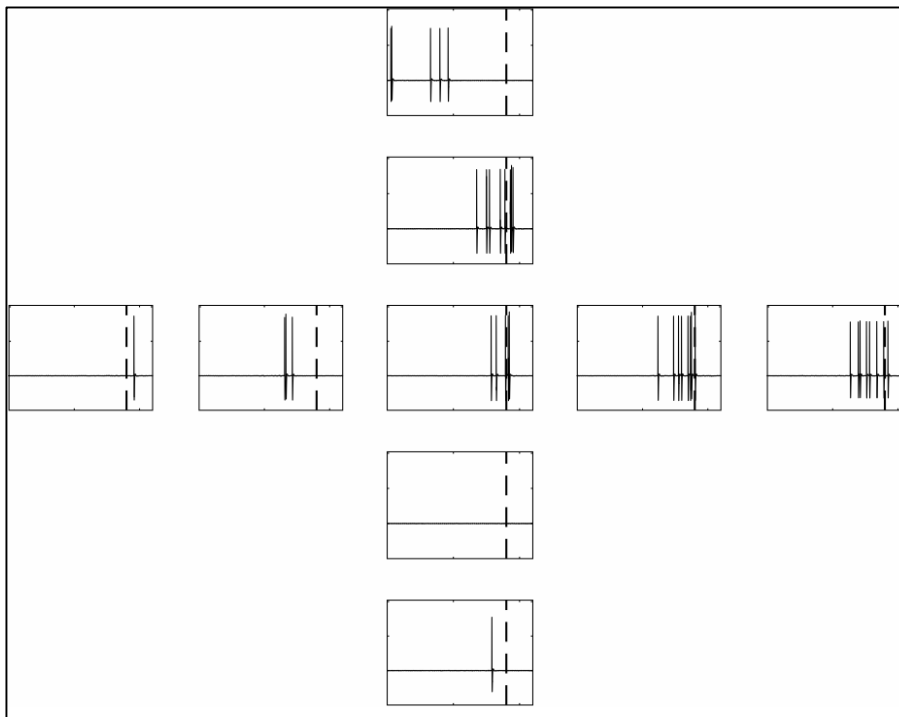
L-MDT3
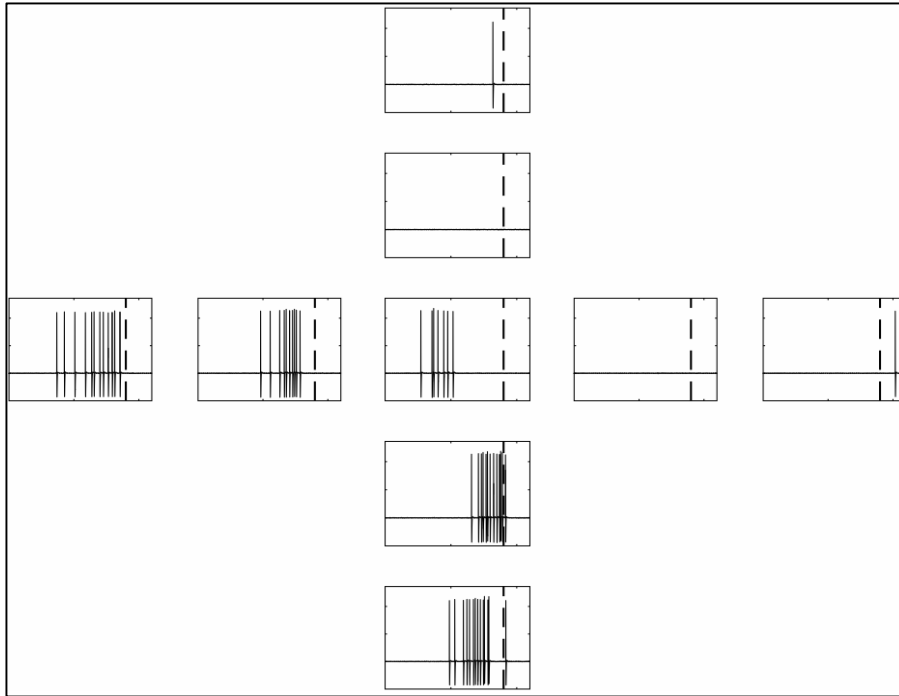
L-DIT3

Figure 3. Action Potentials Relative to Stimulus Position in Representative Cells. Diagrams shown represent stimulus placement in a similar fashion to that shown in Figure 1. The horizontal axis of each box is time, with the vertical dashed line representing the time when the stimulus would make contact with the animal if it were a physical object. The traces represent 1.5 seconds of recording. Each solid vertical line denotes an action potential.

When repeated with many distinct animals and neurons, the following aggregate data were
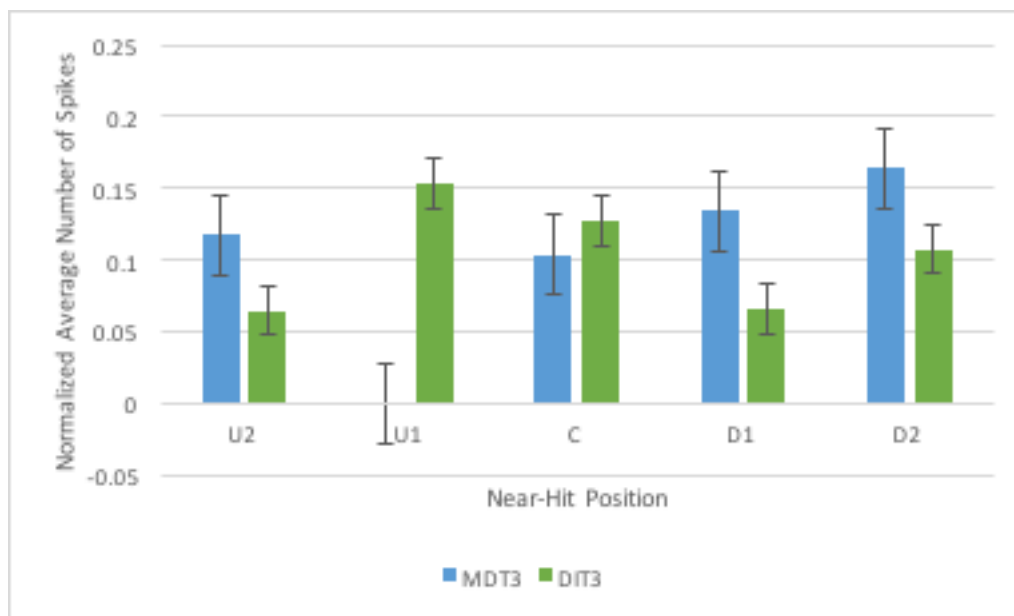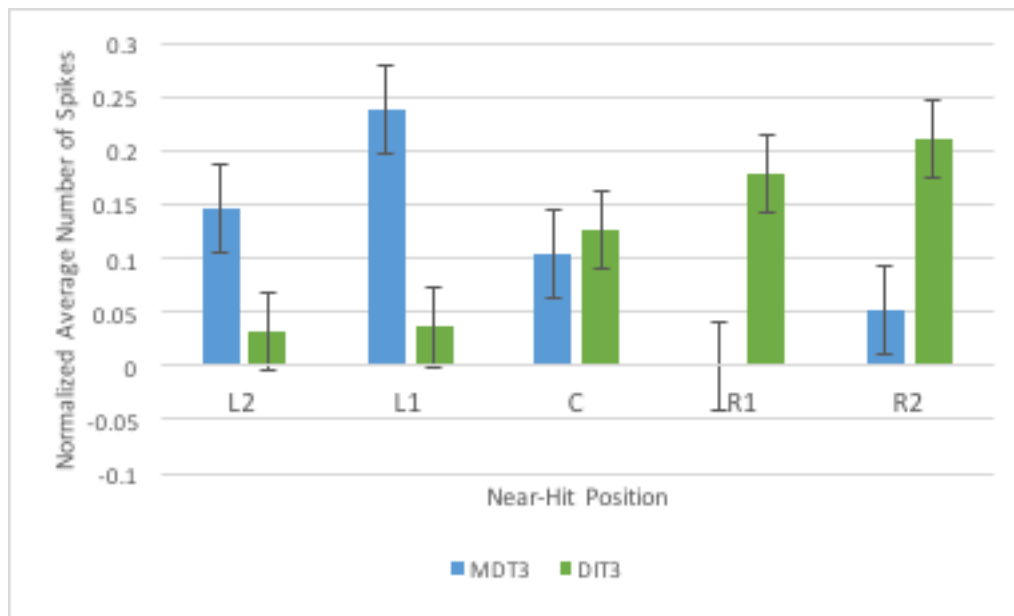
obtained, shown in Figure 4.

Horizontal Miss

Vertical Miss

Figure 4. Aggregate Responses to Near-Hit Looming Objects (n = 10). Shown are the
normalized average number of spikes (action potentials) for each cell type, based on the
location of the stimulus. Stimuli locations are coded in the same fashion as in Figure 1, with **C**

indicating a direct hit at the center of the animal's field of view, **U**, **D**, **L**, and **R** denoting a miss above, below, left of, and right of center, respectively. The numbers after the location codes indicate how far from center the stimulus misses. Values for MDT3 are shown in blue, values for DIT3 are shown in green. Error bars represent standard error.

As seen in Figure 4, over multiple trials the average response patterns matched those seen in the representative cells (Figure 3). Further, there appears to be a distinct difference in the responses of MDT3 and DIT3. The responses of each cell line up with their receptive fields, with MDT3 spiking in response primarily to targets left (ipsilateral) of and below center and DIT3 responding to targets to the right (contralateral). of and above center Additionally, both cells showed some response to on-center stimuli, but these excitations were less frequent and less robust.

Discussion

Our results show that the looming-sensitive TSDNs MDT3 and DIT3 in *Aeshnid* dragonflies are more receptive to targets on a trajectory to miss the animal than those on a collision course. Further, the direction in which each cell is the most excitable aligns with their overall receptive fields. The fact that the cells respond best to stimuli missing in the direction of their receptive fields is not surprising and is what we expected. It makes logical sense that the neurons would be most sensitive to looming objects in the same portion of the visual field that they are sensitive to other types of motion. Both cells being sensitive to the same area would be redundant and counterintuitive.

The result that was more surprising was the difference in response between on and off center targets. Both cells showed a marked decrease in excitation in response to targets in the

center position as compared to a near-hit in their receptive fields. This led us to the conclusion that MDT3 and DIT3 are not only involved in detection of looming objects the animal is on course to intercept. Rather, they can also serve to detect approaching prey which the animal is about to miss. A spike from one cell in the absence of the other would imply that the target is not on the correct trajectory for interception, and must be corrected (with the direction being determined by whether MDT3 or DIT3 is the cell firing). We believe that this information may be used to signal the wings to change position, allowing for a last-second flight path correction to achieve prey interception. However, if both neurons are firing, the calls for "left-down" and "right-up" adjustments would cancel, telling the animal that it is on a collision course with the target, and thus no correction is necessary.

Interestingly, this response to looming objects appears to be unique to MDT3 and DIT3. Similar experiments were previously done on locusts, which have an analogous neuron, the Descending Contralateral Movement Detector, or DCMD. It was found that DCMD responded most robustly to targets on a collision trajectory, with both vertical and horizontal deviations from center resulting in an attenuated response. (Judge and Rind, 1997) A 1999 review article by Rind and Simmons further substantiates these data that DCMD is primarily responsible for collision detection and possibly avoidance.

Judge and Rind argue that DCMD's purpose is for collision detection and possibly prey evasion (Judge and Rind, 1997). This would suggest that, despite the fact that, like the TSDNs MDT3 and DIT3, DCMD responds to looming stimuli; it does so in a different way and for a different purpose. MDT3 and DIT3 are primarily implicated in prey capture mechanisms, while DCMD is involved in evasion. Further, it appears that while DCMD only detects the absence or

presence of a collision, MDT3 and DIT3 work together to indicate if the animal is on a collision course with a target and, if not, what corrections should be made.

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