DISCERNING PREY FROM PREDATOR IN DRAGONFLIES

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ABSTRACT
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The dragonfly is a visual predator that feeds on small flying insects. Because of their high rate of hunting success, dragonflies must have the ability to efficiently discern prey and predator from natural surroundings. In this study, we investigated the extent to which visual flight-control neurons in dragonflies are able to differentiate stimuli that simulate prey items from those that simulate predators. To do this, we presented fourteen sets of rectangular stimuli varying in height, width, speed, and moved in four directions while recording extracellular neuronal responses from the ventral nerve cord. Each of the rectangular stimulus patterns was moved in two ways: (1) along its long axis (a "worm" stimulus) and (2) perpendicular to its long axis (an "antiworm" stimulus). Our preliminary results show that dragonflies respond best to smaller stimuli, particularly the 4° target, with speed having no significant effect on the neural responses. Further research is necessary and ongoing to determine trends related to changes in stimuli size and velocity.

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Introduction

The relationship between prey and predator is one of the oldest and most complex relationships in nature. The inability for one to protect itself from a predator has deadly consequences and the inability to effectively and efficiently hunt has similar results. Evolution of particular features such as paired appendages (Ruvinsky, Oates, Silver, & Ho, 2000), are selected for to help the hunted escape. Theories about what makes something a valuable prey item have been devised, referred to as predator-prey models, and have been offered to explain this seemingly random choice. One of the pioneers of the predator- prey model was Ivlev (Eggers, 1977). Ivlev, who worked with fishes, created a mathematical equation, shown below, to support his hypothesis that the rate of an animal's consumption depends on how hungry it is.

$$r = R\,[1- \exp(-\alpha P)] \qquad (1)$$

In the equation (1), R is maximum amount of food that can be ingested by the predator, P is the prey density, r is the ingested ration and α is a constant. For this equation, Ivlev was criticized (Rashevsky, 1959), for not taking into account factors such as physical and physiological properties of the predator, vision, and effects of hunger. Despite the shortcomings of Ivlev's equation, his efforts helped others create models to better understand prey-predator interactions.

In 1966, Holling (Eggers, 1977) developed another prey-predator model that included variable degrees of hunger as well as the time needed for the predator to hunt, kill and consume its prey before hunting again. Another researcher, Nakamura (1974), building off of both Ivlev and Holling's work, confirmed that hunger was a basic component of predation. The reasons for hunting a particular prey, as summarized by Pyke et al in 1977 (Armstrong & Stamp, 2003) are: 1) the quality of the prey in comparison with other prey 2) quantity of prey available and 3) if capturing the prey maximizes the predator's energy gain.

To be able to escape a predator, one must be able to identify the threat. The three primary ways in which an animal may detect information about its environment are mechanical, visual and chemical (Dicke & Grostal, 2001). In dragonfly larvae, chemical recognition is primarily used to identify its surroundings (Chivers, Wisenden, & Smith, 1996). By adulthood, dragonflies are visual predators that intercept their prey mid-flight. Their prey capture flights are short, often less than 200ms from takeoff to prey interception (Olberg, Seaman, Coats, & Henry, 2007), and highly accurate, with success rates as high as 97% (Olberg, Worthington, & Venator, Prey pursuit and interception in dragonflies, 2000).

Earlier research concerning dragonfly vision highlight the presence of select neurons, known as target-selective descending neurons (TSDNs), located in the ventral nerve cord that respond to stimuli with directional selectivity and size (Frye & Olberg, 1995). These 8 bilateral pairs of neurons fall into one of two general size preferences. Most TSDNs respond to a wide range of sizes (MDT1, MDT2, MDT3, MDT5, DIT2, DIT3), while the two remaining neurons (DIT1, MDT4) respond best to smaller targets. All TSDNs show some directional selectivity while others, such as DIT1 and MDT4, are strongly selective. These feature detectors are believed to steer the dragonfly while it hunts for prey, keeping the image of the prey item in a fixed position on the retina.

Evidence for neurons that respond to targets with particular features are found throughout the animal kingdom. In some insects, such as the hoverfly, there is data that support object selectivity for targets smaller than 1°(Nordström & O'Carroll, 2006). Studies in cats show the presence of neurons with a preference for edges (Pettigrew & Freeman, 1973). Amphibians, particularly the common toad (*Bufo bufo*), have also been studied and are found to have neurons, called T5(2) cells, which are used in prey recognition (Ewert J. , 1980).

Learning about the existence of T5(2) cells came as a result of recording neural responses of the toad to various predators, in the optic tectum region of the brain(Ewert J. , 1980). In 1979, Ewert and Traud tested the behavioral responses of *Bufo bufo* in the presence of known predators. They exposed the toads to predators such as a Buzzard (*Buteo buteo*), a leech (*Hirudo medicinalis*), giant ant (*componotus herculeanus*), hedgehog (*Erinaceus europaeus*) and a ringsnake (*Natrix natrix*). Certain features from the toad's main predators, the hedgehog and ringsnake, were used to create artificial stimuli mimicking them to help determine what characteristics influenced behavior. The parameters of the stimuli included velocity and direction of motion, direction of stimulus, background contrast, stimulus size, and stimulus configuration (Ewert & Traud, 1979).

Researchers determined that the best response was from small targets that moved in a worm-like manner(Ewert & Traud, 1979). This study then inspired further research with only 2-dimensional stimuli that moved in a worm-like manner (moving parallel to the direction of motion), and had a worm-like shape, such as a rectangle, compared with anti-worm stimuli, which was a rectangle moving perpendicular to the direction of motion. The purpose of the study was to determine how the shape of the stimulus and its movement influenced the toad's behavioral response, as measured by turns per minute as well as its neural response, measured in impulses. In nature, toads generally turn toward potential prey and turn away from a potential predator. Therefore it was expected in this study that the more turns per minute, the higher the likelihood that the toad would respond to the stimulus as it would a potential prey item. From these behavioral responses, researchers were able to match them with corresponding neural responses, particularly from T5(2) cells (Ewert J. , 1980). These cells are most responsive to rectangles between 8° and 16° in length, and moving parallel to their long axis. Ewert concluded that the relationship between shape and movement, particularly movement of stimuli in the direction of the long axis, was the most effective stimulus. Separately neither shape nor direction proved most effective, suggesting that effective prey recognition in toads form as a result of adding separate stimulus properties.

The study mentioned above led us to question how dragonflies discern prey from predator. In a similar experiment, we exposed dragonflies to worm and anti-worm stimuli and measured their neural responses. We also manipulated the velocity of the stimuli to determine whether or not this added dimension affected dragonfly response.

Methods and Materials

Animals

Adult male and female dragonflies (family *Aeshnidae*) captured from central New York were used in this study.

Animal Preparation and Electrophysiology

The dragonfly preparation began with chilling the insect, after which the legs were removed. The leg-less insect was then waxed onto a rod with a beeswax/rosin mixture, ventral side up. The cuticle between the prothoracic and mesothoracic area was carefully dissected, exposing the 2 connectives of the ventral nerve cord. One of the two connectives was cut and then allowed to heal for 15minutes. The rod was then placed in a holder, at a 45°, with the dragonfly positioned ventral side up, facing a 600x600 pixel screen, about 16 centimeters away. A suction electrode was then applied to the descending end of the severed connective. The extracellular signal from a bundle of axons was taken up by the electrode that was amplified (AM Systems Model XXXX), digitized (PowerLab by AD Instruments) and recorded on a Macintosh computer for offline analysis. All data were collected at room temperature. The spike signals were sorted in a clustering program (Dataview, by William Heitler).

Stimuli design

On the computer screen one of 7 stimuli were displayed, shown in **Figure 1**. Each stimulus appeared on-screen, starting at the top and making 7 sweeps to the bottom of the screen, **Figure 2**. The stimuli then moved contralaterally (moving in the direction away from the severed nerve-connective), up, and ispilaterally(moving in the direction toward the severed nerve-connective), each making 7 sweeps. Each stimulus moved at a normal speed, 140°/second and then again at a faster speed, 280°/second.

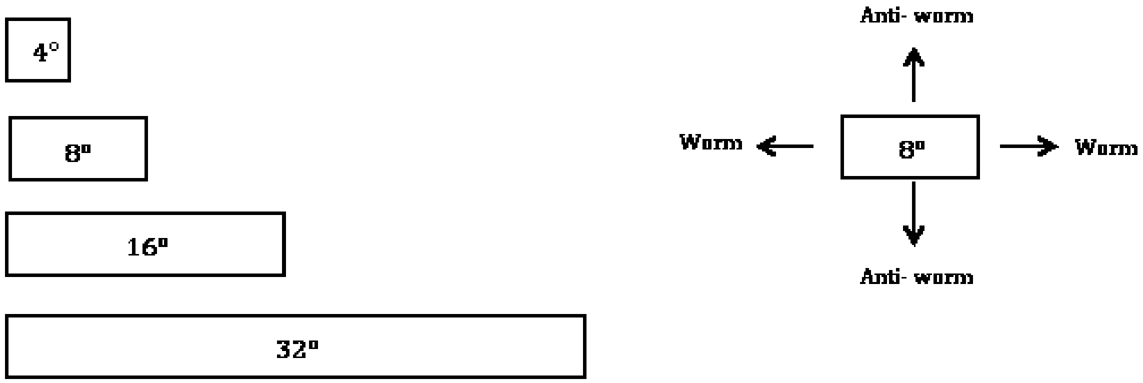

Figure 1. Four types of stimuli presented to the dragonfly. The stimulus was determined to be either worm or anti-worm based on the direction it was moving. When the 8° rectangle on the left moves vertically, it is an anti-worm stimulus because the shape is perpendicular to the motion. When moving horizontally, the stimulus behaves as a worm because its shape is parallel to the direction of movement.

The stimuli presented to the dragonfly were specifically designed to test how pattern expansion either in the direction of movement or perpendicular to the direction of movement affected neural responses. The first stimulus created was a 4° square, used as our control. The worm stimuli we created were based on horizontal growth of their leading edge, that is, increasing the length of the horizontal rectangle (8°, 16° and 32°) while keeping the width (4°) the same. The logarithmic increase in length was meant to test how the neurons responded to both edges and size. In the same way, manipulating the height (8°, 16°, and 32°) of a vertical rectangle while keeping the width (4°) constant was how the anti-worm stimuli were created. Although this was how the stimuli were created and labeled assuming it was only moving horizontally, the worm and anti-worm label varied based on the shape of the stimulus in relation to its motion. An example of this is the stimulus on the left in **Figure 1**. The stimulus was created as a worm, which is that case if it is moved horizontally, but becomes an anti-worm if it moves vertically. Also, the stimuli were black and presented onto a white background.

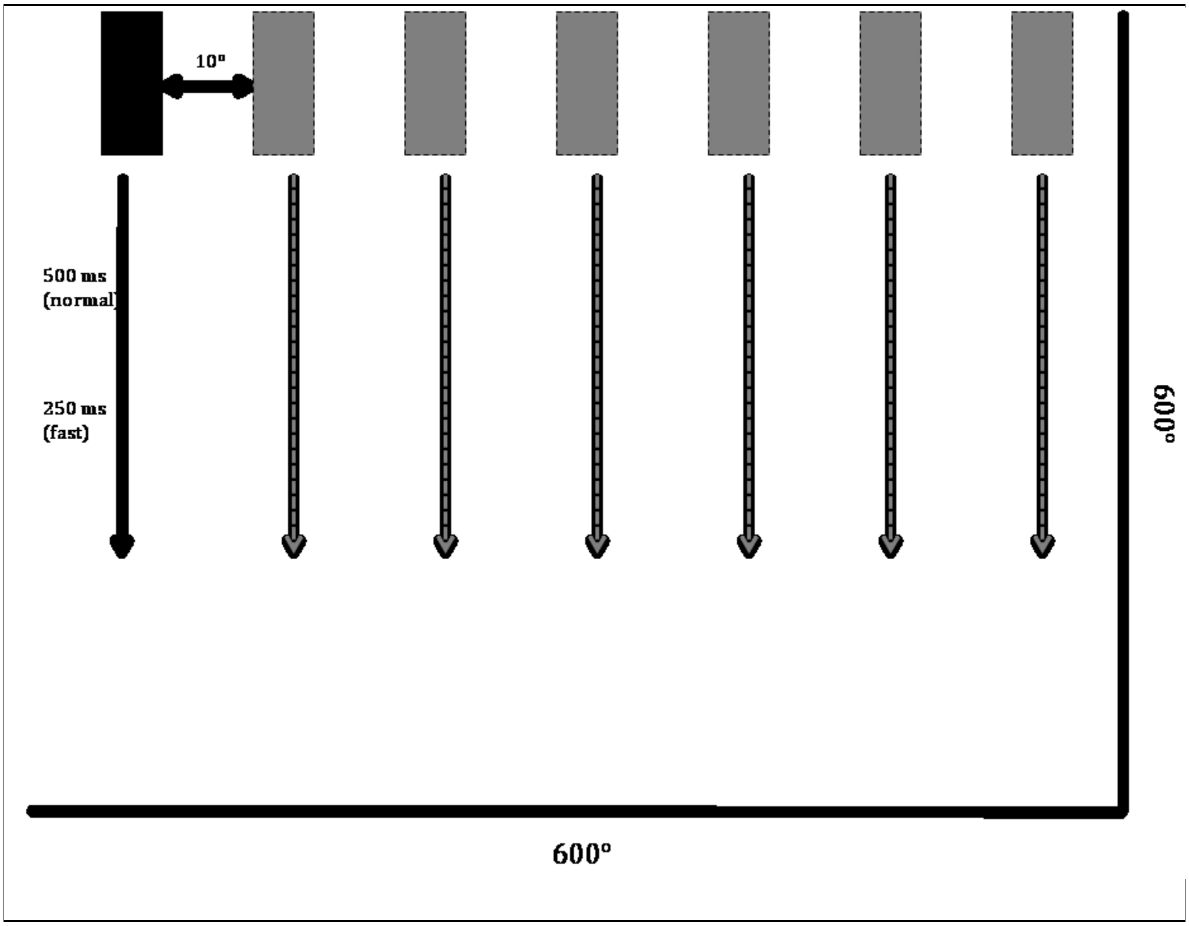

Figure 2. Movement of stimuli on screen. Each stimulus moved in 7 parallel sweeps, 1 movement at a time, 10° apart. The stimuli moving at 140°/second (normal) moved down the screen in 500ms. The stimuli moving at 280°/secong (fast) moved down the screen in 250 ms. This particular example shows downward movement, however, stimuli in each of the other directions (up, ipsilateral, and contralateral) also moved 10° apart in 7 parallel sweeps.

Receptive Field Analysis

To determine receptive field, that is the area of the dragonfly's visual field that issues the best response, we used a 4° circle that moved across screen, with 7 sweeps in 4 directions (up, down, left, right) as depicted in **Figure 2**. This data was then analyzed using a MatLab program that plotted neural activity caused by each stimulus, and then sorted by direction. An example of this is shown in **Figure 3**, where this particular neuron prefers ipsilateral (moving in the direction of the cut nerve connective) movement.

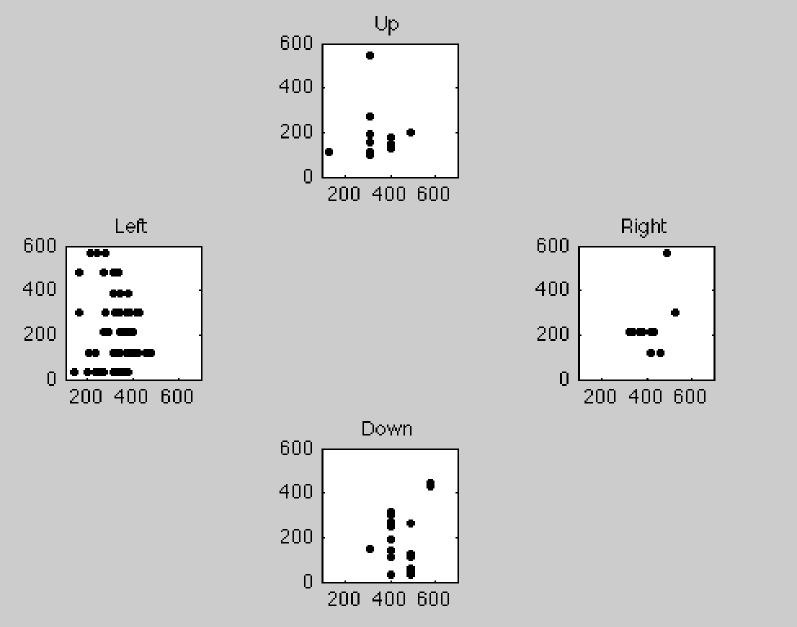

Figure 3. Receptive fields were used to determine directional preference for each unit. Receptive fields such as this was made for each unit to determine directional selectivity. In this particular example, the unit responds best to ipsilateral movement.

Results

We collected data from 18 *Aeshnidae* dragonflies, both male and female, and analyzed the data from 3 of the best trials. The recorded neural responses were classified as TSDNs based on strong responses to the size and direction of target movements. The data plotted below have length or height of stimulus, for worm or anti-worm respectively, on the x-axis, showing progressive stimulus growth. On the y-axis is the spike count, which corresponds to the number of neural responses for that stimulus.

Upward movement

Putatively, we believe that the TSDN corresponds with the pattern of preference for upward movement is DIT3 (Frye & Olberg, 1995). From Frye and Olberg (1995), we know that DIT3 prefers upward movement and looming stimuli. Looming stimuli can be characterized as a target that appears to be approaching. Neurons that respond to looming targets have also been studied in locusts (Rind & Santer, 2004), with results that show the closer the approach of the stimulus, the more observable spike activity. Here, in response to normal speed (140°/second), no consistent trend for stimuli size emerges, with the best worm response to the 8° target moving at the normal speed and the best anti-worm response to the 32° target. Worm data, at both speeds, shows a preference for smaller targets whereas anti-worm data shows preference for increasingly large stimuli (normal speed). There is a noticeable trend for the faster speed (280°/second), in which there is a consistent number of responses to the 4°, 8°, and 32° targets and a response half the spike count for the 16° target for both worm and anti-worm data.

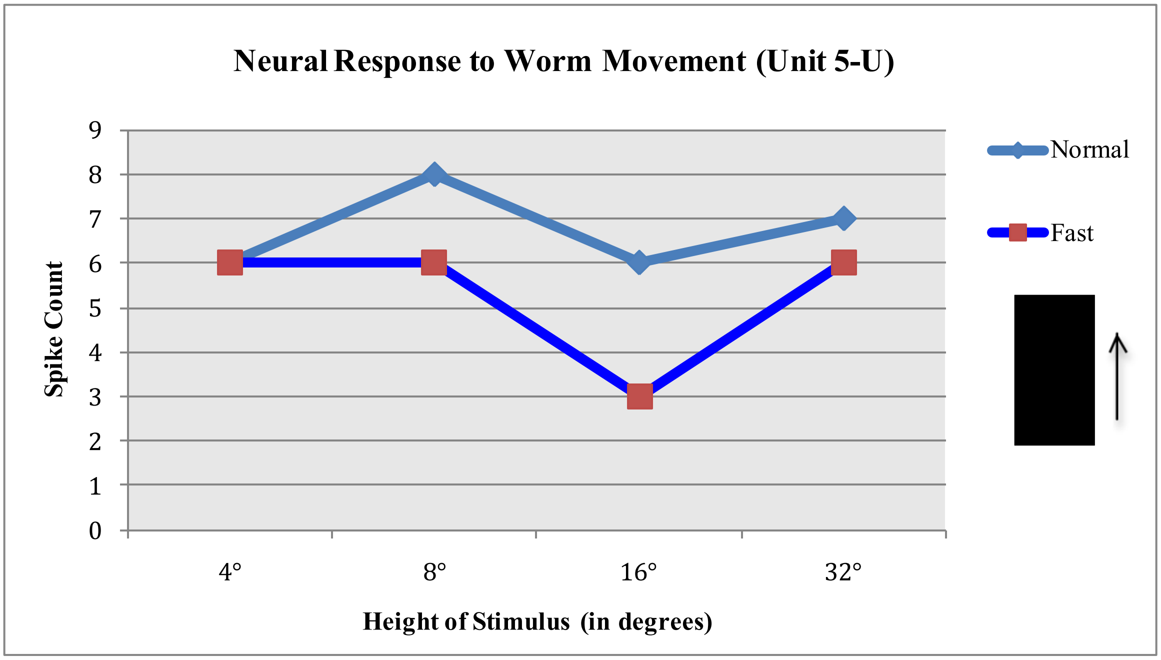

Figure 4. Upward unit response to Worm stimuli. This data suggests a preference for smaller stimuli when they are moving along the long axis.

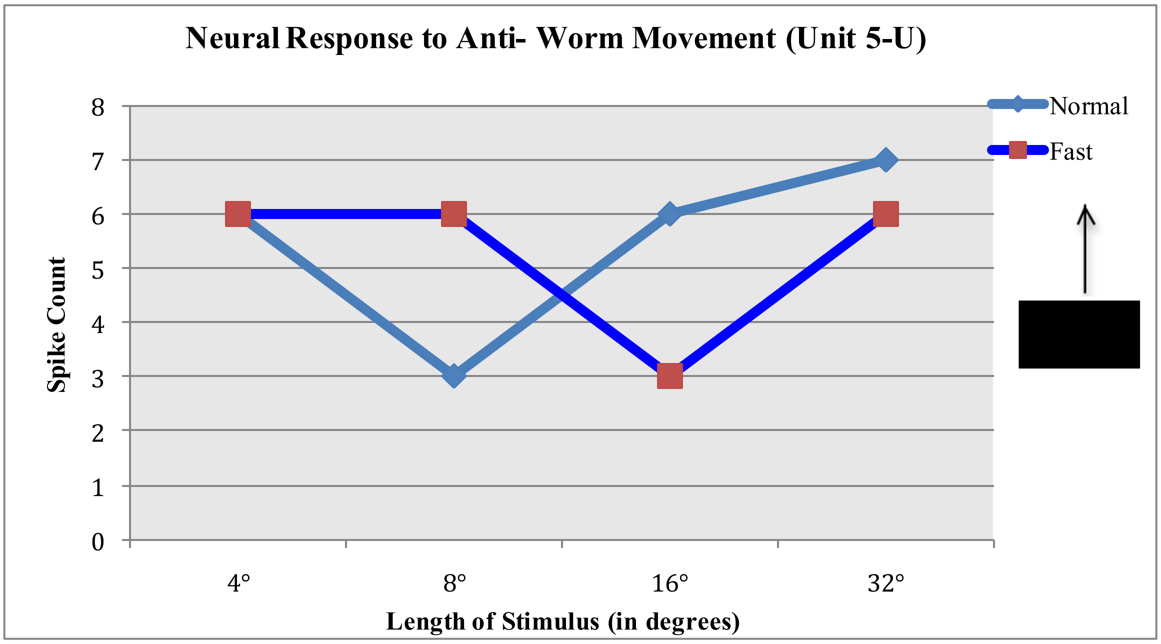

Figure 5. Upward unit response to Anti-Worm stimuli. This data suggests that at normal speeds (140°/second) there is a preference for increasing large targets whereas at the faster speed (280°/second) show no such trend.

Contralateral Movement

The unit that responds best to contralateral movement, defined as moving in the direction opposite of the connective cut, and smaller stimuli we hypothesize to be DIT1 (Frye & Olberg, 1995). DIT1 responds strongly to smaller targets along the midline and contralateral motion. At normal speeds (140°/second) for both worm and anti-worm data we see a fall off response as the target gets larger. More specifically, for the worm stimuli, there are stronger responses to both the 4° and 8° targets whereas in the anti-worm stimuli, there is a dramatic decrease from the activity in the 4° target to the 8° target. As in the upward selective unit, the faster speed (240°/second) had a consistent response in both the worm and anti-worm data; there was a parabolic response to the increasing stimuli. For this unit, neural activity favors the worm stimuli.

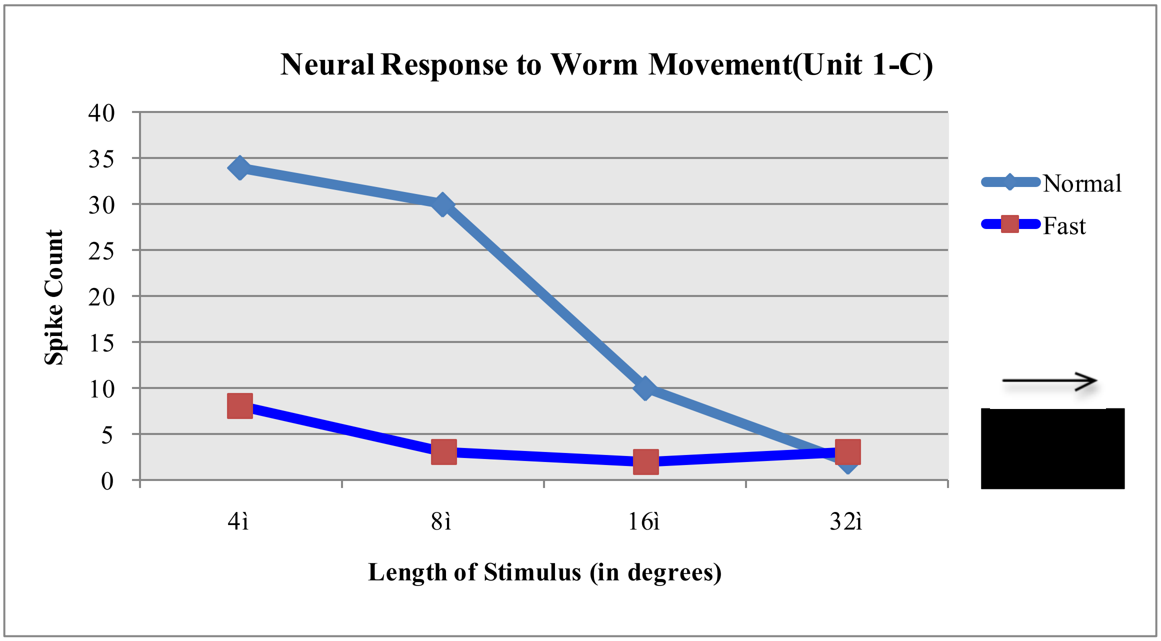

Figure 6. Contralateral unit response to worm stimuli. At the normal speed (140°/second) there is a stronger response than at the faster speed (280°/second). At both speeds there is a decrease in neural activity as the size of the stimulus increases.

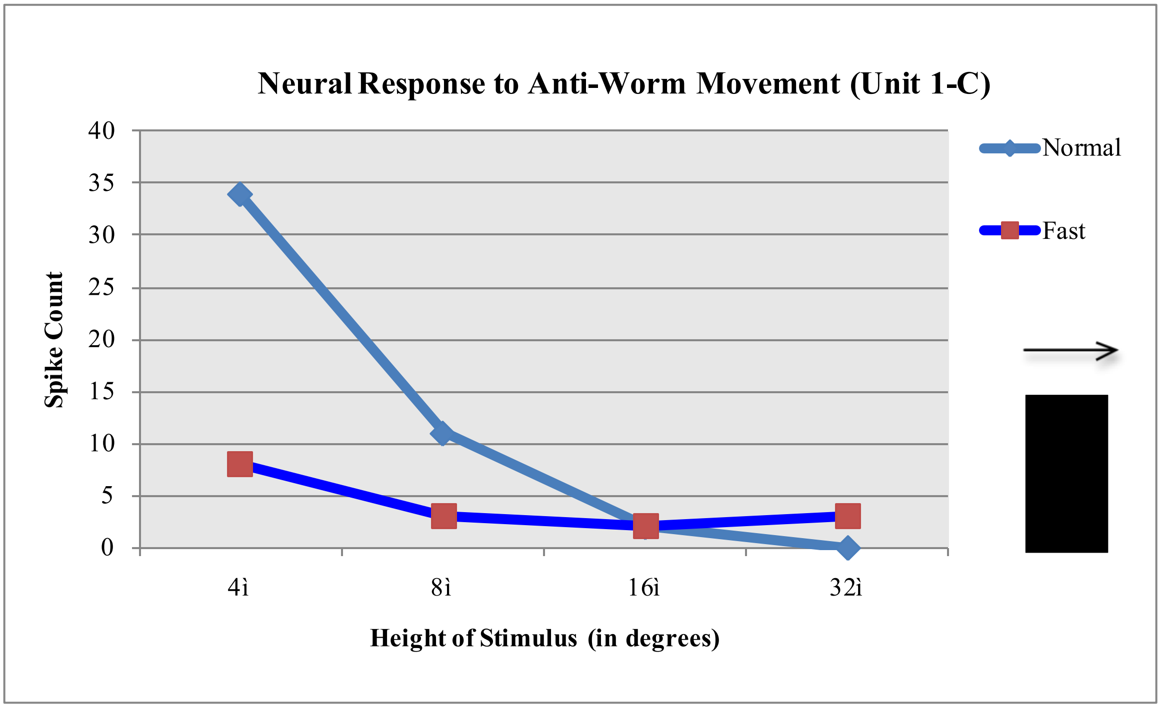

Figure 7. Contralateral unit response to anti-worm stimuli. The contralateral unit, believed to be DIT1, has a decrease in spike count, in both speeds as the height of the stimulus increases.

Ipsilateral Movement

The data from the ipsilateral movement is not clear enough to suggest only one possible TSDN responding, rather, the unit responding may either be MDT2 or DIT2 or some combination of the two. MDT2 responds better to smaller targets, whereas DIT2 responds to a wider range of stimuli size (Frye & Olberg, 1995). However both prefer movements away from the midline and have large lateral receptive fields. At the normal speed (140°/second) for both worm and anti-worm data, there is a decrease in response as the stimulus gets larger. Similar to the contralateral unit, there is a larger decrease from the 4° target response to the 8° target response in the anti-worm data as compared with the worm data. Similarly, for the faster speed (280°/second) there is a decrease in response to increasing target size. The trend for both normal and fast speed in the worm data show a continued decrease to increasing worm size. For the anti-worm data, there is a peak response to the 8° stimulus at the faster speed whereas the normal speed shows a general decline to growing anti-worm stimuli. MDT2 appears to be responding to the worm movement because of the strong selectivity for the smaller targets. However, in the anti-worm movement, it is unclear whether MDT2 is responding on its own, or if there is input from DIT2.

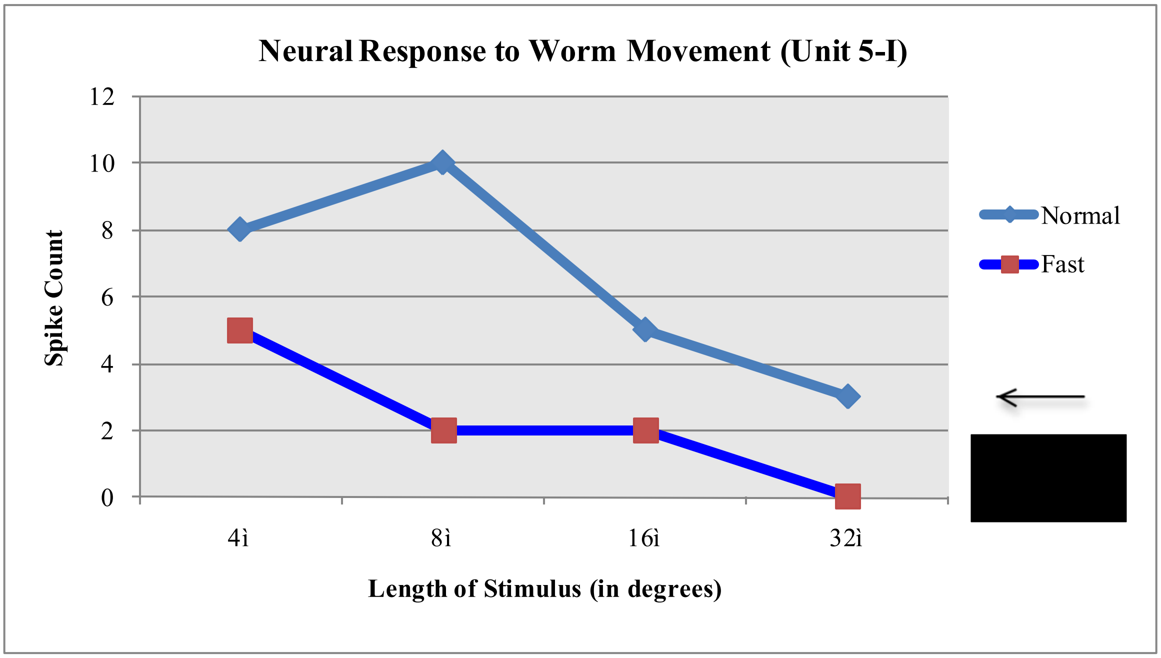

Figure 8. Ispilateral response to worm stimuli. Both speeds have a decline in response to increasing worm size.

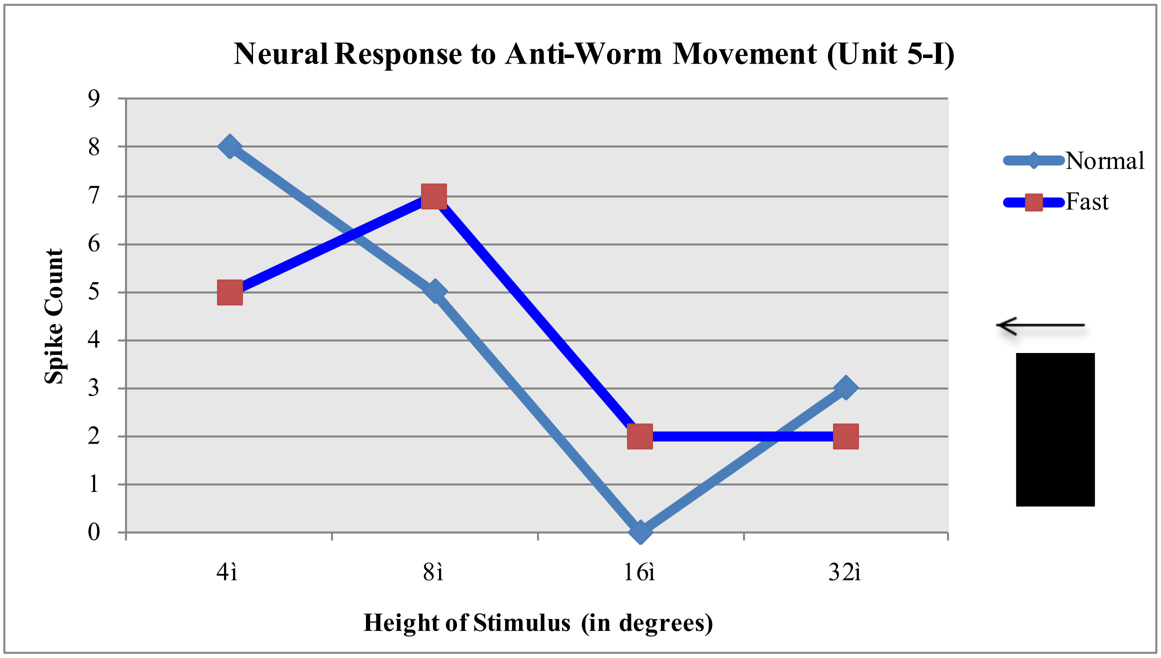

Figure 9. Ispilateral unit response to anti-worm stimuli. The normal speed response immediately declines as the target size increases. In the faster speed, there is first a peak response to the 8° target before there is a decline.

Downward Movement

Based on the unit's preference for downward motion and smaller targets, we hypothesize that MDT4 may be responding (Frye & Olberg, 1995). The normal speed (140°/second) of the stimuli for both worm and anti-worm data show a very similar and almost identical decrease in response to an increasing target size. In both instances, there is about a 25 spike count difference between the 4° stimulus and the 8°. Similarly, the fast speed (280°/second) also has a fall off in response for both worm and anti-worm data, however the decline is not as steep. Overall, for both worm and anti-worm data, there is a consistent decline, and therefore neither stimulus appears to be preferred.

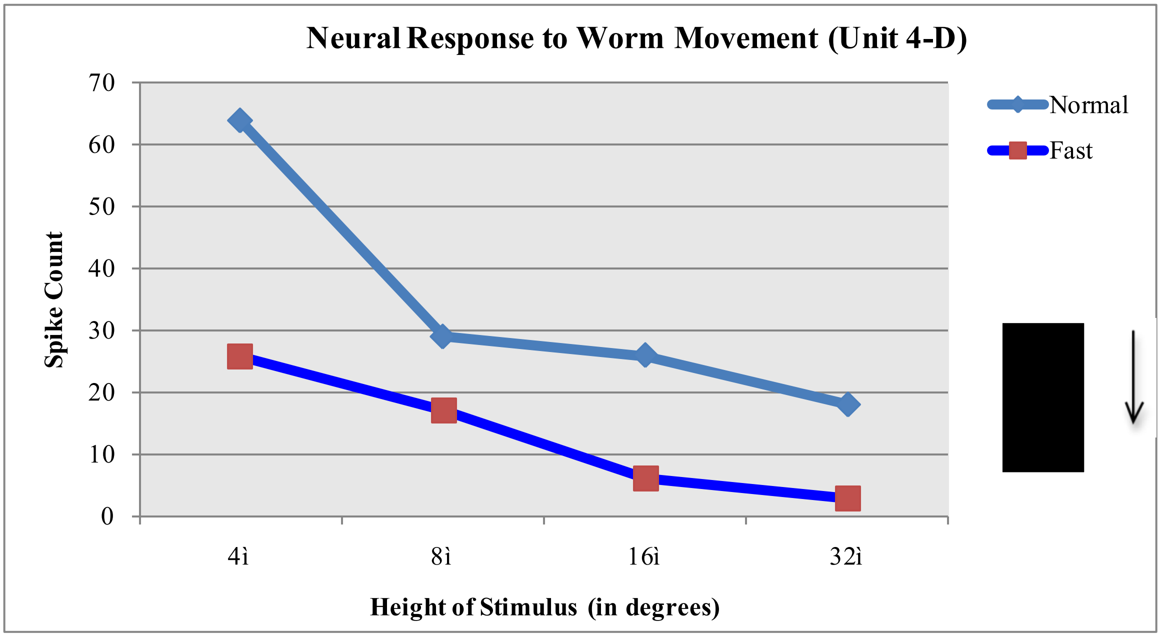

Figure 10. Downward unit response to worm stimuli. At both speeds, the smallest stimulus is preferred. Based on this selectivity of direction and stimuli size, we believe that MDT4 is responding.

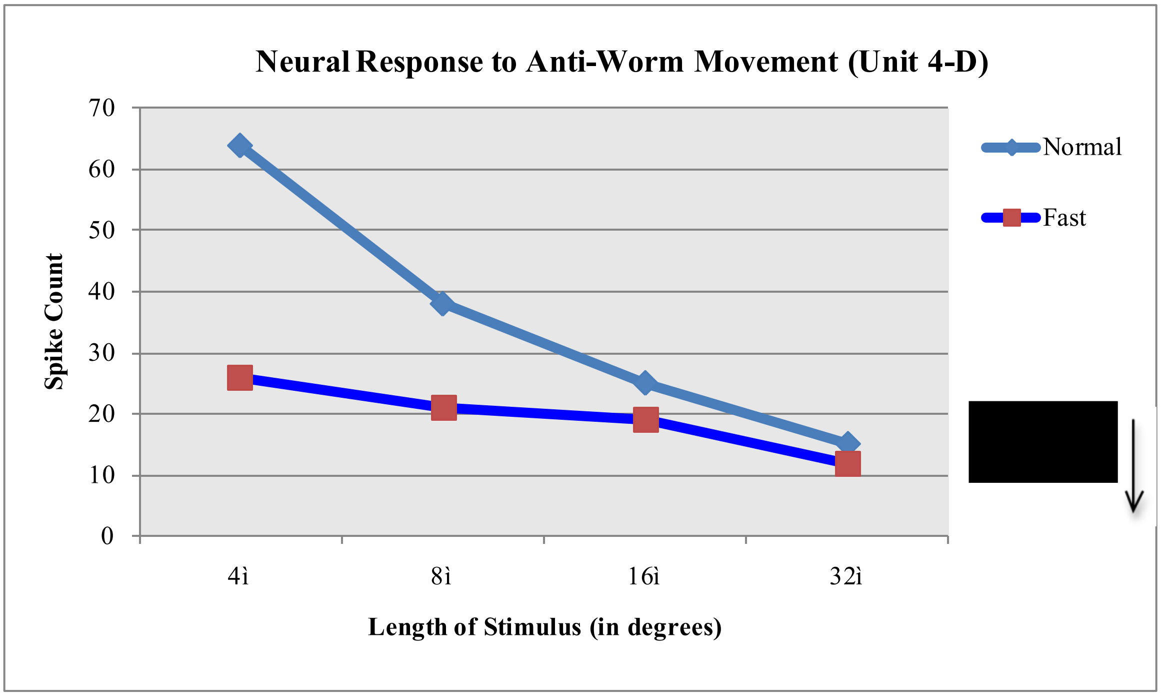

Figure 11. Downward unit response to anti-worm stimuli. As also seen in the worm data for the downward unit, there is a strong preference for the 4° target, regardless of speed, and a fall off response for larger stimuli.

Discussion

For most, but not all of the TSDNs analyzed, we saw a decreased response with expansion in either the direction of movement. Unlike the T5(2) cells in the *Bufo bufo*, dragonfly TSDNs respond best to smaller stimuli. The stimuli more closely resemble prey items for a toad rather than dragonfly prey, and that may be the cause for this variation in response. Dragonflies often eat insects smaller than themselves, including mosquitoes, gnats, flies, and moths (R.Bell & Whitcomb, 1961).

A study in screech owls (Marti & Hogue, 1979) explains why predators in general prefer smaller prey to larger ones, a notion that may provide insight into the neural responses we saw for worm and anti-worm stimuli. A brief summary for a smaller prey preference include: 1) prey species that are smaller tend to be younger and less experienced 2) energy used to capture and kill large prey may not be worth the effort, especially since these prey may have more experience or are stronger 3) there could be a greater risk for injury from a larger item of prey. Dragonflies have two methods of foraging, either by being a hawker or percher; hawkers actively seek prey while perchers sit and wait for prey to come within their vicinity. In a foraging study (Baird & May, 1997) with perching dragonflies, *Pachydiplax longipennis,* there is data that supports the notion that prey of a certain size are preferred. Prey that are too small or large are not sought after because of expenditure of energy required to capture them. Although the dragonflies used in our electrophysiogical experiments are hawkers, there may be a similar behavioral response when preys of various sizes are presented. The dragonfly's TSDNs are hypothesized to steer the dragonfly during prey tracking (Frye & Olberg, 1995), therefore there is a possibility that the stimuli presented here were determined not to be worth taking, since there is less response to both worm and anti-worm rectangles versus that of the 4°square.

In some insects, such as the fly, butterfly and locust, there is data that support the presence of neurons that are either 'fast' or 'slow' (Horridge & Marcelja, 1992) in response to certain frequencies, a factor indirectly studied in this experiment. In our experiment we manipulated the speed of our stimuli, either at normal speed (140°/second) or faster speed (280°/second). The stimuli moving at the faster speed appeared onscreen for a half the amount of time as the normal speed, 250ms for faster and 500 ms for slower. To fairly compare the obtained results at the different speeds, we doubled the spike count for the fast data so that the onscreen time would be the same. As a result, there was a greater response to both worm and anti-worm stimuli for faster speeds than normal speeds, however, the response to 4° targets were still the strongest. From a study by Horridge and Marcelja (1992), we learn that fast neurons respond to a peak frequency at 15-29 Hz while responses of slow neurons peak at 1-10 Hz. With this specialization of cells, we can hypothesize that speed, much like size and direction, is another important factor in an animal's evaluation of prey pursuit.

The ability to differentiate prey from predator is a basic survival skill. Although thousands of years of evolution appears to have selected for animals that can efficiently and effectively use their senses to help them escape from danger, the process by which this occurs remains a mystery. There are several theories to explain how we can identify our environment, and objects in it that may or may not be harmful. These theories include objection recognition, recognition neurons, and spatiotemporal pattern recognizers. The object recognition theory has its basis in the belief that the brain contains a list of feature detecting neurons that recognizes a whole object, even at different angles, instead of a combination of its parts. The theory of recognition neurons (Haber & Hershenson, 1980) involves the brain recognizing certain features and associating them with particular objects to help identify it. The final and most probable hypothesis is that of spatiotemporal pattern recognizer in which a limited group of neurons would all be activated by all perceived objects, but in a different temporal pattern. Such may be the case when *Bufo bufo* and dragonflies perceive stimuli.

Future research on discerning prey from predator may be able to better address some of the shortcomings of this experiment. Perhaps behavior studies can be carried out to get a better understanding of how the dragonfly will respond or interact with such objects instead of only collecting data from its neurons. This behavioral study may be achieved by allowing the animal to move freely. Additionally, studies that manipulate stimuli size, perhaps decreasing stimuli size instead of increasing it are worth considering. Finally, considering how recently the dragonfly has been fed may be another factor worth considering; if the dragonfly has not recently eaten, it may be more apt to respond to a wide range of stimuli sizes.

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