SENSORY CODING IN AN IDENTIFIED MOTION-SENSITIVE
VISUAL NEURON OF THE LOCUST (*Locusta migratoria*)

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By

Glyn Allan McMillan

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ABSTRACT

Visual environments may contain a complex combination of object motion. Animals respond to features of complexity by generating adaptive behavioural responses. One important feature of a complex visual environment is a rapidly expanding object in the visual field (looming) which may represent an approaching predator or an object on a collision path. Many animals respond to looming objects by generating avoidance behaviours (Maier et al. 2004; Santer et al. 2005; Oliva et al. 2007) and neurons involved in the detection and relay of looming stimuli are present in birds (Sun and Frost 1998) and many insects (Simmons and Rind 1992; Hatsopoulos et al. 1995; Wicklein and Strausfeld 2000). One of the most widely studied visual pathways is found in the locust. This visual pathway, which includes the lobula giant motion detector (LGMD) and its post-synaptic target, the descending contralateral motion detector (DCMD), signals the approach a looming visual stimulus (Schlotterer, 1977; Simmons and Rind, 1992; Hatsopoulos et al., 1995). The DCMD descends through the ventral nerve cord and synapses with motorneurons involved in predator evasion and collision avoidance (Simmons, 1980; Simmons and Rind, 1992; Santer et al., 2006).

Previous studies have suggested that this pathway is also affected by more complicated movements in the locust's visual environment. For example, Guest and Gray (2006) demonstrated that the approach of paired objects in the azimuthal position and approaches at different time intervals affect DCMD firing rate properties. In my first objective of this thesis (Chapter 2), I tested locusts with computer-generated discs that traveled along a combination of non-colliding (translating) and colliding (looming) trajectories and demonstrate how distinctly different DCMD responses result from different trajectory types. In addition to estimating the time of collision and direction of object travel, the presence of a discernable peak associated with

the time of object deviation suggests that DCMD responses may contain information related to changes in motion.

Previous studies suggest that LGMD/DCMD encodes approaching objects using rate coding; edge expansion of approaching objects causes an increased rate of neuronal firing (Schlotterer, 1977; Hatsopoulos et al., 1995; Judge and Rind, 1997; Gabbiani et al., 1999). Based on observations of DCMD responses to simple looming objects that showed oscillations in DCMD responses (for example, Fig. 1D Santer et al., 2006) and the fact that bursting occurs in many other sensory systems (Yu and Margoliash, 1996; Sherman, 2001; Krahe and Gabbiani, 2004; Marsat and Pollack, 2006), it was hypothesized that the DCMD may show bursting activity. In my second objective of this thesis (Chapter 3), I tested locusts with simple looming stimuli known to generate behavioural responses in order to identify and quantify bursting activity. Results show that the highest frequency of bursts occurred at intervals of 40-50 ms (20-25 Hz). The behavioural significance of this frequency is related to the average wingbeat frequency of the locust's forewing during flight (~25 Hz; Robertson and Johnson, 1993). Based on previous evidence of DCMD flight-gating (see, for example, Santer et al., 2006), bursting may gate information into the flight circuitry, thereby providing visual feedback that may be modified to generate an avoidance response during flight. Single spiking and bursting occurred throughout object approach up until the late stage of approach, where burst frequency rapidly increased. Results predict that the DMCD may use a bimodal coding strategy to detect looming visual stimuli, where single spiking at the beginning of approach may result in subtle course changes during flight and bursting near the time of collision may initiate an evasive glide.

Taken together, these results illustrate that the encoding of visual stimuli in single neurons is dynamic and likely much more complicated than previously thought.

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LIST OF ABBREVIATIONS

CPG   - Central pattern generator

CNS   - Central nervous system

DCMD   - Descending contralateral motion detector

DIMD   - Descending ipsilateral movement detector

EPSP   - Excitatory post-synaptic potential

IBI   - Interburst interval

ISI   - Interspike interval

LGMD   - Lobula giant motion detector

PNS   - Peripheral nervous system

TOC   - Time of collision

TOD   - Time of deviation

TA90°   - Time at 90° degrees azimuth

VNC   - Ventral nerve cord

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 NEUROETHOLOGY

Neuroethology is a relatively new scientific study of the neuronal mechanisms involved in adaptive animal behaviours (Zupanc, 2004). Neuroethologists seek to discover general principles of how animals respond to features in their sensory environments, both behaviourally and neurologically. One of the first steps in understanding the neuronal control of behaviour is to correlate a neuronal response to a biologically relevant stimulus (Zupanc, 2004).

1.2 GENERAL PRINCIPLES OF SENSORY CODING

1.2.1 Sensory environments

The natural environment of an animal consists of a complex combination of sensory stimuli. These stimuli can include auditory, visual, mechanical, chemical, electrical, and more, in a number of combinations and varying ranges of intensities. Animals have evolved in different ways to detect and respond to different environmental stimuli in a context-dependent manner, where only specific features are needed to be extracted from the environment to survive. For example, the frog's eye contains feature detector neurons that can extract specific features of its visual world such as small, moving dark objects (about the size of a fly) and transmit information

about them to the brain (Barlow, 1953). In a complex sensory environment, specific sensory cues often initiate adaptive behavioural responses (Guest and Gray, 2006). An animal only needs to detect and react to specific features in its sensory environment which are biologically relevant; detection of more than what is needed at a particular time may be maladaptive. For example, images of rapidly approaching objects have been shown to be a dominant visual stimulus in eliciting avoidance reactions in many animal species (Maier et al. 2004; Santer et al. 2005; Oliva et al. 2007; Judge and Rind, 1997). Flying insects, such as locusts, are presented with a complex visual environment that includes self motion (changing scenery as it moves) in addition to object motion (such as conspecifics or potential predators). In such a complex visual scene, detection of approaching objects and rapid maneuverability is highly adaptive. The identification of significant features of an animal's sensory environment is pivotal in understanding and correlating neuronal and behavioural responses.

1.2.2 Sensory coding

The firing properties of a neuron, i.e. the generation of all-or-nothing action potentials, define a neuronal response to a particular stimulus. This is known as sensory coding. Different anatomical structures are responsible for the detection and transduction of different sensory stimuli into an electrical signal. For example, photoreceptors are sensitive to changes in light and through a complicated cascade of biochemical interactions, generate encoded electrical signals that are transmitted and integrated by many neurons, ultimately leading to visual perception. Coded electrical signals (action potentials) are attributable to ion channels, integral cell membrane proteins that facilitate the movement of their ionic substrates across the lipid bilayer of the cell membrane (Hill et al., 2004). Ion channels are passive transporters and the direction of

transport is dictated by the difference in electrochemical potential of the substrate; since ions tend to move from higher to lower concentrations and toward opposing charges (Hill et al., 2004).

How a specific behaviour is generated through sensory coding is of particular interest in neuroethology. For example, certain visual stimuli such as very rapidly approaching objects fire the medial giant neurons that are partly responsible for the tail-flip escape behaviour in the crayfish (Wine et al., 1972). Mating behaviour in the horseshoe crab has been linked to simple patterns of consistent activity in their optic nerve that extend over small ensembles of nerve fibers and contribute to the neural coding of its eye by transmitting robust "neural images" of objects having the size, contrast, and motion of potential mates (Passaglia et al., 1997). In order for the horseshoe crab to find a mate quickly and perform a mating behaviour, it must focus on visual stimuli that match specific visual parameters (Passaglia et al., 1997). While links between behaviour and neuronal responses may be found, the neuronal properties and mechanisms of information transfer in neuronal networks are often not fully understood. Investigations into what neuronal information is transferred leads to the question of how information is transferred.

1.2.3 Rate coding versus time coding

The sequence of all-or-nothing action potentials (spikes) in a given time interval is known as a spike train (Stein et al., 2005). For many years, neuroscientists have debated how spike trains code information about an animal's sensory environment (see Stein et al., 2005). The basic measurement of neuronal activity is its mean firing rate, which is calculated from the total number of spikes in a given period of time and is expressed in impulses/sec or hertz (Israel and Burchiel, 2005). Any variation in the time between two successive spikes in a spike train (the

inter-spike interval or ISI) will cause variation in the rate. Early investigations showed that the intensity of a stimulus is coded as a rate of impulses over time (i.e. rate coded) and variability in the timing was previously interpreted as neural noise (Stein et al., 2005). However, a sequence of action potentials generated at specific time intervals (a time code) may also be important in the flow of neural information (Salinas and Sejnowski, 2001). Therefore a more relevant measurement of the neuronal activity in a neuron may be the firing pattern, which is the signaling code by which neurons communicate (Israel and Burchiel, 2005).

Neural information may be encoded in the precise timing of spikes, rather than neuronal firing rates, and spike time and mean firing rate codes may be used independently to represent different aspects of a stimulus variable (Krahe and Gabbiani, 2004; VanRullen et al., 2005). If information provided by sensory neurons is effectively transmitted only during a certain time window, the timing of spikes may be important in gating processes that control information that goes through at a given time (Salinas and Sejnowski, 2001). However, a spike that may be timed exactly to some internal event may be considered unreliable if the observer is not aware of such an event (VanRullen et al., 2005). There are two binary choices that a neuron will display, where the presence of an all-or-none action potential is represented as a '1' and the absence is represented as a '0'. A signal in a sequence of '100' (spike, no spike, no spike) in a period of 3 ms, would be interpreted as a rate of 1 spike in 3 ms. This rate is the same if the sequence were 010 or 001, so time and rate codes are not mutually exclusive and can be used in the same sensory neurons (Stein et al., 2005). Quantifying the firing pattern of a neuron involves several factors in addition to firing rate, such as the degree of temporal and spatial synchronization of spikes, the degree and duration of possible bursting activity, and the length of inter-spike/inter-burst intervals (Israel and Burchiel, 2005).

The variability in the ISIs of individual neurons is more than 'neural noise' and the timing of each spike in a train may transmit extra information (Stein et al., 2005). Therefore, variability in a firing rate may be an important part of the signal. In sensory neurons, even if variability in spiking may not be part of a sensory signal, it may be an important part of the accurate processing of the signal (Stein et al., 2005). For example, in terms of visual sensory systems, photons of light arrive randomly in time and a single photon can often lead to perception (Pirenne, 1959). The variability in the timing of spikes is related to the irregular arrival of photons and the presence of variability in visual sensory systems may be the result of highly sensitive sensors (Stein et al., 2005). Thus, variability in encoding strategies (known as stochasticity) can be very beneficial in sensory systems. The meaning or relevance of such apparent randomness, however, is not clear in many systems (Tuckwell, 2007).

Examining the ISI distribution of a spike train and determining the frequency of occurrence of the various intervals at which the neuron discharges (plotted in the form of a histogram) may provide information about the fundamental firing properties of the neuron. For example, tonically firing neurons display little variation in ISIs and their histograms are usually unimodal, symmetrical, and exhibit a near normal distribution (Israel and Burchiel, 2005). ISI histograms that are unimodal and positively skewed toward long intervals with no clear firing pattern are typically irregularly firing neurons, while a bimodal ISI distribution will reveal a bursting neuron. The early peak represents the ISI of spikes within the burst, whereas the later peak corresponds to the longer intervals occurring between bursts or non-burst-related spikes (Israel and Burchiel, 2005). Neurons with a bimodal ISI distribution are common (Rowat, 2007) and often represent an oscillatory or bursting property of the cell.

1.2.4 Oscillations and bursting

The output of a neuron may be determined by the firing properties of its inputs, intrinsic mechanisms within the cell, and also by their correlations (Salina and Sejnowski, 2001). Neurons that are sensitive to correlated activity are able to extract more information from their inputs (Salina and Sejnowski, 2001). The most common result of correlated activity are oscillations (rhythmic and endogenous activity of spike(s) within a spike train), and literature showing that neural circuits have oscillatory activity is vast (for review see Salina and Sejnowski, 2001). For example, spikes in the antennal lobe of insects are typically synchronized by 20 Hz oscillations and it has been shown that the disruption of synchrony influences behaviour (Salina and Sejnowski, 2001). Oscillations have also been observed in the mammalian olfactory bulb (Salina and Sejnowski, 2001).

Rather than representing the meaning of neural information, correlations often regulate the flow of information, as they are known to change rapidly as functions of internal events (Salina and Sejnowski, 2001). Neuromodulators may shift the state of neuronal cells, giving rise to oscillatory activity (Salina and Sejnowski, 2001). Experiments in invertebrates have shown that neurons can perform such switching, with highly specific behavioural consequences (Salina and Sejnowski, 2001). For example, a neuron that is part of the pyloric neural network of the lobster stomatogastric nervous system leaves this network and fires exclusively with the cardiac sac network whenever the cardiac sac network is active (Hooper and Moulins, 1989). Sensory inputs control, in part, the long-lasting neuromodulatory influences that are responsible for switching neurons from one network to another (Hooper and Moulins, 1989).

Oscillations may be able to signal the presence of a stimulus in the absence of changes in firing rate (Salina and Sejnowski, 2001). In addition, a particular spike relative to an oscillation

can also carry information (Salina and Sejnowski, 2001). Stein and colleagues (2005) suggest that oscillations can convert a rate code into a temporal code, which may effectively bind neurons into ensembles that function together. For example, in addition to from the information carried by the firing rate, the phase of a spike in a pyramidal cell from the rat hippocampus can encode the number of inhibitory inputs impinging on it (Salina and Sejnowski, 2001). These cells fire according to the location of the animal in the world (producing a place field) and the phase of their spikes relative to an underlying oscillation encodes where the animal is located within this field (Salina and Sejnowski, 2001).

While oscillations are a property of periodicity in a spike train, bursting is defined as a specific period in a spike train which may or may not occur rhythmically (Kaneoke and Vitek, 1996). Bursting activity of neurons consists of periodic clustering of electrical impulses. These brief episodes of high-frequency firing constitute a specific neural code. Bursting occurs in many animal systems and many different nerve and endocrine cells (Krahe and Gabbiani 2004; Sabourin and Pollack, 2009). Burst firing can be the result of high-frequency activation of neurons by sensory input or generated through the activation of intrinsic cellular mechanisms (Krahe and Gabbiani, 2004).

Evidence of oscillatory and bursting activity may be revealed by examining the ISI histogram of a spike train (discussed in section 1.2.3), or by using a power spectral density analysis method. A power spectrum gives the distribution of all frequency components in the signal and their relative contribution to the signal (Israel and Burchiel, 2005). Using an ISI distribution in addition to a power spectral analysis method can provide substantial evidence to determine whether a neuron is bursting.

Bursting has many physiological effects such as avoiding desensitization of receptors (e.g. Zwislocki, 2002), amplifying neurotransmitter secretion (e.g. Li et al., 1998), and relieving presynaptic inhibition (Brody et al., 1997). Burst firing is important in improving the reliability of sensory information transmission across unreliable synapses, enhancing information transfer, and may improve the signal-to-noise ratio of neuronal responses (reviewed in Krahe and Gabbiani, 2004). However it is still unknown whether the role of bursts in sensory coding is related to the first spike in the burst or their internal temporal structure, which may carry additional information (Krahe and Gabbiani, 2004). Bursts can range from tens of milliseconds (common in many neurons) to a few seconds to several minutes. Because bursting encodes two time scales, while periodic spiking encodes only one, bursting is more robust than a single spike and is therefore useful in central pattern generators (CPG).

Sensory bursts are thought to be important in signaling the occurrence of behaviourally relevant sensory events by extracting and signaling important features of a stimulus (Krahe and Gabbiani, 2004; Marsat and Pollack, 2006; Sabourin and Pollack, 2009). For example, sensory burst activity in cells of the geniculate nucleus of mammals is known to signal large light intensity changes typical of moving scenery and pyramidal cells in the electro-sensory lateral line lobe of weakly electric fish show bursts that are associated with electric field distortions caused by moving prey (Krahe and Gabbiani 2004). Although sensory bursts are believed to signal the occurrence of particularly important stimulus features, until recently the behavioural relevance of sensory bursts had not been established in any system. Marsat and Pollack (2006) demonstrated that burst activity in an identified ultrasound-sensitive auditory interneuron reliably signals significant stimulus features of echo-locating bats that use ultrasonic probes to hunt. Crickets respond to ultrasound stimuli with avoidance responses that are triggered by this

interneuron (Marsat and Pollack, 2006). Therefore, it has now been shown that a single interneuron may be able to predict behavioural responses through bursting activity.

1.3 INSECT VISION

Vision is one of the most complex, fascinating, and widely studied systems in animal physiology and the reception of light, transduction into bioelectrical signals, and overall significance in an animal's life are major topics of current biological research (Zupanc, 2004). The most important aspects of visual stimuli that influence an animal's behaviour are the patterns of light, dark and colour, rather than overall light intensity (Hill et al., 2004). Spatial patterns of visual stimuli represent objects in the visual field and temporal patterns of visual stimuli specify movement (Hill et al., 2004). Vision begins in the photoreceptors, where single photons of light are absorbed and neural signals are generated (Yau, 1994).

How an insect accomplishes vision is quite well understood. Insects have compound eyes that are composed of photoreceptors mostly of the rhabdomeric or microvillar type, which are characterized by wide finger-like invaginations of the cellular membrane, called microvilli (Santillo et al., 2006). One of the most commonly studied insect eyes is the compound eye of *Drosophila melanogaster*, which is composed of ~800 single eyes, called ommatidia. Each ommatidium contains 8 microvillar photoreceptor cells (Hardie, 2001). Predators and fast flying insect species have the greatest number of ommatidia for increased visual acuity, movement awareness, and increased depth perception (Burrows, 1996). Locusts, for example, have an apposition type of compound eye, which is most common in day flying insects (Fig. 1.1).
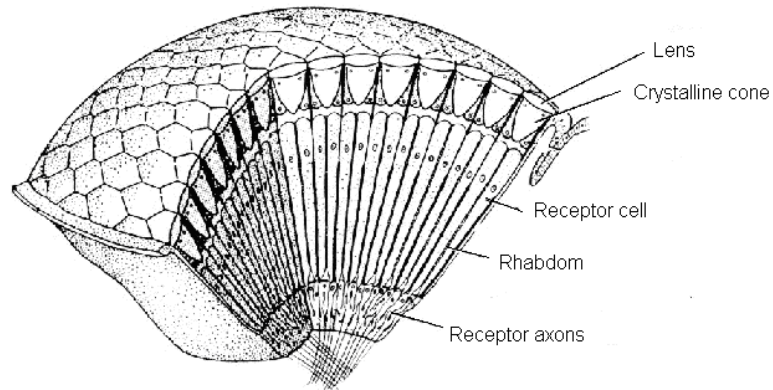
Figure 1.1. The apposition type of compound eye found in locusts. The locust's compound eye is made up of many separate photoreceptors, called ommatidia, and each receptor is at a slightly different angle which permits a wide-angle view of the environment (Burrows, 1996). Each ommatidium is isolated from one another by pigment cells (Burrows, 1996). In the apposition type of compound eye, the lens and crystalline cone form the light gathering apparatus, while the rhabdom is the light sensing apparatus (Burrows, 1996). (Modified from Fig. 7.3 Land and Nilsson, 2002).

Microvillar photoreceptors form a stack of microvilli along one side of the cell and the proteins required for phototransduction are compartmentalized into this region (Hardie, 2001). Direct light is focused by each lens system onto its own rhabdom only (which contain visual pigments such as rhodopsin) and each rhabdom initiates an electrical discharge from a corresponding nerve which contains important information regarding the stimulation (Yau, 1994). These messages are then interpreted by association centers in the protocerebrum of the brain and may be transmitted post-synaptically to the peripheral nervous system of the animal.

Phototransduction is the physiological process by which light is absorbed and converted into an electrical response through the gating of ion channels (Hill et al., 2004). One of the most fascinating components of phototransduction is that it is a process by which a microscopic stimulus (activation of single molecule by a single photon of light) is converted into a macroscopic response (a voltage output contributing to image formation). Phototransduction pathways display a remarkably high chemical amplification. In vertebrate photoreceptors a single

photon activates one rhodopsin molecule, beginning the transduction process and resulting in about 1000 ion channels being gated, while in insect photoreceptors about 10,000 ion channels per absorbed photon are activated (O'Day and Bacigalupo, 1997). Microvillar photoreceptors respond to single photons by opening channels, that are closed at rest (Hardie, 2001). In insects the opening of one channel may be sufficient to generate a detectable response. These unique anatomical and physiological properties of an insect's eye means that insect vision is incredibly sensitive to motion.

1.4 THE LOCUST AS A MODEL SYSTEM

The choice of a model system for study is often difficult for many neuroethologists, as the complexity of behaviour is quite often related to the complexity of an animal's nervous system (Zupanc, 2004). However, some animals have been pressured by natural selection to obtain very complex behaviours, while maintaining a relatively simple nervous system. Insects are a perfect example, as they display many complex behaviours yet their nervous systems are relatively simple and well understood (Zupanc, 2004). One advantage of studying the nervous system of insects is that they have fewer neurons than other animals such as vertebrates. In addition, the properties of a single identified neuron can often yield general properties and mechanisms that are applicable to other systems. In contrast, more complex vertebrate nervous systems often have neurons that are only identifiable as a particular class of neuron, rather than as identified individual neurons.

Insects account for approximately 70-75% of the identified taxonomic animal species on earth (Burrows, 1996) and have evolved some very well adapted nervous systems that are responsible for their continued evolutionary success. One insect in particular, the locust, has evolved a dedicated and well studied collision avoidance neural pathway that is responsible for generating collision avoidance behaviours to avoid predation and continual in-flight collisions with conspecifics (Gray, 2005). The visual system of the locust is paramount to its survival, and acts as a great model system for study.

1.4.1 Locust biology

The migratory locust, *Locusta migratoria* is an Orthopteran insect of the family Acrididae and are found primarily in Africa, but are also distributed eastward to Japan, the Philippines, and Australia (Chapman, 1976). *L. migratoria* are known to be solitarious as well as gregarious and migratory (Matheson et al., 2004). During the solitarious phase, locusts are camouflaged, avoid other locusts (densities typically less than 3 per 100 m^2), move slowly, fly infrequently, and typically fly at night (Matheson et al., 2004). During the gregarious phase when adult locusts aggregate into swarms, there may be 100,000 per m^2, they move quickly, fly frequently, and typically fly during the day (Matheson et al., 2004). Both the behaviour and morphology in the locust visual system varies between the two forms (Matheson et al., 2004).

Locusts are known to form bands as young nymphs and massive swarms as adults. The natural flight speeds of locusts range from 3-6 m/s (Robertson and Johnson, 1993). There have been reports of gregarious swarms exceeding 20 km in width and because swarms of *L. migratoria* can travel great distances in a single day (over 10 km in an hour), they are primarily known for the destruction of agricultural crops. As a result of a combination of locust plagues, a

drought, and a lack of available emergency resources, an estimated 100,000 people were killed between 1931 and 1932 in Africa (Buj Buj, 1995). In the 1930s, there was a push for the formation of a scientific community to fight against the locust. Locust plagues are still catastrophic events for the continent of Africa. Between 1986 and 1989 locusts affected over thirty African countries and in 1988 international aid reached ~250 million dollars and more than 3 million liters of insecticide were used between January and July of that year (Buj Buj, 1995). As a consequence of investigations into creating effective insecticides and other ways in which to prevent future crop destruction, it became increasingly apparent that the locust possessed a robust quality as a standard model system in neuroethology.

1.4.2 *Locusta migratoria* as a model system in neuroethology

Initial investigations into the physiology of locusts occurred primarily because humans often compete for similar food and space, and are also concerned with insect transmitted diseases (both human and crop). The migratory locust, *Locusta migratoria*, has proven to be an excellent model system in the study of neuroethology (Rind and Simmons, 1997). Locusts have been used as a model to study insect development (e.g. Whitington, 1989), environmental effects on nervous system function (e.g. Robertson, 2004), principles of neuronal computation and network modeling (e.g. Gabbiani et al., 2001), robotic engineering (e.g. Yue and Rind, 2005), sensory coding and visual processing (e.g. Faivre and Juusola, 2008), flight and collision avoidance (e.g. Robertson and Johnson, 1993; Mohr and Gray, 2003), and much more.

One of the most popular reasons for studying locust flight and vision, is that flying locusts have evolved the ability to not only avoid predation by birds (such as the fiscal shrike *Lanius collaris humeralis*, and the carmine bee-eater, *Merops nubicus*), but also to effectively

navigate within a swarm. A locust flying in a swarm is surrounded by potential obstacles moving

at different relative speeds and directions (Robertson and Johnson, 1993), yet in such a complex

environment locusts apparently do not collide with each other (Uvarov, 1977). To avoid

predation, collisions with conspecifics, and general navigation through a complex environment,

locusts must produce appropriate collision-avoidance maneuvers (Gray, 2005). Whether

stationary or in flight, it is critical for a locust to determine the time at which looming objects

will collide with it (Robertson and Johnson, 1993). It has been shown that visual stimulation

alone triggers the production of adaptive behavioural responses in the locust (Simmons and Rind,

1992; Judge and Rind, 1997; Gabbiani et al., 1999; Gray et al., 2001). The locust nervous system

contains neurons that may signal the time to collision (TOC) of approaching objects and may

initiate and control avoidance behaviours of flying locusts. This type of system is not isolated in

the animal world, as the nucleus rotundus of the pigeon brain also contains identified collision

avoidance neurons (Sun and Frost, 1998). Although locusts produce relatively complex

behaviours, they have a relatively simple and well understood nervous.

1.4.3 Locust general anatomy and physiology

Locusts, like other members of the class Insecta, have compound eyes, three body regions

(the head, thorax, and abdomen), possess a tracheal system for breathing, three pairs of legs, and

two pairs of wings, forewings and hind wings (Uvarov, 1997; Fig. 1.2).
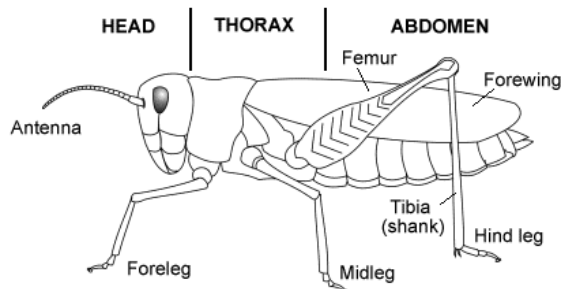
Figure 1.2. Drawing of the general external anatomy of a locust. (http://www.daff.gov.au).

Similar to other insects in the order Orthoptera, locusts develop by gradual metamorphosis (eggs to nymphs to adults), have chewing mouthparts, and leathery forewings called tegmina (Uvarov, 1997). Similar to other members of the family Acrididae, locusts have short antennae (chemosensory organs), an ovipositor (egg layer) in females, an external tympanum (auditory organ) on each side of the first abdominal segment, and three-segmented tarsi (feet) (Uvarov, 1997). Locusts have a segmented body structure, where the thorax (locomotion center) is a fused structure consisting of the prothorax, mesothorax, and metathorax. Each segment bears a pair of legs and the second segment bears a pair of forewings (the tegmina) and the third segment a pair of membranous hind wings. The forewings are responsible for generating flight and steering while the hind wings are primarily for stabilization and lift during flight (Uvarov, 1997). All three legs have similar parts but the hind pair is specially adapted for jumping, since it contains a catapult-like knee joint and is much larger than the first and second pair (Uvarov, 1997).

1.4.3.2 The locust nervous system

Similar to other animals, the nervous system of a locust is divided into the peripheral nervous system (PNS) and the central nervous system (CNS). While in vertebrates the CNS

consists of the brain and spinal cord, the analogous structures in the locusts are the brain and

ventral nerve cord (VNC). The PNS contains the sensory system, which relays information

regarding internal and external environmental events, and the motor system, which relays

commands that control the muscles and elicit behaviours (Burrows, 1996). The VNC runs the

entire length of the locust body along its ventral side and is segmented. Each segment in the

locust VNC is controlled by its own ganglion (a package of neurons containing 10s to 1000s of

neurons) that are joined to each other by interganglionic connectives or are directly fused

together (Burrows, 1996). This creates a chain of linked ganglia that are named for the relative

segment in which they are located in. From anterior to posterior, these ganglia include the brain,

suboesophageal, prothoracic, mesothoracic, metathoracic, seven abdominal ganglia, and the

terminal ganglion (Burrows, 1996).

The largest number of neurons are located in the brain of the locust, primarily because

sensory information from the compound eyes and antennae are processed there. However, other

ganglia also contain a high number of neurons and are responsible for many other forms of

processing and control of behaviours, such as flying, jumping, walking and mating (Burrows,

1996). Although sensory information is processed in various ganglia throughout the locust,

visual information is strictly processed in the brain. Several identified visual neurons are

responsible for relaying visual information to other parts of the body. For example, descending

interneurons, such as the lobula giant motion detector (LGMD) and its post-synaptic partner the

descending contralateral motion detector (DCMD) relay visual information to motor centers

located in the thoracic ganglia (Fig. 1.3; O'Shea and Williams, 1974; Rind, 1984).
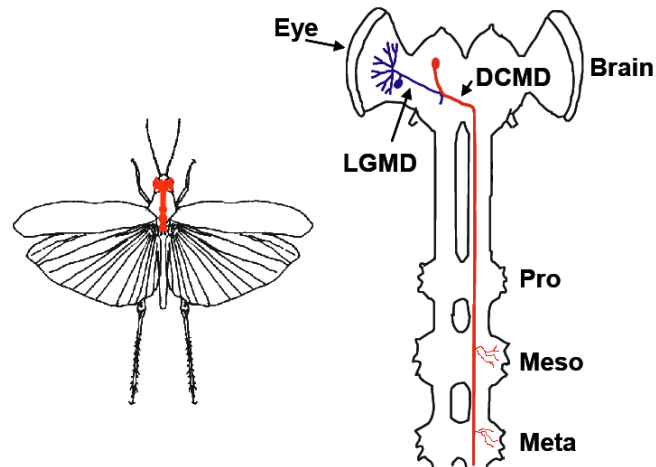
Figure 1.3. Schematic drawing of the eyes, brain, and thoracic ganglia of the locust. The right figure relates to the red area outlined in the whole animal on the left. The lobula giant motion detector (LGMD; blue trace) and its post-synaptic partner the descending contralateral motion detector (DCMD; red trace) are outlined. The DCMD makes synaptic contacts with motor units in the mesothoracic and metathoracic ganglia.

The mesothoracic and metathoracic ganglia contain motor centers that are responsible for the movement of the wings and legs. Therefore the LGMD and DCMD are implicated in initiating avoidance behaviours and modulating existing behaviours, such as flight (Schlotterer, 1977; Rind and Simmons, 1992; Gray et al., 2001; Matheson et al., 2004). Indeed, it has been shown that visual stimulation alone triggers the production of adaptive behavioural responses in the locust (Simmons and Rind, 1992; Judge and Rind, 1997; Gabbiani et al., 1999; Gray et al., 2001). Since the DCMDs are not the only motion-sensitive neurons in a locust's visual system that project to the thoracic ganglia, the DCMDs alone may not be responsible for driving any particular avoidance behaviour (Gray et al., 2001). The descending ipsilateral movement detectors (DIMDs) are also motion-sensitive and make connections to flight neurons, and thus the DCMDs are more likely to be part of a group of descending neurons whose combined activity coordinate flight maneuvers and other avoidance responses (Gray et al., 2001).

1.4.3.3 The LGMD and DCMD

Visual stimuli that reach the ommatidia on the retina of the locust's compound eyes are first integrated (through presynaptic visual afferents) in the three large dendritic fields of the paired lobula giant motion detectors (LGMD) located in the lobula of the brain (Rind, 1984). One of these fields receives excitatory input from many retinotopically arranged fibers sensitive to local motion, while the other two receive local feed-forward inhibition produced by rapid changes in luminance (Rowell et al., 1977). Excitation of the LGMD is mediated through a phasic lateral inhibitory network between incoming afferents (Rowell et al., 1977). Lateral inhibition is a mechanism used by many animals to extend their visual capabilities through, for example, contrast enhancement and edge detection (Tonkin and Pinter, 1996). Simple lateral inhibitory mechanisms found in the locust's ommatidial array have been shown to be similar to those observed in flies and horseshoe crabs (Schlotterer, 1977), as well as in the vertebrate system which also uses lateral inhibition in visual integration (Hill et al., 2004). Inhibitory networks are suitable for edge and motion detection as the networks preferentially inhibit signals moving in one direction (Tonkin and Pinter, 1996). Because these networks are nonlinear, the shape of the output response can vary over a wide range of intensities (Tonkin and Pinter, 1996). The excitation of the LGMD is terminated by a delayed feed-forward inhibition that defines a peak firing rate that occurs near the end of an approaching object (Gabbiani et al., 1999; Gabbiani et al., 2001).

Each LGMD synapses onto an interneuron called the descending contralateral motion detector (DCMD) located in the protocerebrum (O'Shea and Williams, 1974). The synaptic connection between the LGMD and DCMD is so strong that it results in one-to-one presynaptic and post-synaptic spiking activity (Gabbiani et al., 2001). The cell body of the DCMD is the

largest in the brain and its axon is the largest in the VNC (O'Shea and Williams, 1974). The DCMD projects contralaterally through the VNC and synapses with other interneurons and motorneurons located in the mesothoracic and metathoracic ganglia of the locust (O'Shea and Williams, 1974; Rind, 1984).

The LGMD/DCMD system is one of the best described collision-sensitive pathways, although there is some argument about the exact properties and underlying mechanisms of signal transmission (Rind and Simmons, 1997; Gabbiani et al., 1999). Objects that approach along a direct collision course, at a constant velocity, produce a looming visual stimulus. As an object approaches the locust, the edge of the image expands over the retina exciting ommatidia. As more ommatidia are excited, an increase in the LGMD/DCMD firing rate is observed (Fig. 1.4).
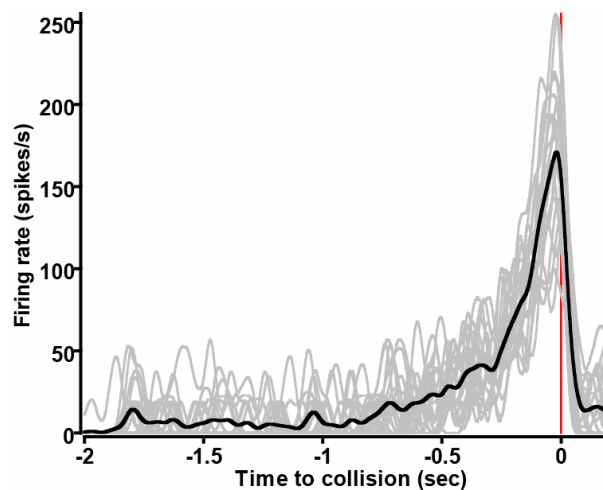
Figure 1.4. Characteristic DCMD response to a looming visual stimulus. This perievent histogram shows the rapid increase in firing rate in response to a looming visual stimulus (a 7 cm black disk approaching at 300 cm/s) from 90°. The black line is the average response of 20 different animals (gray lines). The red line denotes when the time the object would collide with the animal. The data was taken from experiments described in Chapter 2.

The LGMD is believed to act as an angular threshold detector during a looming approach (Gabbiani et al., 1999). The angular threshold of an approaching object is determined by the

angular velocity (speed of edge expansion) and by the area of the retina that is covered by the image, known as the subtense angle (Fig. 1.5).
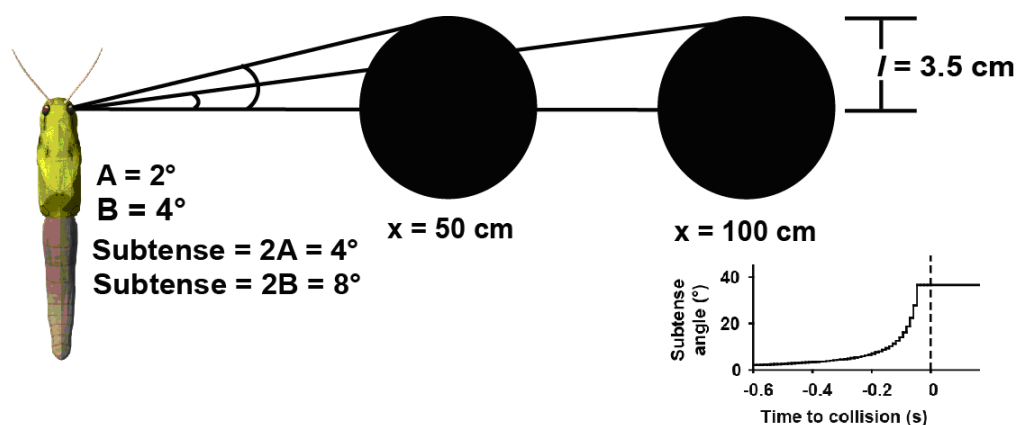
Figure 1.5. Schematic drawing illustrating an important visual stimulus parameter, the subtense angle. Note that both discs are the same size (l denotes the objects half-size). As the object approaches the locust, the area subtended by the object on the eye of the locust (subtense angle) increases, i.e. angle B > angle A. In this example, $l = 3.5$ cm and the disc was 100 cm away and 50 cm away, therefore the disc would subtend 4° and 8°, respectively. The bottom right inset shows a graphical representation of the subtense angle as it relates to an approaching object.

The angle of subtense on the locust's eyes is critical for the selective responses to approaching objects (Rind and Simmons, 1992). While the resolution of individual ommatidia of the locust eye is a 1° subtense, objects that pass through a subtense angle of 10° generate collision-avoidance manoeuvres in tethered flying locusts (Robertson and Johnson, 1993).

The computation performed by the LGMD is thought to involve first a calculation of angular velocity of the edges of an expanding object on the retina and the size subtended by the object at the retina (Fig. 1.5), then a multiplication and conversion of the object's angular velocity and subtense angle into a firing rate (Gabbiani et al., 2001). It is thought that the post-synaptic multiplication of converging excitatory and inhibitory inputs onto the LGMD produces the observed peak firing rate (Guest and Gray, 2006). This peak firing rate occurs with a fixed

delay after a looming object reaches a fixed threshold angular size and before the time of perceived collision (Guest and Gray, 2006). Both angular size and angular edge velocity of an approaching object are considered to be nonlinear functions of time, depending only on the ratio between the object's half size and its approach speed (Gabbiani et al., 2001). Peak firing rate during object approach is sensitive to object size and speed, but unaffected by object shape (Guest and Gray, 2006). Guest and Gray (2006) found that angular threshold computation is invariant to object shape, no matter how complex the object is.

The LGMD/DCMD also displays habituated responses to repeatedly presented visual stimuli (Rowel, 1971; Bacon et al., 1995; Gray, 2005). It is believed that habituation in the LGMD/DCMD pathway occurs at the afferent synapses onto the LGMD (Matheson et al., 2004; Gray, 2005). Apparently the DCMD habituates more strongly in solitarious locusts and it is believed that the relative resistance to habituation in gregarious locusts may allow for the sensitivity required to avoid colliding with another locust in a swarm (Matheson et al., 2004).

A characteristic DCMD response to a looming approach includes an increasing firing rate that peaks before the perceived time of collision. However, there is little known about how complexity within the visual scene influences responses of looming-sensitive neurons (Guest and Gray, 2006). Given that complicated trajectories of objects of various sizes move through the locust's visual field in its natural environment, the ability for the DCMD to encode more complicated motion seems quite possible. Although habituating to objects that are repeatedly presented in the same trajectory (Gray, 2005), the DCMD remains sensitive to the same object on a new trajectory (in the same time frame that resulted in a habituated response; Guest and Gray, 2006). Localized habituation to repeated approaching stimuli along the same trajectory suggest that the LGMD/DCMD pathway is sensitive to approaches of individual objects within a

complex visual scene (Guest and Gray, 2006). In addition, the DCMD can respond to the same

object on a new trajectory (Gray, 2005) and it is also sensitive to translating objects along its

visual field (Pinter et al., 1982; Judge and Rind, 1997; Gray et al., 2001). These recent findings

predict that the LGMD/DCMD should be able to respond to approaches of multiple objects

approaching from different trajectories and objects approaching on a complex trajectory.

While many studies have outlined characteristic responses of the DCMD to looming

trajectories, several models fail to fully explain non-linear responses (Rind and Simmons 1997;

Gabbiani et al. 1999). DCMD responses have always been described as being rate coded but

recent studies have shown that sensory neurons are able to use more than one form of

information coding (Marsat and Pollack, 2006). The timing of spikes, in addition to the

frequency, may carry extra information regarding the approach of an object. Many questions

remain unanswered regarding the exact encoding properties and transmission mechanisms of the

LGMD/DCMD pathway. To fully explore these questions requires well designed and

biologically relevant visual stimuli.

1.5 STIMULUS DESIGN

Many studies that have examined locust collision avoidance and looming responses of

motion-sensitive neurons used computer-generated stimuli. These stimuli incorporate aspects of

objects thought to be biologically relevant (Gray, 2005). Computer-generated simulations of

looming objects stimulate the DCMDs similarly to real looming objects and they also produce

stereotypical collision-avoidance behaviours in tethered flying locusts (Gray et al., 2001).

Although studies that involve simple stimuli alone may not tell us how a neuron responds to

more complex stimuli encountered in the locust's natural environment, well designed simple

stimuli have been pivotal in revealing the neuronal basis of sensory processing (Felsen and Dan, 2005). The complexity of natural stimuli also presents a major difficulty in the analysis of a stimulus-response relationship (Felsen and Dan, 2005). The major advantage of using artificial stimuli is that they are easily parameterized and ideal for correlating a neuronal response with a particular stimulus parameter (Felsen and Dan, 2005). For example, object parameters like the angle of subtense, angular velocity, angular acceleration, time to collision, and relative retinal expansion velocity can be easily observed using computer-simulated stimuli. The object size, shape, and velocity used in this study are biologically relevant, and have been shown to produce behaviourally relevant reactions from locusts (see Gray, 2005; Gray et al., 2001; Guest and Gray, 2006).

In this study, each object was scaled to real world coordinates and designed to approach at 3 m/s, which is the natural flight speed of locusts (3-6 m/s; Baker et al., 1981). Robertson and Johnson (1993) found that if avoidance could be affected in the duration of one wing beat (after a reaction had been initiated), the minimum time needed from detection of the obstacle would be around 115 ms (a latency of 65 ms for neural processing and 50 ms for the wing beat). At an approach velocity of 3 m/s an obstacle would need to be detected 35 cm away (115 ms at 3 m/s) and would require a dimension greater than 6.2 cm for successful avoidance (Robertson and Johnson, 1993). Given that the spacing between individuals in dense swarms range from 30 cm to 9 m (Waloff, 1972; Uvarov, 1977) and the average wing span of locusts is around 11 cm and the pectoral diameters of predatory birds are around 5-7 cm (Robertson and Johnson, 1993), the choice of using an object of 7 cm in diameter traveling 3 m/s in this study was biologically relevant.

1.6 OBJECTIVES

For the two major objectives and experiments outlined in this thesis, I used a similar experimental setup and recording procedure. A typical setup can be seen in Figure 1.6.
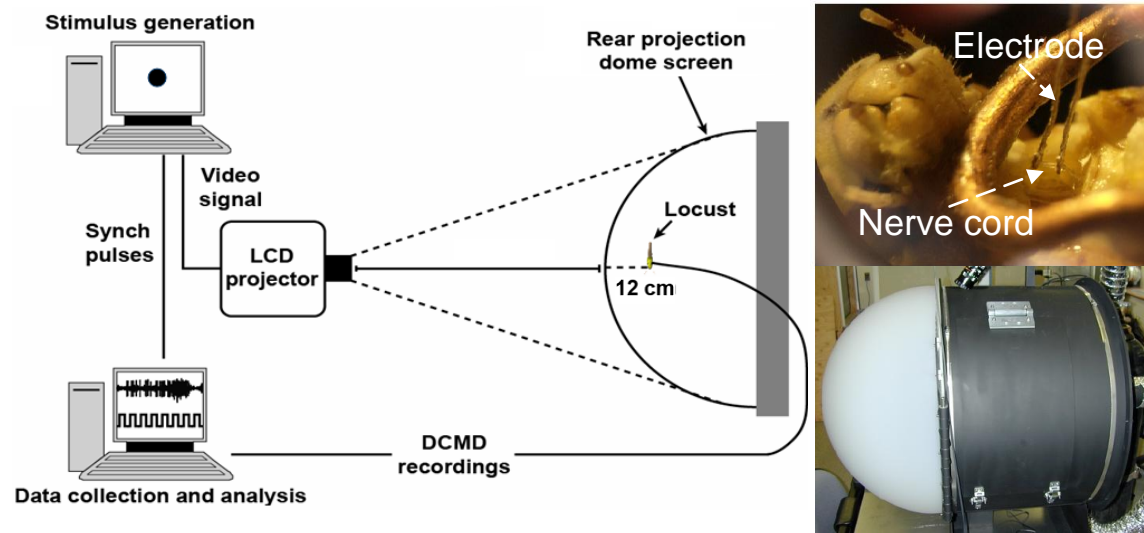
Figure 1.6. General experimental setup for both objectives. Left, visual stimuli were presented onto a rear projection dome screen by an LCD projector and DCMD recordings and video synch pulses were collected and analyzed. A tethered locust was positioned with the right eye centered at 90° with the dome apex. Top right, a photograph of a mounted locust preparation showing the silver hook recording electrodes on the ventral nerve cord, anterior to the prothoracic ganglia. Bottom right, photograph of the flight simulator used in both objectives.

This setup allowed for the fine control of computer-generated visual stimuli and precise quantification of neuronal firing and various stimulus parameters. All methods are discussed in detail in Chapters 2 and 3.

A locust's visual environment consists of a complex combination of translating, receding, and looming visual stimuli that are produced by both self motion and object motion (Gray, 2005). In a natural visual scene, a locust will be presented with multiple objects traveling through its visual field at various trajectories (Uvarov, 1977). The first set of experiments was designed to examine the effects of complex object approach on the firing frequency of the

DCMD. The visual stimuli tested included translating (front to back and non-colliding), looming (head-on approaches) and a combination of translating and looming trajectories with a deviation component. Results and detailed descriptions can be seen in Chapter 2.

Observations of DCMD responses to single approaches to individual animals have led to the idea that bursting activity may also occur in the LGMD/DCMD pathway. No studies have investigated such an observation. Sensory neurons can encode stimuli in many different ways and temporal coding may be more important in sensory systems than previously thought (Salinias and Sejnowski, 2001). Bursting activity in the DCMD may be important if the timing of the bursts were correlated with a post-synaptic event, such as the rhythmic movement of a locust's wings during flight. The central pattern generators (CPGs) involved in locust flight maintain the basic flight rhythm of the wings and are known to be modified by sensory input once the rhythm is initiated (Burrows, 1996). My second experiment was designed to investigate the exact firing properties the DCMD by using a simple visual stimulus (a looming 7 cm black disc) presented multiple times, to multiple animals. Results and detailed descriptions can be seen in Chapter 3.

Findings from these experiments are expected to provide insight into the biophysical mechanisms of looming-sensitive neurons, in addition to the broader question of how visual stimuli are coded and what is the neuronal basis of sensory processing.

CHAPTER 2

A SINGLE MOTION SENSITIVE NEURON ENCODES

CHANGES IN OBJECT APPROACH

2.1 ABSTRACT

Two identified locust neurons, the lobula giant movement detector (LGMD) and its post-synaptic partner, the descending contralateral movement detector (DCMD), constitute one motion sensitive pathway in the visual system that responds preferentially to objects that approach on a direct collision course and are implicated in collision-avoidance behaviour. Previously described LGMD and DCMD responses suggest that this pathway is also affected by more complicated movements in the locust's visual environment. For example, the approach of paired objects in the azimuthal position and approaches at different time intervals affect DCMD firing rate properties (Guest and Gray 2006). To test whether locusts are able to respond to more complicated motion, locusts were presented with computer-generated discs travelling along a combination of non-colliding (translating) and colliding (looming) trajectories. Distinctly different responses to different trajectories demonstrate that the DCMD may encode complicated aspects of a visual stimulus. Object motion initiated in the frontal visual field generated a larger peak response relative to object motion initiated in the posterior visual field and response amplitude varied with the distance from the eye. In addition to estimating the time of collision and direction of object travel, the presence of a discernable peak associated with the time of

object deviation suggests that DCMD responses may contain information related to changes in motion. Encoded within the DCMD firing rate, subtle and dramatic changes in rate may provide crucial cues that allow the animal to perform suitable avoidance behaviours or continually modify an existing behaviour, such as flight.

2.2 INTRODUCTION

Natural environments contain dynamic and complex combinations of sensory stimuli. Many animals simplify this complexity by extracting salient sensory cues and appropriately modulating their behaviours. In complex visual environments, the detection and response to certain visual stimuli, such as an approaching predator, increases survivability. Looming objects, approaching at constant velocity on a direct collision course, produce an expanding image on the retina and trigger avoidance responses in many animals, such as monkeys (Maier et al., 2004), crabs (Oliva et al., 2007), and locusts (Santer et al., 2005). Neurons responsible for the detection and relay of looming visual stimuli have been characterized in birds (Sun and Frost, 1998) and many insects (Simmons and Rind, 1992; Hatsopoulos et al., 1995; Wicklein and Strausfeld, 2000). While research on looming-sensitive neurons has provided insight into mechanisms that underlie responses to simple shapes traveling along simple trajectories (Gabbiani et al., 2002, 2005) or multiple local motion stimuli (Krapp and Gabbiani, 2005), little is known about how these systems work in complex natural environments (Guest and Gray, 2006).

Gregarious locusts, such as *Locusta migratoria*, provide an ideal system for studying neural mechanisms of complex motion coding. Locusts in swarms (~0.8 m apart; Waloff, 1972)

must manoeuvre in a complex visual environment consisting of multiple objects traveling along various, and often changing, trajectories. Locusts respond to motion within their environment (such as images of predators or conspecifics) by manoeuvring during flight (Robertson and Reye, 1992) or initiating a jump while on the ground (Gibson, 1979). Descending visual input from identified interneurons is implicated in initiation and modulation of these avoidance behaviours. The lobula giant movement detector (LGMD) and its post-synaptic partner, the descending contralateral movement detector (DCMD), constitute one motion sensitive pathway in the visual system that responds preferentially to looming objects (Schlotterer, 1977; Simmons and Rind, 1992; Hatsopoulos et al., 1995; Judge and Rind, 1997). The LGMD receives converging input from an array of photoreceptors and network of neurons (O'Shea and Williams, 1974) and transmits this information to the DCMD in a 1:1 spiking ratio (O'Shea and Rowell, 1975a) via mixed chemical and electrical synapses (O'Shea and Williams, 1974; Killman et al., 1999). In turn, the DCMD descends along the contralateral side of the body and synapses with interneurons and motorneurons in the thoracic ganglia that control the wings and legs (Burrows and Rowell, 1973; Simmons, 1980b; Pearson et al., 1985; Boyan, 1989). Thus, this pathway is ideally suited to evoke or control directed behaviours.

Previous experiments using complex scenes presented to locusts (Rind and Simmons, 1992) did not provide quantitative descriptions of how DCMD activity is modulated by different levels of complex object motion. Results from presentations of equally timed or closely timed paired object approaches as well as objects with compound shapes, strongly suggested that DCMD responses could encode more complicated object motion (Guest and Gray, 2006). To test this possibility, we presented locusts with a virtual black disc with different trajectory parameters (direction, proximity and course changes). Trajectories with looming components evoked

characteristic and consistent DCMD responses (i.e. time and amplitude of peak firing relative to projected collision). We found that DCMD responses were affected by direction and proximity (higher peak amplitude in response to closer trajectories and trajectories initiated in the anterior visual field) as well as changes in course trajectory (producing a peak associated with a deviation change). Results suggest that the DCMD encodes complicated visual stimuli through the modulation of its firing rate.

2.3 MATERIAL AND METHODS

2.3.1 Animals

Twenty adult male *Locusta migratoria* were obtained from a crowded colony maintained in the Department of Biology at the University of Saskatchewan (25-28°C, 12hr:12hr light:dark). Locusts selected for experiments (carried out at room temperature ~25°C) were at least 3 weeks past the imaginal molt.

2.3.2 Preparation

After removing the legs and clipping the wings, a rigid tether was attached to the ventral surface of the thorax using 3M™ Vetbond™ Tissue Adhesive 1469SB (3M Animal Care Products, St. Paul, MN). A small patch of ventral cervical cuticle was removed to expose the underlying paired connectives of the ventral nerve cord anterior to the prothoracic ganglia. The exposed tissue was then bathed in a drop of locust saline (147 mmol NaCl, 10 mmol KCl, 4 mmol $CaCl^2$, 3 mmol NaOH, 10 mmol Hepes, pH 7.2) and the preparation was transferred to

the recording stage. Neuronal recordings were obtained from the left ventral nerve connective

using a bipolar silver wire hook electrode insulated with a mixture of Vaseline and mineral oil.

The entire preparation was rotated so that the locust was oriented dorsal-side up with its

longitudinal axes perpendicular to the apex of the rear projection screen and the right eye was

aligned with the azimuthal and elevation axes of the dome apex (Fig. 2.1A). In this orientation 0°

was directly in front of the locust, 180° was directly behind, and 90° was aligned with the center

of the eye. The preparation was left for ~30 minutes in front of a projected white visual field

(background luminance $= 430$ cd/m^2) before the experiment started to allow the animal to

acclimate to the experimental setup. To prevent confounding effects of neural habituation, the

interval between each presentation was at least 2 minutes. Preparations that produced no

discernable neural responses to local motion (hand waving across the projection screen) were not

used for experimentation.

2.3.3 Visual stimuli

The procedure used for visual stimulus generation and data acquisition was similar to that

used by Guest and Gray (2006). Visual stimuli were created using the Vision Egg visual stimulus

generation software (A. Straw; http://visionegg.org/) on a Python programming platform and

represented as 1,024 x 1,024 pixel portable network graphics (png) files. Individual pixel sizes

were approximately 0.7 mm, which corresponds to a visual subtense angle of ~0.4°, which is

below the 1° resolution of individual ommatidia (Horridge, 1978). The visual stimulus, a 7 cm

diameter black disc traveling 300 cm/sec, was scaled in real-time at 85 frames/sec (fps) and

projected onto a specialized rear projection dome screen using a Sony VPL-PX11 LCD data

projector. To account for the distortion due to projection onto the curved surface of the screen,

correction factors were embedded in the Vision Egg code. A 1.2-ms TTL pulse included in each video frame and the vertical refresh synchronization pulse (Vsynch) from the video card (NVIDIA GeForce4 Ti4200 128 MB) were used to align physiological recordings with either the projected time of collision (TOC) or, for non-looming trajectories, the time when the object had reached 90° in the azimuthal plane (the center of the right eye). The final frame of each presentation was determined using the last TTL pulse, which indicated when the object had disappeared from the screen. The corresponding Vsynch pulse determined the start time of the rendering of this frame. The luminance values and Michelson contrast ratio (0.48) were the same as those used by Guest and Gray (2006).

All visual stimuli were presented at 0° elevation and modified within the azimuthal plane (Fig. 2.1B). Different trajectories were based on optically and behaviourally relevant retinal image sizes, i.e. visual subtense angles. Based on the subtense angle when the disc was at 90° azimuth, three distances from the longitudinal plane of the locust's eye were used: 400 cm, 80 cm, and 40 cm (1°, 5°, and 10° visual subtenses at 90° azimuth, respectively). At the furthest distance (400 cm), the diameter of the disc was close to the spatial resolution of a single ommatidium (~1°; Horridge, 1978). Approaching objects that pass through a 10° subtense angle (~40 cm away and the closest distance used here) have been shown to initiate an avoidance behaviour in tethered flying locusts (Robertson and Johnson 1993). The 80 cm distance (5° subtense at 90° azimuth) was used as an intermediate distance. Discs on direct collision trajectories approached from 400 cm at 45°, 90° and 135° azimuth, stimulating the anterior, center and posterior visual fields respectively. Translating trajectories (no collision component) were based on the three distances used (400 cm, 80 cm, and 40 cm). To test the effects of trajectory changes during object motion we presented combinations of translating motion that

switched to looming. For one combination, motion began in the anterior or posterior visual field and travelled along a translating trajectory at one of the distances described above. Motion from the anterior region switched to looming at 45° azimuth and motion from the posterior region switched to looming at 135° azimuth. Another combination included motion that began in the anterior or posterior region, translated through 90° azimuth, and switched to looming at either 135° azimuth (from initial anterior motion) or 45° (from initial posterior motion). To test the effect of direction, each trajectory used had a corresponding mirrored direction of motion. In total, the experimental presentation sequence consisted of three looming, six translating, and eighteen translating to looming trajectories (Fig. 1B). To control for potential effects of the length of each experiment, the first and last approaches were direct looms from 90°. All other trajectories were presented randomly for each animal. The times of deviation and times that the object was at 90° azimuth (TA90°) for the most complicated trajectories are shown in Table 2.1.

Table 2.1. Comparison of times of stimulus deviation and peak DCMD responses

	A400 cm (sec)	A80 cm (sec)	A40 cm (sec)	P400 cm (sec)	P80 cm (sec)	P40 cm (sec)
90°	-0.3716	-0.0278	-0.0419	-0.1126	-0.1778	-0.0709
Ant45°/Post135°	0.2056	0.0276	0.0425	0.3446	-0.0524	-0.0355
Ant135°/Post45°	0.3141	0.1451	0.0319	-0.0919	0.1491	0.0389
Ant135°/Post45° TA90°	-0.3217	-0.0604	-0.0317	-0.0357	-0.1224	-0.0537

The difference between median times of peak firing (TOP) for each distance (400, 80, and 40 cm) and direction (anterior (A) and posterior (P)) associated with a deviation to looming (TOD) or associated with the time the stimulus was 90° azimuth (TA90°) are displayed (see Results section). Numbers represent the difference between the TOD/TA90° and the median TOP. Different trajectory types are explained in Methods section.

2.3.4 Spike sorting and DCMD identification

For each presentation, neuronal activity from the left cervical connective, pulses synchronized with each frame of the stimulus, and Vsynch pulses from the video card were recorded continuously and stored for analysis. Recorded activity was amplified with a differential AC amplifier (A-M Systems, model No. 1700, gain = 10,000) and sampled at 25 kHz. An RP2.1 enhanced real-time processor (Tucker-Davis Technologies, Alachua, FL) with Butterworth filter settings of 100 Hz (high pass) and 5 kHz (low-pass) was used to store the data to disk.

DCMD activity in the cervical connective was identified using threshold analysis in Off-line Sorter (Plexon, Dallas, TX). The characteristically large amplitude DCMD spikes were easily isolated using this method (Fig. 2.1A).

Figure 2.1. Experimental setup (A) and stimulus configuration (B). A) Computer-generated objects (7 cm black disc) were presented onto a rear projection dome screen. A tethered locust was positioned onto a recording stage with its right eye perpendicular to the dome apex. Neuronal recordings were taken from the left ventral nerve connective and DCMD activity was isolated using threshold sorting (DCMD spikes were clearly larger than background neuronal activity). Neuronal recordings, video card pulses, and pulses inserted into each stimulus frame were collected and analyzed offline. B: Three types of object motion were designed: Looming, translating, and combinations of translating with deviations to looming. We presented three simple looming trajectories at azimuthal angles of 45, 90, and 135° and six simple translating trajectories that traveled from the anterior or posterior visual field at three different distances perpendicular to the longitudinal axis of the locust's eye (400 cm, 80 cm, and 40 cm; see text for details). We also presented eighteen trajectories that contained combinations of translating objects that deviated to looming. Motion along these trajectories began in the anterior or posterior visual field at the three distances described above and deviated at azimuthal angles of 45, 90, and 135° (see text for details). This combination of visual stimuli allowed us to test the effect of complicated visual environments on DCMD responses by manipulating direction, proximity, and deviations during object motion.

Spike times were exported to Neuroexplorer spike train analysis software (NEX

Technologies, Littleton MA) and spike times were transformed into peristimulus time histograms

using a 1-ms bin width and smoothed with a 50-ms Gaussian filter (for example see Fig. 2.2).
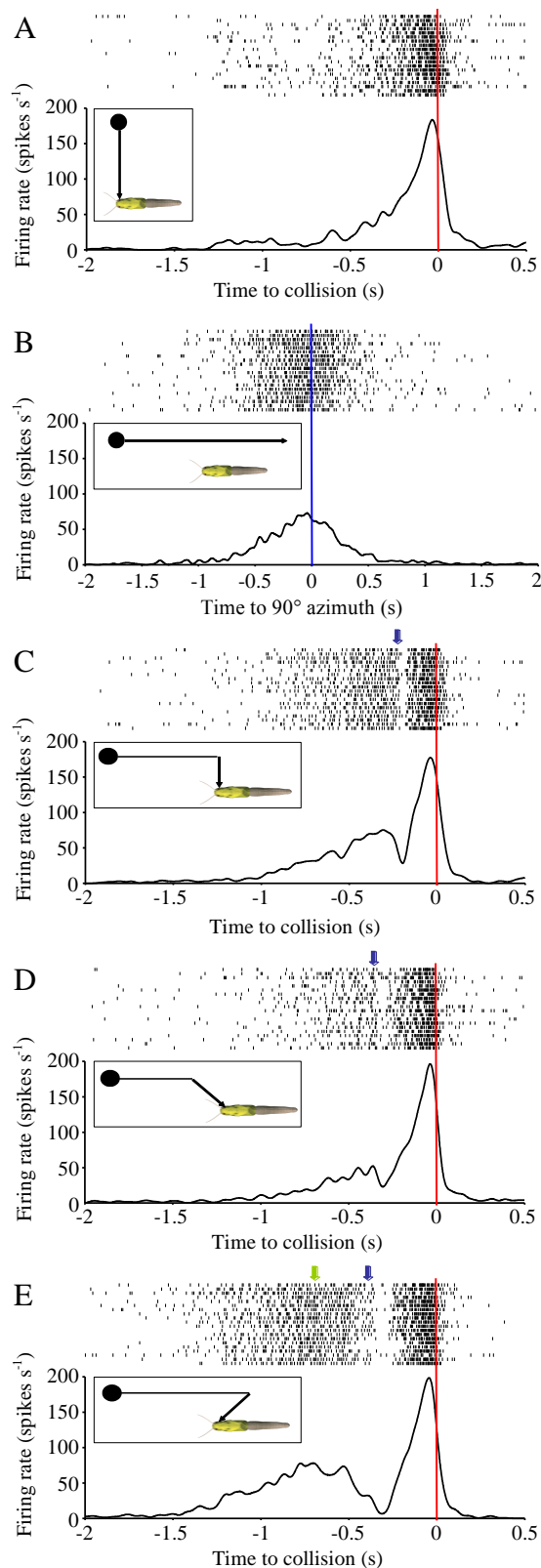
Figure 2.2. Sample DCMD peristimulus time histograms (PSTH) showing responses to different categories of object motion. Each panel shows rasters from each animal (top, n= 20) and the mean peristimulus time histogram (bottom) in response to a single trajectory category. For clarity, mirrored approaches are not shown. A) Looming from 90° azimuth with motion initiated 400 cm from the eye. B) Translating. C) Translating to looming at 90° azimuth. D) Translating to looming at 45° azimuth. E) Translating to looming at 135° azimuth after passing through 90° azimuth. For panels B-E motion was initiated at 80 cm from the longitudinal plane of the eye. In response to all trajectory categories presented the firing rate increased after the start of object motion. In response to a purely translating trajectory (B), the firing rate decreased after TA90° whereas in response to all translating to looming trajectories (C-E) the firing rate decreased following a trajectory change (TOD, blue arrows), producing a valley, and subsequently increased during the looming phase of motion. Thus, each valley was associated with a change to a looming trajectory. Insets in each panel show the trajectory category (not to scale). The green arrow in E shows TA90°. The red lines in A, C, D, E represent TOC and the blue line in B represents TA90°.

Depending on the trajectory type, DCMD activity was quantified by measuring the time and amplitude of a peak firing rate associated with collision, deviation, and/or when the disc passed through 90° azimuth. Pulses produced from the video card and frame times representing each stimulus were used to determine the time of the last presentation frame. The last frame was used to calculate TOC, the time of object deviation (TOD), and time at which the object was at the centre of the locust's right eye (TA90°).

2.3.5 Data analysis and statistical analysis

DCMD firing parameters in response to different motion trajectories were tested for significant differences at $P < 0.05$ using SigmaStat 3.0 and plotted using SigmaPlot 10.0 (Systat Software Inc., Richmond, CA, USA). Statistical tests comparing the effects of distance and direction for parametric or nonparametric data are indicated in appropriate sections of the results. In some cases a t-test or Mann-Whitney rank sum test were used to compare parameters of looming trajectories and a 2-way ANOVA or Friedman RM ANOVA on ranks (Student Newman-Keuls for significance of difference) were used when comparing complicated trajectories.

2.4 RESULTS

2.4.1 DCMD responses to looming objects

Looming discs evoked consistent and characteristic DCMD responses where the spike rate increased during object approach and peaked near TOC (Fig. 2.2A). The first and final approach in a sequence presented to each animal were used to control for the duration of each experiment. We found no significant differences in time of peak relative to TOC (Mann-Whitney rank sum test, $n = 20$, $P > 0.05$), peak amplitude of the firing rate (t-test, $n = 20$, $P > 0.05$), total number of spikes during approach (Mann-Whitney rank sum test, $n = 20$, $P > 0.05$), or the peak width at half height (t-test, $n = 20$, $P > 0.05$). Thus, experiment duration did not affect DCMD responses.

To compare DCMD responses to objects that loomed in different regions of the locust's visual field, discs were presented at azimuthal angles of 45, 90, and 135°. There were no significant differences in the peak time relative to TOC, number of spikes, or peak width at half height. Approaches from 90°, however, evoked a significantly higher mean peak amplitude compared to a 45° approach (1-way ANOVA, $F_{57,2} = 7.14$, $n = 20$, $P < 0.05$).

There were no significant differences in the amplitude of the peak firing rate that occurred near TOC when data from the 45, 90, and 135° looming trajectories were compared to all the translating and deviating to looming approaches at the same azimuthal angles of deviation. Significant differences were found in the time of peak firing between the 45° looming trajectory and the posterior 45° deviation at 40 cm and the anterior 45° deviation at the 80 cm distances (Friedman RM ANOVA on ranks, $\chi_6^2 = 20.31$, $n = 20$, $P = 0.002$). Significant differences also were found when comparing the time of peak between the 90° looming

trajectory and the posterior 90° deviation at 80 cm (Friedman RM ANOVA on ranks, $\chi^2_6 =$ 21.62, $n = 20$, $P < 0.001$). No significant differences were found when comparing the time of peak firing for the 135° looming trajectory and 135° deviation trajectories.

No significant differences were found in the amplitude of TOC-associated peak firing associated across all trajectories that contained a looming component. The only significant differences in the time of peak firing were found in the posterior 90° deviation at 40 cm, which occurred significantly earlier than the posterior and anterior 90° deviations at 80 cm, posterior 135° deviation at 400 cm, and anterior 90° deviation at 400 cm (Friedman RM ANOVA on ranks, $\chi^2_{17} = 58.241$, $n = 20$, $P < 0.001$). These findings suggest that the stimulation history within the approach did not significantly affect the final amplitude and timing of peak DCMD firing.

2.4.2 DCMD responses to translating trajectories

We compared DCMD responses to translating objects that were initiated from the anterior or posterior visual field and at 3 different distances from the locust's eye (Fig. 2.1B). Regardless of distance or direction of travel, the DCMD responded to translating objects with an increasing spike rate that peaked close to the time that the disc passed through 90° azimuth (TA90°) (Fig. 2.3A).
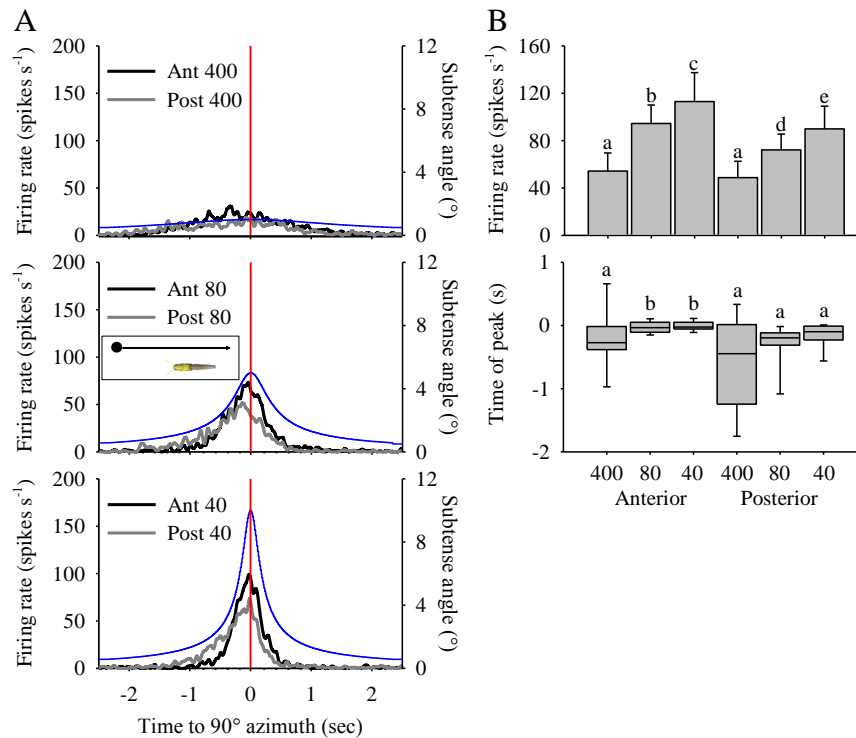
Figure 2.3. Mean DCMD responses and statistical summary for responses to translating trajectories time aligned to TA90°(t = 0 sec, red lines in A). A) All six response profiles show responses to mirrored trajectories set at different distances from the longitudinal plane of the locust's eye. Black lines represent responses to motion starting in the anterior visual field and gray lines represent responses to motion starting in the posterior visual field. Blue lines in each panel represent the change in the disc's subtense angle during translation. The inset in the middle panel represents the trajectory category (not to scale). B, top panel) Motion initiated in the anterior visual field and objects that traveled at closer distances generated higher peak DCMD responses. B, bottom: While the time of peak firing generally occurred before TA90° in response to all six translating trajectories, peak firing occurred earlier in response to motion initiated in the posterior visual field. Bars are means +/- SD (B, top) and box plots are medians with 90th and 10th percentiles (B, bottom), n=20 locusts. Different letters represent significance differences. See text for statistical details.

We found a significant effect of distance (2-way ANOVA, $F_{2,114} = 84.70$, $n = 20$, $P < 0.001$) and direction (2-way ANOVA, $F_{1,114} = 28.85$, $n = 20$, $P < 0.001$) and a significant interaction effect (2-way ANOVA, $F_{2,114} = 3.24$, $n = 20$, $P = 0.043$) on peak DCMD firing (Fig. 2.3B). Anterior approaches and approaches at closer distances resulted in higher peak firing rates.

For each translating trajectory, time of peak firing generally occurred before the disc passed through 90° azimuth and variation about the median was much lower for anterior approaches (Fig. 2.3B, 80 cm and 40 cm). The time of peak firing occurred significantly later for the 40 cm and 80 cm anterior translating trajectories (Friedman RM ANOVA on ranks, $\chi_5^2 = 25.00$, $n = 20$, $P < 0.001$), although times of the other translating trajectories were not significantly different (Fig. 2.3B).

2.4.3 DCMD responses affected by translating to looming trajectories

Based on the results from responses to translating trajectories, it is possible that the DCMD may encode information regarding the relative distance and direction of approach of visual stimuli. Consequently, DCMD responses to different translating to looming trajectories were compared based on distance and direction. To test the effect of distance, similar trajectories with the same starting direction but different starting distance were compared. To test the effect of direction, mirrored trajectories that had a different direction of travel but similar trajectories were compared.

Generally, most objects that deviated to a collision course evoked an observable decrease in the DCMD firing rate that produced an early peak near TOD (see, for example, Fig. 2.2C and D). In most cases, anterior approaches and trajectories at closer distances resulted in higher and earlier TOD associated peak firing rates. Translating to looming trajectories where the object passed TA90° before deviation resulted in a discernable peak associated with passing the centre of the eye that was similar in both amplitude and time peak responses to the translating trajectories (see, for example, Fig. 2.2E). In addition, we observed a decreased firing rate (valley)

after TOD, a quantifiable peak before TOD, and a final peak associated with the time of perceived object collision (TOC).

2.4.4 DCMD responses to changes in distance and direction of complicated motion

Translating trajectories that deviated to looming at 45°, 90°, or 135° azimuth had equal total stimulus times, times of object deviation (TOD), times of collision (TOC). Mirrored trajectories differed with respect to the relative region of the visual field in which motion occurred. Features of these complicated trajectories, such as relative distance, direction of motion, and deviations to a looming trajectory, were related to the time and amplitude of peak firing within DCMD responses that were associated with trajectory changes or collision.

Anterior and posterior approaches that deviated at 90° (Fig. 2.4A) resulted in significantly higher TOD-associated peak firing rates at closer distances and significantly higher firing rates were evoked during anterior approaches starting 40 cm from the eye (Fig. 2.4B, top; Friedman RM ANOVA on ranks, $\chi^2_5 = 81.83$, $n = 20$, $P < 0.001$). TOD-associated peak firing times were less variable for anterior approaches, specifically at 80 cm and 40 cm distances (Fig. 2.4B, bottom). Distance significantly affected the time of peak firing for both directions of travel and all but the responses to motion initiated at 400 cm were affected by direction (Fig. 2.4B, bottom; Friedman RM ANOVA on ranks, $\chi^2_5 = 57.66$, $n = 20$, $P < 0.001$). Although the time of peak firing occurred before TOD for each trajectory (see Table 1, 90° TOD-TOP), anterior approaches resulted in a later TOD-associated peak, with the exception of responses to approaches starting at the furthest distance.
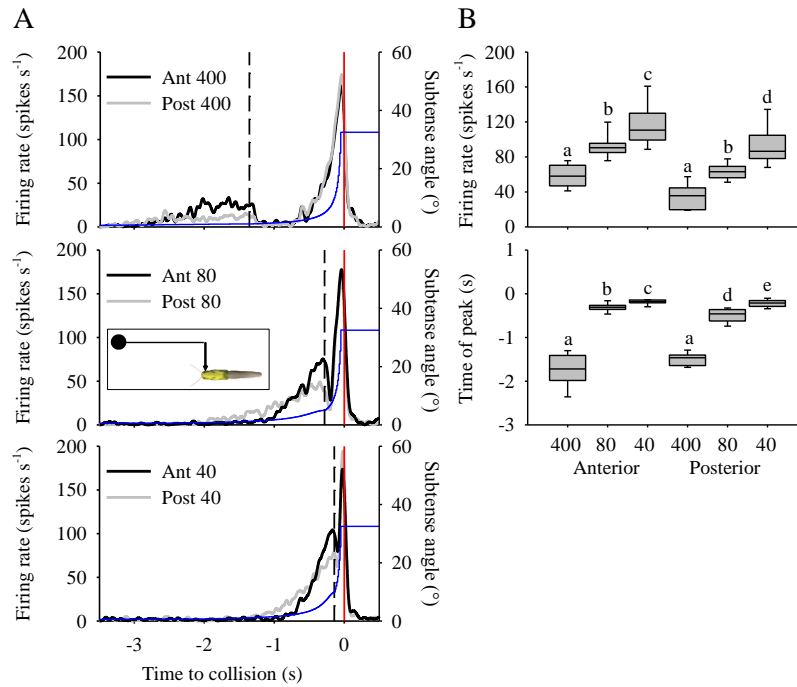
Figure 2.4. Mean DCMD responses and statistical summary for responses to translating to looming trajectories with deviations at 90° and time aligned to TOC (red lines in A). A) All six response profiles show responses to mirrored trajectories set at different distances from the longitudinal plane of the locust's eye. Black lines represent responses to motion starting in the anterior visual field and gray lines represent responses to motion starting in the posterior visual field. Blue lines in each panel represent the change in the subtense angle during motion. The dashed lines represent TOD. The inset in the middle panel shows the trajectory category (not to scale). B, top panel) Deviations at closer proximities generated higher early peak DCMD responses, while a direction effect was only found at the closest distance (40 cm). B, bottom) The time of each early peak occurred near TOD in response to each trajectory and displayed a significant proximity effect. Motion initiated in the posterior visual field generated peak times that occurred significantly earlier in responses to motion initiated in the anterior visual field. Box plots are medians +/- 90th and 10th percentiles (B), $n = 20$ locusts. Different letters represent significance differences. See text for statistical details.

Anterior and posterior translating approaches that deviated to looming at 45° and 135° respectively, also evoked an early, TOD-associated peak in the firing rate (Fig. 2.5A).
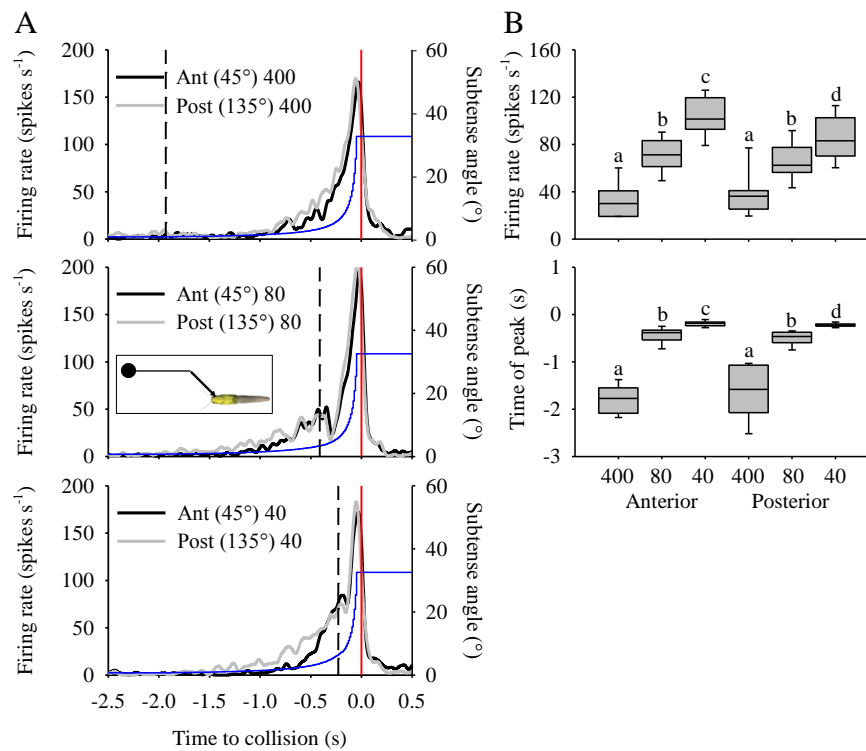
Figure 2.5. Mean DCMD responses and statistical summary for responses to translating to looming trajectories with deviations at 45° azimuth from motion initiated in the anterior visual field and 135° azimuth from motion initiated in the posterior visual field. Responses were time aligned to TOC (red lines in A). A) All six response profiles show responses to mirrored trajectories set at set at different distances from the longitudinal plane of the locust's eye. Black lines represent responses to motion starting in the anterior visual field and gray lines represent responses to motion starting in the posterior visual field. Blue lines in each panel represent the change in the subtense angle during motion. The dashed lines represent TOD. B, top panel) Deviations at closer distances generated higher early peak DCMD responses, while a direction effect was only found for the closest distance (40 cm). B, bottom: The time of each early peak occurred around TOD for each trajectory and displayed a significant effect of distance. Motion initiated in the posterior visual field generated peak times that occurred significantly earlier than motion initiated in the anterior visual field only at the closest distance (40 cm). Box plots are medians +/- 90th and 10th percentiles (B), $n = 20$. Different letters represent significant differences. See text for statistical details.

Significantly higher TOD-associated peaks occurred in response to motion initiation at closer proximities and the peak was affected by direction only in response to motion initiated at 40 cm (Fig. 2.5B, top; Friedman RM ANOVA on ranks, $\chi_5^2 = 51.51$, $n = 20$, $P < 0.001$).

Counter to responses from deviations at 90° azimuth, median times of peak firing occurred slightly after TOD for anterior directions deviating at 45° and the posterior 400 cm 135° deviation (see Table 1, TOP-TOD, Ant45°/Post135°). Significantly later peak times occurred at closer object deviations and a directional effect was found for motion initiated at 40 cm, where the posterior approach generated a close but significantly later peak time [Fig. 2.5B, bottom (Friedman RM ANOVA on ranks, $\chi_5^2 = 46.34$, $n = 20$, $P < 0.001$)].

Representing the most complicated trajectories used in this experiment, anterior and posterior approaches that deviated to looming at 135° and 45°, respectively, contained three important times of interest: TA90°, TOD, and TOC. Average response profiles show that these trajectories resulted in three peaks in the DCMD firing rate (Fig. 2.6A), where the earliest peak was associated with TA90°, the TOD generated a second, though less distinct peak (and valley after TOD), and the final and largest peak occurred near TOC.
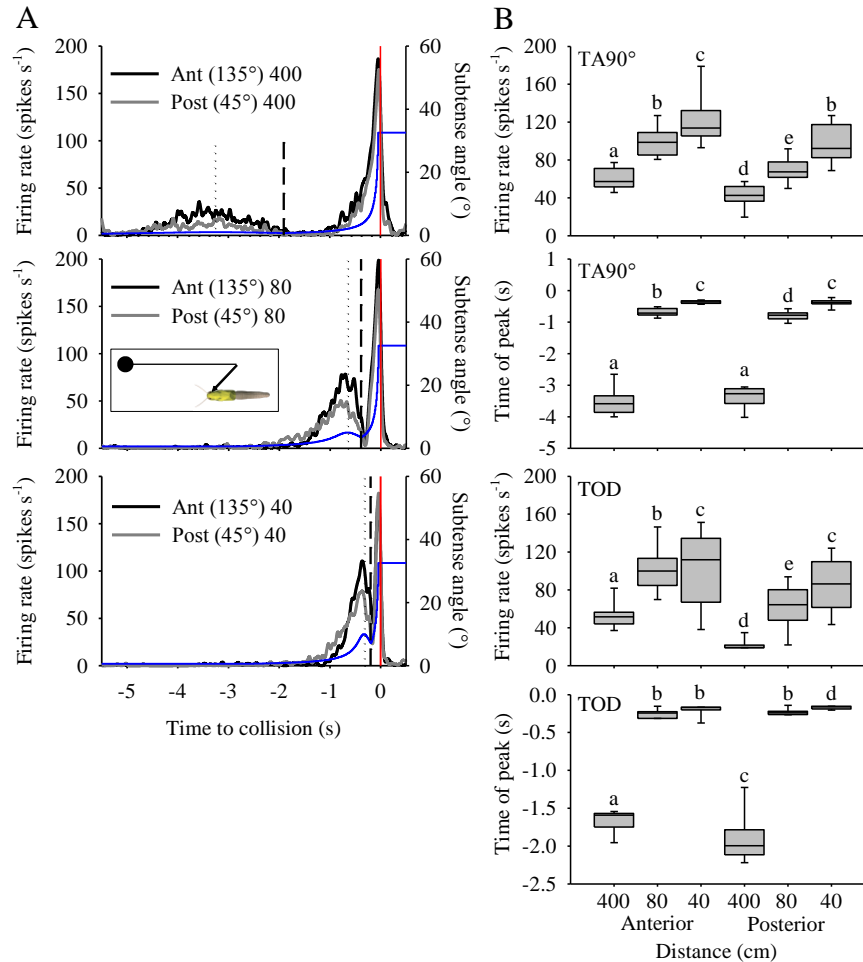
Figure 2.6. DCMD responses and statistical summary for translating to looming trajectories with deviations at 135° and 45° azimuth after passing through 90° azimuth. Responses time aligned to TOC (red lines in A). A) Profiles show responses to mirrored trajectories set at different distances from the longitudinal plane of the locust's eye. Black lines represent responses to motion starting in the anterior visual field and gray lines represent responses to motion starting in the posterior visual field. Blue lines in each panel represent the change in subtense angle during motion. Dotted lines represent TA90° and dashed lines TOD. B, 1st panel) Motion initiated in anterior visual field and closer distances generated higher peak DCMD responses associated with TA90°. B, 2nd panel) Each TA90°-associated peak occurred around TA90° for each trajectory and displayed a significant effect of distance. There was little effect of direction on the time of peak, with the exception of responses to the intermediate distance (80 cm). B, 3rd panel) Deviations to looming at closer distances generated higher TOD-associated peak responses, while a direction effect was found in all but the closest distance (40 cm). B, 4th panel) Time of each TOD-associated peak occurred around TOD for each trajectory and, with the exception of motion initiated in the anterior, displayed a significant effect of distance. Motion initiated in the posterior visual field generated significantly later and earlier peak times than motion initiated in the anterior visual field at the furthest (400cm) and closest (40 cm) distances, respectively. Box plots are medians +/- 90th and 10th percentiles (B), $n = 20$. Different letters represent significant differences. See text for statistical details.

Similar to responses to purely translating discs, approaches at closer proximities and anterior approaches resulted in significantly higher peak firing rates associated with TA90° [Fig. 2.6B, 1st panel (Friedman RM ANOVA on ranks, $\chi_5^2 = 80.80$, $n = 20$, $P < 0.001$)]. Timing of the TA90°-associated peak was affected by distance but a directional effect was only found at the 80 cm distance [Fig. 2.6B, 2nd panel (Friedman RM ANOVA on ranks, $\chi_5^2 = 91.29$, $n = 20$, $P < 0.001$)]. The time of peak firing in response to each trajectory occurred before TA90° (see Table 1, ANT135°/Post45° TA90°). Peak firing rates associated with TOD were significantly higher at closer proximities and approaches from the anterior, with the exception of responses to motion initiated at 40 cm (Fig. 2.6B, 3rd panel; Friedman RM ANOVA on ranks, $\chi_5^2 = 48.60$, $n = 20$, $P < 0.001$). With the exception of responses to posteriorly initiated motion at 400 cm, the time of peak firing occurred after TOD (see Table 1, ANT135°/Post45°). Peak times were significantly later in response to closer distances and approaches from the anterior at 400 cm and 40 cm [Fig. 2.6B, 4th panel (Friedman RM ANOVA on ranks, $\chi_5^2 = 55.347$, $n = 20$, $P < 0.001$)].

2.4.5 DCMD encodes deviations to a loom via a drop in firing rate

As Fig. 2.7 illustrates, up to the point of TA90° both translating and translating to 90° looming trajectories evoke similar DCMD response profiles, which are affected by the initial direction of motion.
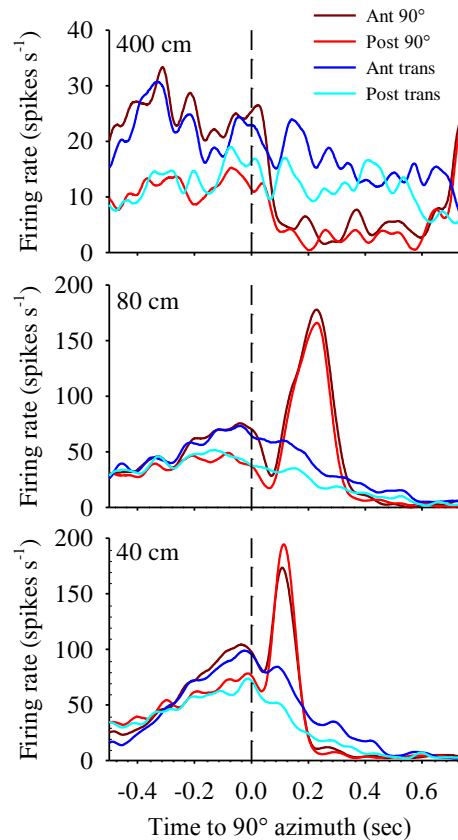
Figure 2.7. Mean DCMD responses to translating and translating with deviation to looming, time aligned to TA90° and scaled to illustrate the effect of deviation to looming. The 1st, 2nd, and 3rd panels represent responses from trajectories at 400 cm, 80 cm, and 40 cm, respectively, and include responses to motion initiated in the anterior and posterior visual fields. Following a deviation to looming (Ant 90° - dark red line, Post 90° - light red line), the firing rate rapidly dropped regardless of distance or direction of travel. Although the translating trajectories (Ant trans – dark blue line, Post trans – light blue line) also produced a decrease in firing rate, the decrease was more gradual and failed to produce a subsequent fast rise to peak indicative of a response to looming. The time taken to regain this looming response was affected by the distance of deviation, with the shortest time occurring at the closest distance (40 cm). The dashed line represents TA90°.

Deviation to looming evoked a rapid drop in the firing rate that was dependent on the distance and direction of each trajectory (Fig. 2.7). Moreover, initiation of a TOD-associated peak took longer to develop when the trajectory change occurred further away and the peaks were larger at shorter deviation distances (compare Fig. 2.7 1st, 2nd, and 3rd panels).

2.5 DISCUSSION

This is the first study to quantify and compare DCMD firing in response to translating and complicated object motion. The DCMD is sensitive to object motion anywhere within a wide visual field (Rind and Simmons, 1997) and has been tested with a variety of moving visual stimuli (Rowel, 1971; Rind and Simmons, 1992; Krapp and Gabbiani 2005). However, previous work has not provided a quantitative description of how DCMD activity is modulated by complex visual motion. Guest and Gray (2006) found that the DCMD is sensitive to complex motion using simultaneously or closely timed paired object approaches as well as objects with compound shapes, supporting our hypothesis that a single motion sensitive pathway may encode aspects of complex motion. We demonstrate that DCMD responses are shaped by the distance and direction of object motion and are strongly modulated by deviations to a looming trajectory.

2.5.1 DCMD responses to purely looming objects

DCMD responses to a purely looming disc, an increasing firing rate that peaked near TOC (Fig. 2.2A), were consistent with previous reports (Gabbiani et al., 1999; Gray et al., 2001; Matheson et al., 2004; Gray, 2005; Guest and Gray, 2006). Gabbiani et al. (2001) found no difference in the time of peak firing for squares approaching from 45, 90, and 135° azimuth. Conversely, Guest and Gray (2006) reported that a disc approaching from 45° produced a significantly earlier peak time than an approach from 135°, though the difference was small (11.5 ms). Here we found no difference in the time of peak firing between responses to 45, 90, and 135° looms. However, looming from 90° evoked a higher peak firing rate than a loom from 45°. Although different regions of the locust eye were stimulated (i.e. the anterior (45°), posterior

(135°), or a combination of both (90°)) the final subtense angle after object motion ceased was identical. Perhaps the difference in area stimulated during each approach may explain the observed result. Additionally, differences in stimulus design may also be attributable, such as the final subtense angle of the object.

2.5.2 DCMD sensitive to direction and distance in translating trajectories

Our translating visual stimuli showed a directional and proximity effect where closer objects traveling from anterior to posterior generated the largest peak firing rates. The peak occurred before TA90° but was delayed in response to motion initiated in the anterior visual field (Fig. 2.3). It was recently reported that the highest firing rates in response to local motion objects were found for stimuli presented in the posterior region of the visual field (Krapp and Gabbiani, 2005; Peron et al., 2007) and that presentations in the frontal region are less robust with a greater latency in response (Krapp and Gabbiani, 2005). Previous studies using extended translating stimuli, such as sweeping bars in different directions over the eye did not show directional sensitivity in the DCMD (Krapp and Gabbiani, 2005). However, directional selectivity has been shown in other animal systems, such the lobula plate interneurons in many fly species (see Krapp et al., 1998) and neurons in the accessory optic system in pigeons (see Wylie and Frost, 1999).

Possible differences with our results may relate to the stimulus presented. Peron et al. (2007) used a stimulus that was a disc rotating on a circular path around the mid point of the eye, which is not truly translating, while Krapp and Gabbiani (2005) used a stimulus whose angular size remained constant throughout the presentation. Conversely, our translating stimuli took on a more 'natural' appearance as they were designed with real world coordinates and translated across the horizontal axis of the eye where the subtense angle gradually increased and decreased

around TA90° (Fig. 2.3A). Our results show that the directional sensitivity of the DCMD may be correlated with the relative density of ommatidia, assuming the number of local inputs alone is dominant in forming the response. However, even though the density of ommatidia is highest in the anterior and gradually decreases towards the posterior region of the eye (Krapp and Gabbiani, 2005; Peron et al., 2007), there is an apparently weak negative correlation between ommatidia density and DCMD sensitivity along the horizontal axis (Krapp and Gabbiani, 2005). This is in part explained by thicker dendrites sampling the posterior field of view (Peron et al., 2007), which is assumed to generate a more robust response. Thus, we cannot negate the possibility that other biophysical factors and network properties pre-synaptic to the LGMD/DCMD and not simply the number of input stimulations shape these responses. Results presented here are in accordance with previous reports (Krapp and Gabbiani, 2005; Guest and Gray, 2006). The LGMD/DCMD pathway is not a linearly and uniformly summing unit (as predicted in an early biophysical models by Edwards (1982), Rind and Bramwell (1996), and Gabbiani et al. (2001)), as it displays nonlinear responses to different visual stimuli. The responses shown here, and given the contrast in results from other studies testing translating stimuli, suggest a greater complexity in the integration and signaling of this visual pathway.

2.5.3 The effect of complicated trajectories on DCMD responses

All 18 translating to looming trajectories were different in some way (direction, distance, and angle of deviation), yet the final peak firing rate associated with TOC was generally not affected. This result emphasizes the ability of the DCMD as a collision detector neuron, which is able to reliably predict a collision regardless of previous presentation history. However, results demonstrate that the DCMD is more than just a simple looming detector.

Overall response profiles clearly illustrate the difference in DCMD responses to looming (Fig. 2.2A), translating (Fig. 2.3A), and translating to looming object trajectories (Fig. 2.4A, 2.5A, and 2.6A). Responses to looming objects have a much faster rise to peak and also a much higher firing rate, while the opposite is observed for translating trajectories. More complicated object trajectories generated another distinct response where early peak firing occurred before a deviation. Deviations that occurred at the furthest distance (400 cm), especially at the 45° and 135° azimuthal angles, did not always generate a distinct peak around the TOD, resulting in a larger variation in times of peak firing. However, closer object deviations to looming consistently resulted in a noticeable drop in firing rate. The average ommatidial resolution is around 1° (Horridge, 1978) and some deviations occurred at lower angles of visual subtense, e.g. at a 45 or 135° deviation at 400 cm distance, the angle subtended by the disc was < 1°. Object motion at angular distances below 1° may result in some animals not receiving any visual input and therefore no information would be transferred downstream from pre-synaptic visual networks.

Complicated approaches generated an early peak and valley associated with the time of deviation and the final peak responses associated with TOC were not affected by the immediate stimulus history. Whether a translating to looming trajectory occurred at 45, 90 or 135° azimuth, there was a TOD-associated peak that was affected by direction and distance, where closer distances and anterior travel generated higher peak firing rates. Closer distances generate a relatively larger angle of subtense, which results in stimulation of more ommatidia, and higher associated peak firing rates. Responses to the most complicated trajectories presented here illustrate not only the effectiveness of the DCMD in signaling an impending collision but also its ability to encode a deviation regardless of previous visual stimulation history. While the

biological significance of deviation detection is as of yet unknown, these responses may be better understood once we understand the role of the DCMD downstream. It has been shown that the DCMD is directly involved in flight-gating, specifically with the forewing elevator muscle motorneuron, MN84 (Santer et al., 2006). Responses to stimuli presented here caused a modulation in the firing rate that may affect the overall flight rhythm, which would ultimately result in behavioural consequences.

Previously reported responses to approaching objects have shown that an increased rate of object expansion on the retina results in an increased DCMD firing rate (Gabbiani et al., 1999, 2001; Judge and Rind, 1997; Guest and Gray, 2006). While consistent with translating responses, we found that a rapid decrease in firing rate occurs following a deviation change to looming, even though the object is rapidly expanding (Fig. 2.7). Translating responses display a gradual drop in rate associated with a decreased area of subtense and rate of edge expansion. The rapid drop in firing rate following a deviation may be a response to edge expansion into previously stimulated areas of the retina (i.e. a response linked to local synaptic habituation). Habituation occurs at localized synapses (Rowel, 1971; Bacon et al., 1995; Gray, 2005), which allows the eye to remain sensitive to multiple objects in the environment (Guest and Gray, 2006) or the same object traveling into new areas of the visual field (Gray, 2005). The edges of the looming object after deviation would briefly expand within a habituated array of ommatidia, resulting in a brief transiently decreased DCMD response. However, edge expansion in the visual field beyond this habituated area of the translating trajectory would stimulate a nonhabituated array of ommatidia, thus providing new input to the LGMD/DCMD pathway. The time when the object deviates to the time the object's edges are stimulating new ommatidia clearly depends on the direction and distance of the visual stimulus (Fig. 2.7) and is most likely linked to network properties that

underlie local habituation. Perhaps after a deviation to looming, and a short habituation time, the visual stimulus is interpreted as novel, which may arouse the nervous system and dishabituate the DCMD response (Bacon et al., 1995). However, previous studies have shown that a new trajectory or object presented during a habituated period did not dishabituate DCMD responses (Rowell, 1971; Bacon et al., 1995; Gray, 2005). Determining whether the DCMD is dishabituated following a rapid increase in the angular size of the object or as a result of edge expansion into new areas of the retina requires further investigation. It is also not known if these responses are due to a sudden change in the expected motion of an object.

The relationship between stimulation parameters and DCMD responses can vary depending on trajectory and complexity (Gray, 2005). A general linear model developed by Gabbiani et al. (2001) showed that the relationship between $l/|v|$ does not explain approaches from 0° and 45° azimuth and textured objects with multiple expanding edges. In addition, paired object approaches resulted in sublinear DCMD responses (Guest and Gray, 2006). Therefore, quantifying the stimulus based on subtense angle and edge expansion alone may adequately describe DCMD responses to simple looming and translating trajectories. It seems inadequate, however, in fully explaining responses to more complicated trajectories. Further experiments are needed to quantify DCMD responses to other complicated visual stimuli, with different $l/|v|$ values, in addition to understanding and modeling pre-synaptic network modulations that underlie DCMD responses.

The complicated trajectories presented here were intended to emulate the attacks of an aerial predator while tracking (the translating component) and engaging (the looming component) a prey locust. Some species of bird specialize in capturing locusts while in flight, such as the Carmine bee-eater (Fry et al., 1992). Choosing these types of visual stimuli is

reasonable considering that locusts presumably evolved a high level of sensitivity to looming

objects as an adaptation to predation (Schlotterer, 1977; Rind and Simmons, 1992, 1997;

Hatsopoulos et al., 1995; Gabbiani et al., 1999). Although an aerial predator's deviation would

not be as instantaneous as the course changes presented here, our purpose was to test what

response changes in trajectory would evoke without the potential properties of a nonlinear

stimulus. Due to potential adverse effects of presenting multiple visual stimuli over a relatively

long period of time, each animal was subjected to an initial and final looming trajectory. The

habituating effects of repeat visual stimulation (Bacon et al., 1995; Stern, 1999; Gray, 2005) and

the chance of a reduced response due to an extended period of experimentation did not affect

overall DCMD responses, thereby providing confidence in our results.

Results shown here provide evidence that simple nervous system networks are able to

encode complicated stimuli by extracting important features of sensory stimuli. Many other

visual systems extract features to reduce complexity. The horseshoe crab, for example, focuses

on visual stimuli that match specific visual parameters (size, contrast, and motion of potential

mates) in order to find a mate quickly and perform a mating behaviour (Passaglia et al., 1997).

Results presented here suggest that the LGMD/DCMD pathway is more than just a simple

looming detector and may encode complicated motion by extracting specific visual features. The

relative distance of object motion significantly affected DCMD responses in every trajectory

type tested. Evidence of directional sensitivity is shown by the differences in the time and peak

firing rates from different approach directions, demonstrated specifically in translating

trajectories and shown in different distances for more complicated trajectories. The DCMD also

responds to object deviations that occur in different times and space. Taken together, these

results provide clear evidence that the LGMD/DCMD pathway is able to encode complicated

aspects of complex visual environments. Previous investigations into the role of the DCMD support the idea that this pathway is important in predator avoidance. In designing our stimuli we therefore took the point of view of the predator that may track and attack a prey locust. However, it would be beneficial to look at the locust's point of view, using other complicated trajectories that emulate the natural scene of a flying locust. Deriving a link between complicated visual stimuli, neuronal activity, and active avoidance responses is crucial to elucidating important aspects of complex visual environments and general principles of sensory coding.

CHAPTER 3

BIMODAL CODING IN A DESCENDING MOTION

SENSITIVE VISUAL NEURON

3.1 ABSTRACT

The locust visual system contains an identified motion-sensitive pathway that responds preferentially to objects approaching on a direct collision course and descends throughout the body with lateral projections that synapse with motorneurons involved in predator evasion and collision avoidance (Simmons, 1980; Simmons and Rind, 1992; Santer et al., 2006). Previous studies have explained the encoding strategy of this pathway's interneuron, the descending contralateral movement detector (DCMD), as rate coding; edge expansion of approaching objects causes an increased rate of neuronal firing (Schlotterer, 1977; Simmons and Rind, 1992; Hatsopoulos et al., 1995; Judge and Rind, 1997) that peaks after a certain retinal threshold angle is exceeded (Gabbiani et al., 1999, 2001, 2002; Matheson et al., 2004). Based on observations of DCMD responses to rapidly approaching (looming) objects that showed oscillations in the averaged firing rates and tight clustering of spikes in raw traces (see, for example, Fig. 2 Gabbiani et al., 1999; Fig. 7A Guest and Gray 2006; Fig. 1 Money et al., 2006; Fig. 1D Santer et al., 2006) we hypothesized that bursting may be present. Bursting occurs in many sensory systems (Yu and Margoliash, 1996; Sherman, 2001; Krahe and Gabbiani, 2004; Marsat and Pollack, 2006), yet no studies have rigorously investigated such an observation in the locust

visual pathway. We tested locusts with simple looming stimuli known to generate behavioural responses in order to identify and quantify possible bursting activity and demonstrate that the DCMD does show evidence of bursting. Although it is not known whether a quantitative link between bursting and behaviour exists, the frequency of bursting in the DCMD could have behavioural implications. We found the more frequent and shorter inter-spike intervals (ISIs) occurred from 1-8 ms while the longer and less frequent ISIs occurred from 40-50 ms. A subsequent burst analysis showed that the majority of inter-burst intervals occurred at 40-50 ms (or 20-25 Hz), which is the average wingbeat frequency of a flying locust (~25 Hz, Robertson and Johnson, 1993). Although preliminary, results described here suggest that the DCMD employs a bimodal coding strategy to detect looming visual stimuli.

3.2 INTRODUCTION

Neuroscientists have long debated how spike trains code information about an animal's sensory environment (Stein et al., 2005). Early investigations have shown that the intensity of a stimulus is coded as a rate of impulses over time (i.e. rate coded) and variability in the timing was interpreted as neural noise (reviewed in Stein et al., 2005). A sequence of action potentials generated at specific time intervals (i.e. time coded) may also be important in the flow of neural information (Salinas and Sejnowski, 2001). Spike times and firing rates may be used independently to represent different aspects of a stimulus variable (VanRullen et al., 2005), are not mutually exclusive, and can be used in the same sensory neurons (Stein et al., 2005). If information provided by sensory neurons is effectively transmitted during a certain time window,

the timing of those spikes may be important in gating processes that control information that goes through at a given time (Salinas and Sejnowski, 2001).

Images of rapidly approaching (looming) objects have been shown to be a dominant visual stimulus in eliciting avoidance reactions in many animal species (Maier et al., 2004; Santer et al., 2005; Oliva et al., 2007) and neurons responsible for the detection and relay of looming stimuli are present in birds (Sun and Frost, 1998) and many insects (Simmons and Rind, 1992; Hatsopoulos et al., 1995; Wicklein and Strausfeld, 2000). Visual stimulation alone triggers the production of adaptive behavioural responses in the locust (Simmons and Rind, 1992; Judge and Rind, 1997; Gabbiani et al., 1999; Gray et al., 2001). An identified motion-sensitive neuron, the lobula giant motion detector (LGMD) and its post-synaptic partner the descending contralateral movement detector (DCMD), reacts preferentially to looming visual stimuli (Schlotterer, 1977; Rind and Simmons, 1992; Gray et al., 2001). The DCMD likely contributes to object avoidance as it is known to excite thoracic interneurons and motorneurons involved in flight steering and jumping (Burrows and Rowell, 1973; Matheson et al., 2004). The DCMD has been proposed to gate information into the flight rhythm to modify a course during flight (Reichert and Rowell, 1985, 1986; Reichert et al., 1985; Santer et al., 2006). However, since the DCMDs are not the only motion-sensitive neurons in the locust's visual system that project to the thoracic ganglia, the DCMDs alone may not be responsible for driving any particular avoidance behaviour (Gray et al., 2001; Santer et al., 2006).

The DCMD is one of the best described collision-sensitive neurons and although there is some argument about the exact properties and underlying mechanisms of signal transmission (Rind and Simmons, 1997; Gabbiani et al., 1999), a typical response to looming visual stimulus is an increased firing rate that peaks before collisions, with the peak thought to signal the time of

collision (TOC) (Gray et al., 2001). We observed rhythmic oscillations in the DCMD firing rate in the beginning of responses to simple looming stimuli and hypothesized that it may reflect bursting activity. To test this hypothesis we presented twenty locusts with thirty repetitions of a simple looming visual stimulus using a computer generated black 7 cm diameter disc.

Bursting activity (brief episodes of high-frequency firing of neurons that constitute a specific neural code) is known to occur in many sensory systems, including mammalian auditory (Eggermount and Smith, 1996) and visual (Sherman, 2001) systems, weakly electric fishes (Krahe and Gabbiani, 2004), and auditory neurons in insects such as crickets (Marsat and Pollack, 2006) and locusts (Eyherabide et al., 2008). Burst activity is important in signaling the occurrence of some behaviourally relevant sensory event. For example, bursts in pyramidal cells in the electro-sensory lateral line lobe of weakly electric fish are associated with electric field distortions caused by moving prey (Oswald et al., 2004). It has also been shown that bursts reliably predict behavioural responses, a link that was previously not established. Marsat and Pollack (2006) demonstrated that burst activity in an identified ultrasound-sensitive auditory interneuron (AN2) of crickets signals stimulus features of echo-locating bats that use ultrasonic probes to hunt. Crickets respond to ultrasound stimuli with avoidance responses that are triggered by AN2 (Nolen and Hoy, 1984). Marst and Pollack (2006) proposed that isolated spikes in the AN2 neuron may play a role in encoding cricket song and contributes to behavioural responses to these stimuli, while bursting may be used when avoiding bats, given that only bursts elicit strong behavioural responses. Thus, multiple modes of encoding in single neurons may have context-dependent behavioural implications.

Although preliminary, this study is the first to describe bursting in the DCMD. Similar to other sensory neurons (e.g. the AN2 in crickets; Marsat and Pollack, 2006), the DCMD may use

two modes of coding to detect sensory stimuli. Although the AN2 is an auditory descending neuron and the DCMD a visual descending neuron, both trigger avoidance responses. Given the number of post-synaptic neurons downstream from the DCMD, elucidating what information is useful to post-synaptic neurons may be context-dependent.

3.3 MATERIAL AND METHODS

3.3.1 Animals

We used twenty adult male *Locusta migratoria* that were over 3 weeks past the imaginal molt obtained from a crowded colony maintained in the Department of Biology at the University of Saskatchewan (25-28°C, 12hr:12hr light:dark). Experiments were carried out at room temperature (~25°C).

3.3.2 Preparation

Locusts were prepared for experimentation by removing the legs and clipping the wings. A rigid tether was attached to the ventral surface of the thorax using 3M™ Vetbond™ Tissue Adhesive 1469SB (3M Animal Care Products, St. Paul, MN) and a small incision was cut into the ventral cervical cuticle to expose the underlying paired connectives of the ventral nerve cord anterior to the prothoracic ganglia. A drop of locust saline (147 mmol NaCl, 10 mmol KCl, 4 mmol $CaCl_2$, 3 mmol NaOH, 10 mmol Hepes, pH 7.2) was used to bath the exposed tissue and prevent desiccation during experimentation. The preparation was transferred to the recording stage where neuronal recordings were obtained from the left ventral nerve connective using a

bipolar silver wire hook electrode. The nerve connective and electrode were insulated with a mixture of Vaseline and mineral oil. To position the locust so that the right eye was aligned with the azimuthal and elevation axes of the dome apex, the preparation was rotated dorsal-side up with its longitudinal axes perpendicular to the apex of the rear projection screen (Fig. 2.1A).

Before experimentation, the preparation was left for a minimum of 30 minutes in front of a projected white visual field (background luminance = 430 cd/m^2) to allow the animal to acclimate to the experimental setup. Each visual stimulus was presented in intervals ≥ 2 minutes to prevent confounding effects of neural habituation. Any preparation that failed to produce a reliable neural response to motion (hand waving across the projection screen) was not used for experimentation.

3.3.3 Visual stimuli

The procedure used for visual stimulus generation and data acquisition was similar to that used by Guest and Gray (2006). Vision Egg visual stimulus generation software (A. Straw; http://visionegg.org/) on a Python programming platform was used to create visual stimuli, which were represented as 1,024 x 1,024 pixel portable network graphics (png) files. Individual pixel sizes on screen were approximately 0.7 mm, which corresponds to a visual subtense angle of ~0.4°, below the 1° resolution of individual ommatidia (Horridge 1978). The visual stimuli (7 cm diameter black disc traveling 300 cm/sec) were scaled in real-time at 85 frames/sec (fps) and projected using a Sony VPL-PX11 LCD data projector onto a specialized rear projection dome screen. Correction factors were embedded in the Vision Egg code to account for the distortion due to projection on the curved surface of the screen. A 1.2-ms TTL pulse included in each video frame and the vertical refresh synchronization pulse (Vsynch) from the video card (NVIDIA

GeForce4 Ti4200 128 MB) were used to align the DCMD recordings with the projected time of collision (TOC). The final frame of each presentation was determined using the last TTL pulse, which indicated when the object had disappeared from the screen. The corresponding Vsynch pulse determined the start time of the rendering of this frame. The luminance values (430 cd/m^2) and Michelson contrast ratio (0.48) were the same as those used by Guest and Gray (2006). Thirty looming stimuli were presented 90° in the azimuthal plane relative to the right eye of each locust at a starting subtense angle of 1° (400 cm away) and final subtense of 32.5° (12 cm away).

3.3.4 Spike sorting and DCMD identification

For each presentation, neuronal activity from the left cervical connective, pulses synchronized with each frame of the stimulus, and Vsynch pulses from the video card were recorded continuously and stored for analysis. Recorded activity was amplified with a differential AC amplifier (A-M Systems, model No. 1700, gain = 10,000) and sampled at 25 kHz. An RP2.1 enhanced real-time processor (Tucker-Davis Technologies, Alachua, FL) with Butterworth filter settings of 100 Hz (high pass) and 5 kHz (low-pass) was used to store the data to disk.

DCMD activity in the cervical connective was identified using threshold analysis in Off-line Sorter (Plexon, Dallas, TX). The characteristically large amplitude DCMD spikes were easily isolated using this method (Fig. 2.1A). Spike times were exported to Neuroexplorer spike train analysis software (NEX Technologies, Littletonm MA) for analysis.

3.3.5 Burst analysis

Based on the consistency within trials (N = 30 looming approaches) and between animals

(*n* = 20) (Fig. 3.1) we grouped all the responses (600 approaches in total) and performed our

analysis using Neuroexplorer.
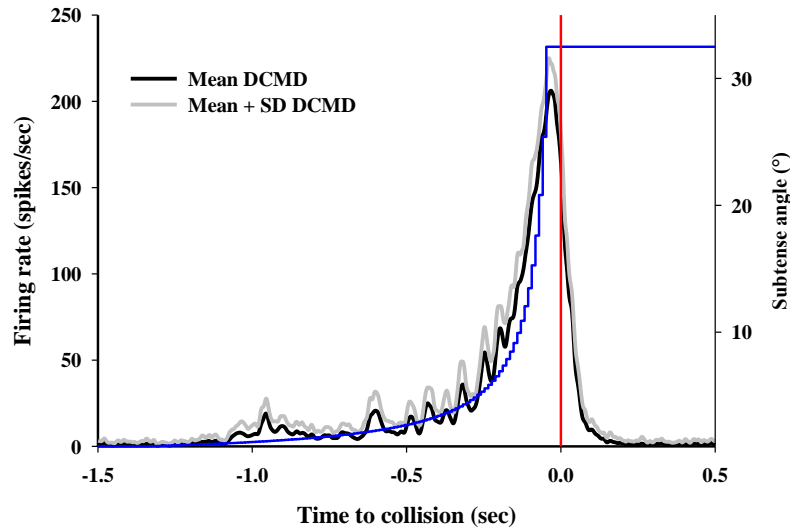
**Figure 3.1.** Mean DCMD responses (black line) plus the standard deviation (SD, gray line), time aligned to the time of collision (red line) with an overlay of the change in subtense angle over time (blue line).The DCMD response follows the change in subtense angle and peaks before the time of collision and after object motion stops. Response were very consistent between animals and within animals, resulting in a low SD about the mean. Variability in the firing rate is observed up to ~0.2 sec before the time of collision while the firing rate rapidly and consistently increases shortly before collision. Data from 20 animals with the mean response from 30 approaches for each animal. Bin width of 1 ms and Gaussian smoothed at 11.76 ms (according to the frame rate of stimulus presentation).

Previous studies have shown that low DCMD firing rates 200 ms before collision may

trigger avoidance steering responses (Gray 2005; Matheson et al. 2004). Therefore, we isolated

and analyzed DCMD activity from the beginning of object approach up to 200 ms before the

time of collision (TOC), from 200 ms prior to TOC until TOC, and finally the entire response

while the visual stimulus was presented.

Analysis of the ISI distribution is commonly used to determine if burst activity is present in single neurons since a bursting neuron will display a bimodal ISI distribution. A limit value is used to separate isolated spikes from spikes grouped into bursts based on the shape of the ISI distribution (see, for example, Metzner et al., 1998; Gabbiani and Krapp, 2006; Marsat and Pollack, 2006; Oswald et al., 2007). Although spike number is variable within a burst, the minimum number of spikes in a burst is typically two (see Marsat and Pollack, 2006). Figure 3.2 displays a large variability in the ISIs during object motion. Within this variability two clusters were observed, where one was relatively constant another displayed a gradual decrease in ISIs as the object approached collision (Fig. 3.2). The fan-shaped ISI distribution and constant ISI distribution illustrates variability in DCMD responses to simple looming objects.
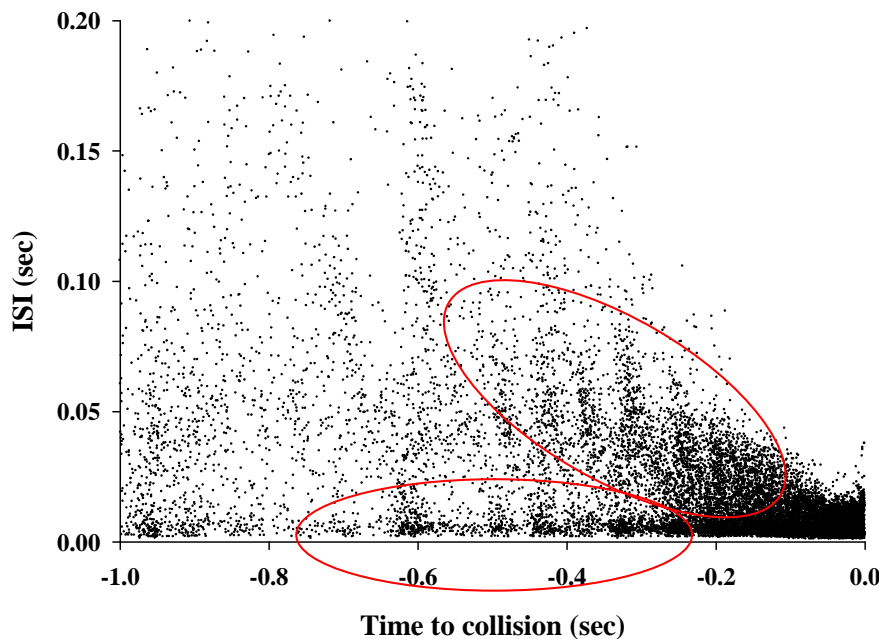
Figure 3.2. Inter-spike intervals (ISIs) calculated from raw spike times from all 600 presentations (20 animals x 30 presentations for each). Most ISIs are distributed from 200 ms before the time of collision (red line). Two ISI clusters are highlighted within the red; short ISIs occurred consistently throughout the approach while another cluster appears to begin around 500 ms before collision and decreases up to collision. This fan-shaped ISI distribution highlights the variability in spike times during a looming approach.

We plotted an inter-spike interval (ISI) distribution histogram which also showed variability in DCMD firing; the time range from stimulus start to 200 ms before TOC yielded a strong bimodal ISI distribution; the most frequent ISIs occurred around 1-8 ms and the second most frequent around 30-50 ms (Fig. 3.3B).
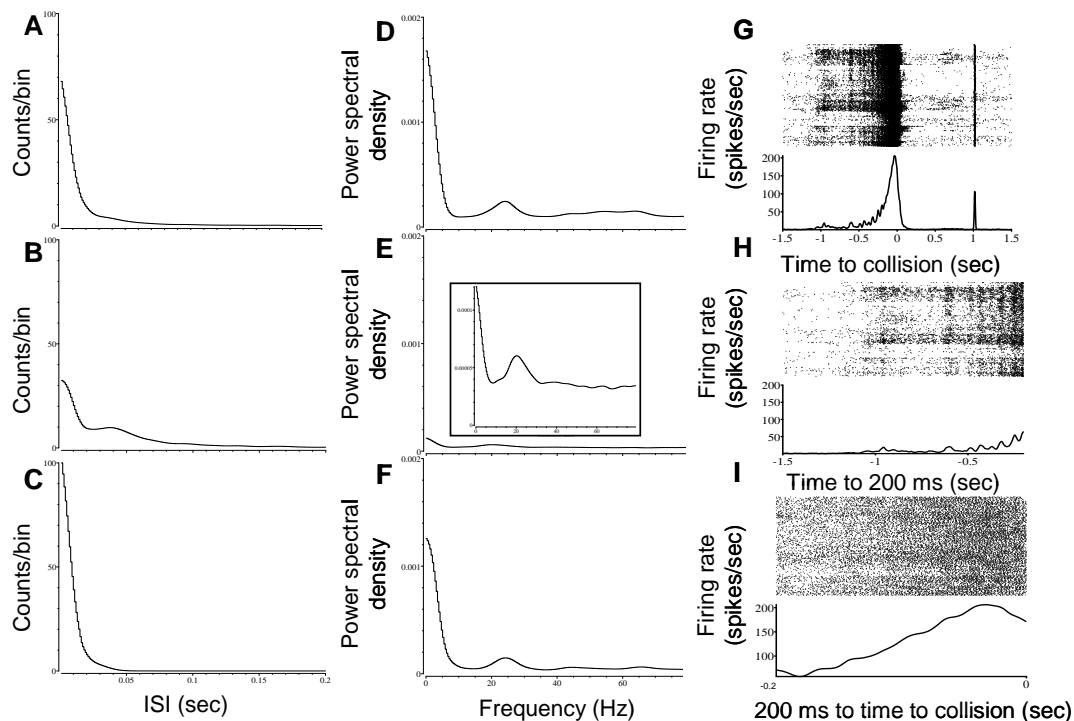
Figure 3.3. Inter-spike interval (ISI) histograms and power spectral density analysis, and DCMD responses for three intervals of time for DCMD responses to a looming visual stimulus. A, D, G represent the entire DCMD response while the stimulus was active. This resulted in a weakly bimodal ISI distribution (A), a bimodal power spectral density with a smaller peak around 25 Hz (B), and a typical DCMD response showing a high amount of fluctuations in the firing rate during the beginning of approach (G). B, E, H represents DCMD activity from start of stimulus approach to 200 ms before collision. This resulted in a strong bimodal ISI distribution, with an early distribution occurring between 1-8 ms and a longer distribution between 40-50 ms (B), a strong bimodal power spectral density with a lower peak value around 25 Hz, and a fluctuating DCMD response (H). C, F, I, represents DCMD activity from 200 ms before collision up to collision. This resulted in a strong unimodal distribution (C), a strong bimodal power spectral density with a lower density peak occurring around 25 Hz, and a DCMD response that is dominated by high frequency spiking that peaks before the time to collision. Each row of rasters (G, H, I) represents the response of one animal to a single looming approach (600 total). Bin-width set at 1 ms, Gaussian smoothed at 11.76 ms (aligned to the frame rate of the stimulus.

The ISI distribution for the entire response while the stimulus was active and from 200 ms to TOC failed to show a clear second grouping around 30-50 ms (Figs. 3.3A and 3.3C). In support of these findings, power spectral density analyses also displayed a bimodal distribution for each interval (Figs. 3.3D, 3.3E, and 3.3F). The ISI distributions can be related to the raster plots of DCMD responses during each interval (Figs. 3.3G, 3.3H, and 3.3I). Hypothesizing that 30-50 ms may be the inter-burst interval (IBI) and 1-8 ms may be ISI within each burst, we developed a burst detection assay (Fig. 3.4).
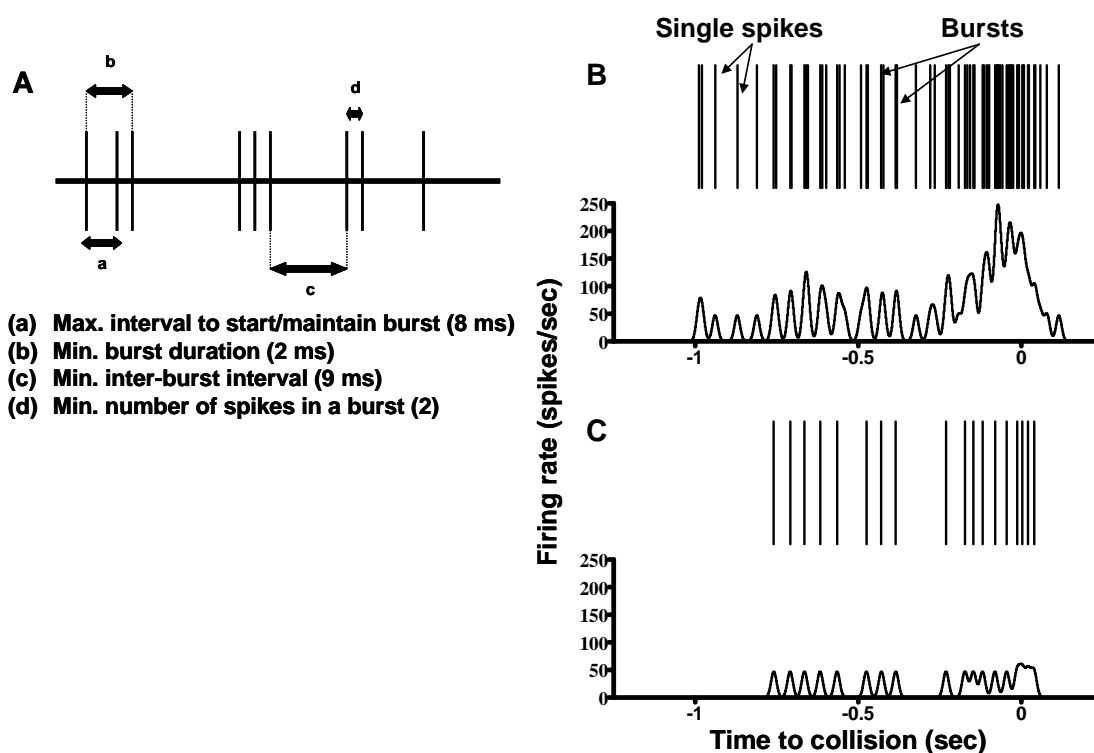
Figure 3.4. Burst isolation methods. A, Burst analysis parameters based on the average inter-spike interval (ISI) distribution of DCMD activity during a looming approach. A burst must have at least 2 successive spikes occurring at a minimum duration of 2 ms as it is physiologically impossible to observe an ISI below 1 ms between 2 spikes from a single neuron. ISIs below 8 ms were treated as ISIs within a burst and a minimum inter-burst interval of 9 ms was used to separate individual bursts. B, example of a DCMD spike train displaying single spikes and bursts of spikes. C, Burst analysis result isolating DCMD bursts within the spike train.

As a result, all spikes were classified as isolated spikes or grouped into bursts of two or more spikes with ISIs less than 8 ms.

3.3.6 Statistical analysis

Significance of different DCMD firing parameters was assessed at $P < 0.05$ using SigmaStat 3.0 and plotted using SigmaPlot 10.0 (Systat Software Inc., Richmond, CA, USA). Statistical tests used to compare various parameters of the DCMD spike train were a t-test for parametric data and a Mann-Whitney Rank Sum Test for non-parametric data. Specific tests used are indicated in appropriate sections of the results.

3.4 RESULTS

3.4.1 DCMD responses to simple looming stimuli

Typical DCMD responses (Gabbiani et al., 1999; Gray et al., 2001; Rind and Simmons, 1992; Guest and Gray, 2006) were generated when we presented a locust with a looming visual stimulus (i.e. an increasing spike rate that reached a peak near the TOC). This response was very consistent between animals and between stimulus presentations (Fig. 3.1). Variability in the overall response was observed to increase up to 200 ms before TOC, after which the firing rate steadily increased. To show that DCMD responses were consistent throughout the entire experiment, the first and last presentations for each animal were compared. No significant differences were found in the time (t-test, $t_{38} = -0.32$, $n = 20$, $P > 0.05$) and amplitude (t-test, $t_{38} = 0.50$, $n = 20$, $P > 0.05$) of peak firing rate, peak width at half height (Mann-Whitney, $U_{38} = 252$

($T = 358$), $n = 20$, $P > 0.05$), and total number of spikes (t-test, $t_{38} = 0.61$, $n = 20$, $P > 0.05$). For all presentations for every animal, the mean ± standard deviation for peak amplitude was $317 ±$ 43 spikes/sec, time of peak was $50 ± 39$ ms before TOC, number of spikes was $52 ± 16$, and the peak width at half height was $28 ± 13$ ms. These values are typical of responses to smaller disks, which usually generate a higher peak firing rate, shorter peak duration, and a peak closer to collision relative to larger discs (Gray et al., 2001; Gray, 2005; Guest and Gray, 2006). The ratio of the half size of a symmetrical object (l) and the absolute velocity $|v|$ may be used to calculate a single value that relates to the increase in angular subtense during a looming approach (see Gabbiani et al., 1999). Based on the properties of our stimulus (objects half-size (l) = 3.5 cm and absolute velocity ($|v|$) = 300 cm/s, $l/|v|$=12 ms) responses presented here were similar with what was previously reported for similar $l/|v|$ values (see Guest and Gray, 2006).

Using the raw spike times, ISIs were manually calculated by taking the difference between two successive spikes from all 600 spike trains and comparing against the stimulus duration (Fig. 3.2). The majority of ISIs were short and occurred close to TOC, however a fan-shaped distribution representing a large variability in ISIs exists, specifically during the beginning of approach (Fig. 3.2). Two clusters also appear in the ISI over time plot where one cluster is a relatively constant cluster of short ISIs and the second begins around 50 ms intervals and decreases over time (Fig. 3.2). The results from the ISI distribution histograms and power spectral density analyses showed a clear distribution of spikes occurring at a frequency of ~25 Hz (Fig. 3.3).

3.4.2 DCMD response displays evidence of bursting

The first reason for looking at bursting in the DCMD comes from observations of DCMD responses to simple looming objects (see, for example, Fig. 3.1 here; Fig. 1E Rind, 1996; Fig. 2 Gabbiani et al., 1999; Fig. 1A Gabbiani et al., 2001; Fig. 7A Guest and Gray 2006; Fig. 1 Money et al., 2006; Fig. 1D Santer et al., 2006). Second, evidence that sensory neurons encode behaviourally relevant stimuli using bursts has been proposed for other systems (Guido et al., 1995; Lesica and Stanley, 2004; Oswald et al., 2004; Marsat and Pollack, 2006; Eyherabide et al., 2008; Sabourin and Pollack, 2009). Examining the ISI distribution of a spike train and determining the frequency of occurrence of the various intervals at which the neuron discharges (plotted in the form of a histogram) may provide information about the fundamental firing properties of the neuron. Evidence of oscillatory and bursting activity may also be revealed using a power spectral analysis method (Israel and Burchiel, 2005).

We separated the entire DCMD spike train into three intervals for analysis. The first separation includes DCMD activity up to 200 ms before TOC, the second is activity from 200 ms before TOC up to TOC, and the third included the entire spike train in which the stimulus was active. Results from the power spectral method analysis for all three intervals showed a bimodal distribution where a clear distribution is present around 25 Hz (Figs. 3.3D, 3.3E, and 3.3F). This bimodality is also strongly illustrated in an ISI histogram for DCMD activity up to 200 ms before TOC (Fig. 3.3B). A strong unimodal ISI distribution was observed from 200 ms to TOC and the ISI histogram representing the entire spike train while the stimulus moving showed a slight bimodal distribution (Fig. 3.3C).

The ISI over time distribution (Fig. 3.2) reflects the inversed trend of the perievent histogram of the mean DCMD burst response (Fig. 3.5), where a strong relationship between the burst frequency and subtense angle exists.
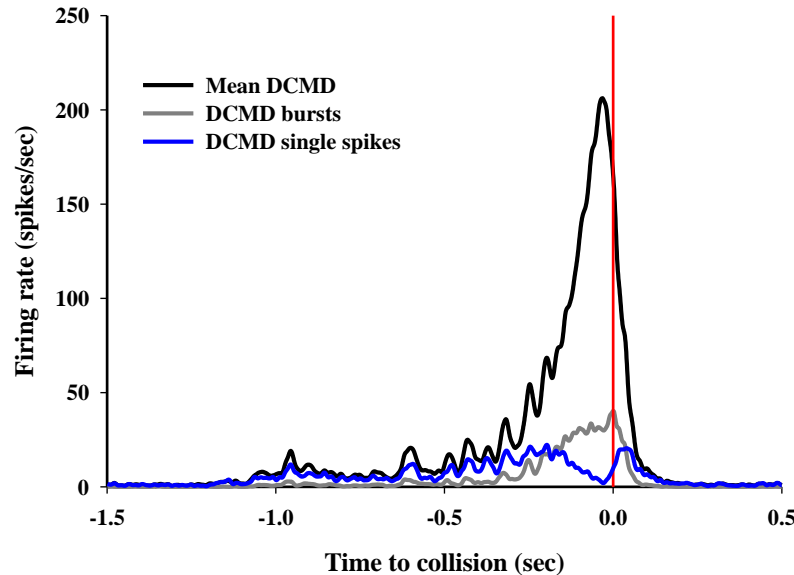
Figure 3.5. Mean DCMD (black line), DCMD bursts (gray line), and DCMD single spikes (blue line) time aligned to the time of collision (red line). Mean DCMD response shows an increase in firing rate with a peak response before collision. DCMD burst firing rate (represented by the first spike at the beginning of each burst) increases during object approach and peaks at the time of collision and after object motion stops. DCMD single spiking (filtered from the DCMD response with DCMD burst intervals) shows a decrease in firing rate ~0.2 sec before collision. Almost all spikes after ~0.2 sec before collision are within bursts. Data represents 20 animals with the mean response from 30 approaches for each animal. Bin width of 1 ms and Gaussian smoothed at 11.76 ms (according to the frame rate of stimulus presentation).

Near the end of object approach almost all spikes were within bursts, illustrated by the plotted single spike data (Fig. 3.5). Isolated single spikes increased up to 200 ms before collision but decreased soon after (Fig. 3.5). Similar to the mean DCMD response (plotted in both Figs. 3.1 and 3.5), burst frequency also increased as object size increased during approach, however the peak burst frequency occurred at TOC rather than before (Fig. 3.5). The inter-burst interval

(IBI) distribution histogram for all intervals displayed a unimodal distribution with the most

frequent distribution around 40-50 ms (20-25 Hz) (Fig. 3.6).
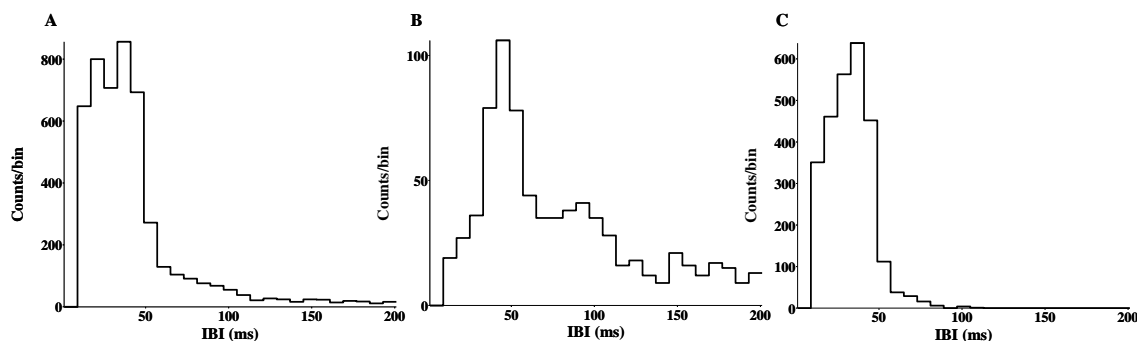
Figure 3.6. Inter-burst interval (IBI) histogram of DCMD bursts for the entire spike train (A), up to 200 ms before collision (B), and from 200 before collision to collision. For all time intervals (A, B, and C), the highest count of IBIs occurred at 40-50 ms intervals. Up to 200 ms before collision (B) the IBIs are more defined. Data not smoothed and binwidth set at 8 ms.

Approximately 70 % of all spikes within the entire response were found within bursts.

However, 90 % of spikes before TOC were within bursts and up to 200 ms before TOC only 36

% were within bursts, displaying the predominance of single spiking early in the approach and

bursting near the end of approach. The number of spikes in a burst was on average 4 ± 3 SD for

the entire response, however the spike number increased from 3 spikes ± 1 SD during the

beginning of approach (up to 200 ms before TOC) to 5 spikes ± 4 SD during the final approach

up to TOC. The mean duration of bursts for the entire response was highly variable, displaying

11 ms ± 12 SD durations, however the mean duration of bursts changed from 7 ms ± 3 SD from

the beginning of approach to 13 ms ± 5 SD from 200 ms to TOC, which again shows that a high

number of spikes near the TOC were contained within bursts. Although the internal components

of the bursts were relatively variable throughout the approach of the object, the ISI distribution

within all bursts was strongly unimodal and constant, displaying peak ISIs around 4 ms (Fig.

3.7).
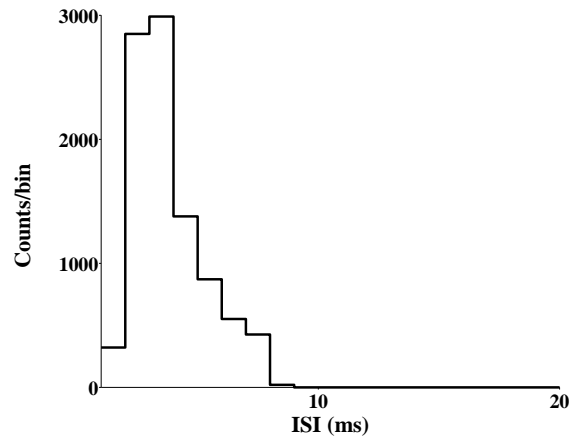
Figure 3.7. Inter-spike interval (ISI) histogram of spikes within the bursts. The ISIs within each burst remained relatively constant with the highest counts occurring at ISIs of 3 - 4 ms. Data not smoothed and binwidth set at 1 ms.

Because the frequency of spikes within a burst did not change over time (~270 ± 80

spikes/sec), the internal temporal structure of each burst was not analyzed here and only the first

spike in each burst was considered.

3.5 DISCUSSION

Many studies have suggested that the role of the LGMD/DCMD pathway is in collision

avoidance (Simmons and Rind, 1992; Judge and Rind, 1997; Gabbiani et al., 1999; Gray et al.,

2001; Santer et al., 2006), encodes looming objects via a rate code (Schlotterer, 1977;

Hatsopoulos et al., 1995; Judge and Rind, 1997), and implied that DCMD activity may gate

information regarding an impeding collision into post-synaptic motorneurons (Reichert et al.,

1985; Santer et al., 2006). The goal of this paper was to see if bursting was present in DCMD responses to looming visual stimuli. Based on our preliminary results, there is evidence to suggest bursting does exist and considering that bursts occurred around the average wingbeat frequency of a flying locust (25 Hz, Robertson and Johnson, 1993), there may be behavioural implications. The DCMD descends from the brain, connects to forewing muscle motorneurons in the thoracic ganglia (Simmons, 1980; Santer et al., 2006), and has thus been implicated in collision avoidance since the forewings likely contribute the most to avoidance behaviours during flight (Roberston and Reye, 1992). Bursting activity in the DCMD may be a way of gating information into the flight circuitry with a possible role of an underlying rate code that acts to modify flight rhythm, effectively altering forewing asymmetry and generating a collision avoidance behaviour.

There are limitations to the technique we used to test DCMD responses. Although it is not a natural visual stimulus, many studies that have examined locust collision avoidance and looming responses of motion-sensitive neurons used computer-generated stimuli by incorporating aspects of objects thought to be biologically relevant (Gray, 2005). Computer-generated simulations of looming objects stimulate the DCMDs similarly to real looming objects and they also produce stereotypical collision avoidance behaviours in tethered flying locusts (Gray et al., 2001; Guest and Gray, 2006). Although studies that involve simple stimuli alone may not tell us how a neuron responds to more complex stimuli encountered in the locust's natural environment, well designed simple stimuli have been pivotal in revealing the neuronal basis of sensory processing (Felsen and Dan, 2005). Natural stimuli also have limitations as they offer less flexibility than artificially designed computer generated images, which are much easier to quantify and manipulate at the experimenter's discretion. We only used a single type of visual

stimulus, therefore future studies should address this by using variable sizes and different trajectory types. Given the goal of this study, these various types of visual stimuli were not needed for comparison, as our goal was to provide preliminary evidence of bursting in DCMD responses.

One cannot negate the possibility that the results presented here were affected by averaging so many trials together and the bursts we quantified were stochastic events. Variability in spike firing times exists in many neurons and even if variability in spiking may not be part of a sensory signal, it may be an important part of the accurate processing of the signal (Stein et al., 2005). Variability in the timing of bursts means that each burst may arrive at different times during the phases of each wing beat, consequently generating a slightly different behavioural response. Although DCMD responses alone may not reliably predict the behaviour of the locust, variability in behavioural responses to similar visual stimuli may allow the locust to remain elusive if being attacked by an aerial predator (Santer et al., 2006). Quantification of responses in post-synaptic motorneurons and behavioural trials using similar stimuli used here will ultimately reveal which components of the DCMD spike train are important in affecting avoidance behaviours.

Based on results shown here, the LGMD/DCMD pathway may employ a bimodal firing property in order to encode aspects of a looming visual stimulus. Analysis of the DCMD spike train using a power spectral density method displayed a peak frequency of 25 Hz and an ISI histogram distribution resulted in a bimodal distribution with shorter ISIs representing the interval between spikes within a burst and the longer ISIs corresponding to intervals between bursts. The burst analysis displayed a unimodal IBI distribution (Fig. 3.6) where peak IBIs occurred around 40 ms, or 25 Hz, roughly the average wingbeat frequency during locust flight

(Robertson and Johnson, 1993). Bursting in the DCMD may provide the encoding link in gating information into post-synaptic motorneurons or the flight central pattern generator (CPG). Santer et al (2006) found that high frequency DCMD spikes caused summing EPSPs in a forewing elevator motorneuron (MN84) and occurred with the onset of the MN84 burst that elevates the forewings into a gliding posture. Santer et al. (2006) also proposed that DCMD activity may directly affect the flight CPG during a glide.

Burst frequency increases with stimulus intensity (Fig. 3.5). Therefore, it is likely that DCMD bursts encode some aspect of the visual stimulus. However, bursting may simply aid in the transfer of visual information, affectively gating an underlying rate code into the flight circuitry. In this light, the rapid decrease in single spiking and a predominance of bursting near TOC may be an artifact of our burst analysis, as all spikes occurring close to TOC are of very low ISIs and were classified as bursts. Another interpretation to test is whether bursting results from a local flow field created partially by the approaching visual stimulus. In addition to other sensory input, continuous and regular bursting information provided by the DCMD encoding the flow field during flight may allow a flying locust to maintain a basic flight rhythm. A future study testing the locusts to flow field motion using translating bars expanding past the periphery, for example, would yield some beneficial results in better describing this possible relationship.

Results from our ISI distributions (Fig. 3.2) and histograms (Fig. 3.3) suggest that rate coding alone may not define DCMD responses to simple looming stimuli. However, bursting also may not define these responses, as single spiking occurred irrespective of bursting (Fig. 3.5). After 200 ms before TOC, the DCMD response is characterized by a rapid increase in burst frequency and decrease in single spiking (Fig. 3.5). Locusts perform steering avoidance behaviours around 200 ms before the TOC (Matheson et al., 2004; Gray, 2005) and an evasive

glide if the DCMD firing rate is still increasing beyond this time (Santer et al., 2006). In many nocturnally flying insects, such as moths, high frequency spiking occurs when an attacking bat is near and a similar last chance avoidance behaviour is performed (Triblehorn and Yager, 2005). We found that single spiking increases up to and decreases after 200 ms before TOC, whereas burst frequency gradually increases up to 200 ms before TOC followed by a rapid increase up to TOC (Fig. 3.5). These results predict that low burst rates (and relatively high single spikes rates) before 200 ms may elicit responses that result in subtle trajectory changes, while the high frequency bursting (and low single spike frequency) near the TOC may result in an evasive glide.

The true relationship between DCMD bursting and single spiking is, as of yet, unclear. Other sensory neurons involved in generating avoidance behaviours (such as the cricket's AN2), may use two modes of encoding in a context dependent manner (Marsat and Pollack, 2006). For example, the AN2 neuron was proposed to encode threatening bat cries using bursting, while single spiking may encode non-threatening calls from conspecifics (Marsat and Pollack, 2006). Bursting may overshadow single spikes after the object that is approaching appears to be threatening, thus generating an evasive glide. Alternatively, single spiking may only modify the flight rhythm slightly, thereby generating a less pronounced avoidance behaviour. These predictions need to be tested with threatening and non-threatening looming visual stimuli representing, for example, another conspecific in a swarm (non-threatening and non-colliding objects with relatively low final subtense angles) and an approaching predator (threatening with relatively high final subtense angles).

Given the variability in DCMD responses and use of only a single stimulus type, we cannot confidently say what the role of bursting is in encoding an approaching object, or if bursting is only important in flight gating, without further experimentation. Understanding what

information post-synaptic motorneurons take from the DCMD spike train during a looming

presentation is also required to properly elucidate the role of this visual pathway in collision

avoidance. Although burst-encoded sensory stimuli has been linked to behavioural roles in other

insects (Marsat and Pollock, 2006), it is necessary to establish a link in the timing of DCMD

bursts in a behavioural context. Understanding the downstream role of sensory neurons in a

behavioural context is imperative in the proper interpretation of a sensory signal, as the timing of

a spike to some internal event may be considered unreliable if the observer is not aware of such

an event (VanRullen et al., 2005). A clearer understanding of the role of sensory neurons, such as

the LGMDs/DCMDs, in affecting post-synaptic neurons and the role of those affected neurons in

generating behaviours may ultimately provide the big picture required to understand what is a

reliable sensory code. Future studies examining the role of bursting in encoding visual stimuli

will not only aid in understanding what information the DCMD conveys but also provide more

clues into the general principles of sensory coding and information transfer.

CHAPTER 4

GENERAL DISCUSSION

4.1 SENSORY ENCODING IN THE DCMD

Results from both Chapters 2 and 3 illustrate the complexity a single neuron can display in encoding visual stimuli. Results presented here have not been reported previously. Chapter 2 presented the LGMD/DCMD pathway to complicated object motion. The DCMD was able to discriminate between the direction of initial object motion and relative distance for almost all non-looming to looming deviations. A drop in firing rate that followed a deviation to a loom in the visual stimuli also provided evidence to suggest the DCMD may encode complicated aspects of complex visual environments. This is thought to be related to the adaptation or habituation of local photoreceptors. Differences in looming, non-looming, and non-looming to looming trajectories show that this single motion sensitive pathway encodes aspects of a complex visual environment.

Results from Chapter 3 show another level of complexity in DCMD responses. The DCMD displayed a bimodal encoding strategy in response to a repetitive stimulation of a simple looming disc. An analysis of the ISI distribution clearly demonstrated two distributions of ISIs; shorter ISIs corresponded to spikes within a burst and the longer ISIs corresponded to intervals between bursts. Further analysis showed that bursting and single spiking occurs in the DCMD. Bursts occurred in an average frequency of 25 Hz, which is the average wing beat frequency of

the locust forewings (Robertson and Johnson, 1993). Although the relationship between single spiking and bursting in encoding an approaching object remains unknown, burst frequency increased as the object approached which suggests that bursting is affected by the stimulus. However, bursting may be involved in carrying information and gating into the flight circuitry. DCMD bursting frequency increases rapidly after 200 ms before TOC and up to TOC, but the single spiking frequency drops after 200 ms before TOC. Locusts have shown avoidance behaviours after 200 ms before TOC and an evasive glide if the object is still approaching after 200 ms (see discussion in Chapter 3). Thus, it may be that coding in the DCMD is context-dependent, where single spiking signals a non-threatening visual stimulus and the bursting signals a threatening object. Although further experiments are required to quantify these predictions, results from Chapter 3 provides the first preliminary evidence to demonstrate bursting in the DCMD.

4.2 IMPLICATIONS IN BEHAVIOUR

The DCMDs synapse with interneurons and motorneurons involved in generating avoidance behaviours and are implicated in generating avoidance behaviours (Simmons, 1980; Simmons and Rind, 1992; Santer et al., 2006). Therefore, it is likely that any variation in DCMD firing patterns would affect behavioural responses in locusts. As discussed in Chapter 3, bursting in the DCMD may act to gate information into the flight circuitry. It may be that bursts of spikes in the DCMD directly affects motorneurons involved in elevating forewings, which, for example, have been shown to generate bursts of activity to generate the power necessary to lift the wing

(see Fig. 1D Santer et al., 2006). It would be also be beneficial to observe behavioural responses to trajectories presented in Chapter 2. Some of these stimuli were meant to represent the trajectory of an aerial predator on an attacking course. Perhaps the deviations would cause behavioural responses, such as slight shifts in course trajectory or an evasive glide. Differences in DCMD responses to different directions and distances, may be important in signaling where an object is at any particular time and in any given space, therefore allowing the animal to quickly predict whether it is threatening and potentially avoid it. Without further experiments that directly link DCMD activity, post-synaptic motorneuron responses, and behavioural responses, the affect of these types of stimuli on behaviours remains unknown and we can only predict what may happen based on fragments of the whole picture.

4.3 FUTURE DIRECTIONS

The locust visual pathway acts as a great model system in neuroethology to test many different stimulus variables and elucidate general principles of sensory coding. As it is well defined and easily accessible, this system has also been pivotal in aiding in descriptions from neuronal responses to changes in temperature and other environmental stresses (for example, Money et al., 2006) to generating neuronal network models used in robotics and car manufacturing (for example, Yue et al., 2006). The idea that a single neuronal pathway can yield so much useful scientific data is quite impressive, but it is not surprising, as insects have evolved relatively simple and compact nervous systems yet they can generate remarkably complex behaviours using ingenious methods. A great example in this sense are collision avoidance

behaviours during flight, which not only require the coordinated activity of many dynamic

neuronal pathways and sensory feedback networks, but also require rapid detection and relay to

be effectively generated. Reducing this complexity into easily quantifiable and discrete

components is required before the overall picture can be seen. This led into my thesis work on

testing the ability of locusts in detecting and encoding different visual stimuli.

In Chapter 2, I showed how a single neuron could encode different visual stimuli by

modifying its firing rate, a modification that may be generated by local synaptic adaptation in the

photoreceptors that initiate the electrical code. In Chapter 3, I discussed that the LGMD/DCMD

pathway may employ a bimodal encoding strategy in detecting approaching objects, where both

bursting and single spiking exist and were affected by the visual stimulus. These findings

provide evidence to suggest that the DCMD is more than just simple looming detector and

employs a complicated coding strategy. However, further experimentation is required to solidify

these findings.

Identified visual neurons, such as the DCMD, allow researchers to test various

experimental variables, such as complicated visual environments (discussed in Chapter 2) against

a reliable response. Comparing general encoding strategies of the DCMD with other visual

systems is necessary to determine the reliability of DCMD responses to different experimental

variables that could be applied to more complex animals, such as vertebrates. One major future

experiment should utilize a closed feedback system where wing kinematics, DCMD recordings,

and various visual stimuli may be tested and quantified. This would help to identify many

important features of visual environments that affect behavioural responses. A test using

repeated stimuli of different complexities and types is also required to identify whether bursting

exists for different types of motion, not only looming.

It is difficult to compile a concise list of future experiments when there are so many studies that need to done to not only quantify DCMD responses to various visual stimuli, but also to correlate neuronal and behavioural responses. Future studies examining the role of the DCMD in encoding visual stimuli correlated with behavioural responses will not only aid in understanding what information is important in affecting post-synaptic neurons but also provide more clues into the general principles of sensory coding, information transfer, and animal physiology.

REFERENCES

Bacon J, Thompson KSJ, and Stern M. Identified octopaminergic neurons provide an arousal mechanism in the locust brain. *J. Neurophys.* 74:2739-2743, 1995.

Buj Buj A. International experimentation and control of the locust plague. Africa in the first half of the 20th century. *Nature et environ.* 3:93-105, 1995.

Burrows M. The neurobiology of an insect brain. Oxford university press inc., New York, USA, 1996.

Burrows M and Rowell CHF. Connections between descending visual interneurons and metathoracic motoneurons in the locust. *J. Comp. Physiol* [A]. 85:221-234, 1973.

Brody DL, Patil PG, Mulle JG, Snutch TP, and Yue DT. Bursts of action potential waveforms relieve G-protein inhibition of recombinant P/Q-type Ca^{2+} channels in HEK 293 cells. *J. Physiol*. 499:637-644, 1997.

Chapman RF. A biology of locusts. Southhampton, Camelot press Ltd. pp 67, 1976.

Edwards DH. The cockroach DCMD neurone I. Lateral inhibition and the effect of light-and dark-adaptation. *J. Exp. Biol.* 99:61-90, 1982.

Eggermount JJ and Smith GM. Burst-firing sharpens frequency-tuning in primary auditory cortex. *NeuroReport.* 7:753–757, 1996.

Eyherabide HG, Rokem A, Herz AVM, Samengo I. Burst firing is a neural code in an insect auditory system. *Front. Comput. Neurosci.* 2:3, 2008.

Faivre O and Juusola M. Visual coding in locust photoreceptors. *PLoS ONE* 3(5): *e2173. doi:10.1371/journal.pone.0002173*, 2008.

Felsen G and Dan Y. A natural approach to studying vision. *Nat. Neurosci*. 8(12):1643-1646, 2005.

Fry CH, Fry K and Harris A. Kingfishers, bee-eaters and rollers. Princeton: Princeton University Press, 1992.

Gabbiani F, Cohen I, and Laurent G. Time-dependent activation of feed-forward inhibition in a looming-sensitive neuron. *J. Neurophysiol.* 94:2150-2161, 2005.

Gabbiani F and Krapp HG. Spike-frequency adaptation and intrinsic properties of an identified, looming-sensitive neuron. *J. Neurophysiol*. 96:2951-2962, 2006.

Gabbiani F, Krapp HG, Koch C, and Laurent G. Multiplicative computation in a visual neuron sensitive to looming. *Nature*. 420:320-324, 2002.

Gabbiani F, Krapp HG, and Laurent G. Computation of object approach by a wide-field motion-sensitive neuron. *J. Neurosci.* 19:1122-1141, 1999.

Gabbiani F, Mo CH, and Laurent G. Invariance of angular threshold computation in a wide-field looming-sensitive neuron. *J. Neurosci.* 21:314-329, 2001.

Gibson JJ. The ecological approach to visual perception. Boston, MA: Houghton Mifflin, 1979.

Gray JR. Habituated visual neurons in locusts remain sensitive to novel looming objects. *J. Exp. Biol*. 208:2515-2532, 2005.

Gray JR, Lee J-K, and Robertson RM. Activity of descending contralateral movement detector neurons and collision avoidance behaviour in response to head-on visual stimuli in locusts. *J. Comp. Physiol.* [A]. 187:115-129, 2001.

Guest BB and Gray JR. Responses of a looming-sensitive neuron to compound and paired object approaches. *J. Neurophysiol*. 95:1428-1441, 2006.

Guido W, Lu SM, Vaughan JW, Godwin DW, and Sherman SM. Receiver operating characteristic (ROC) analysis of neurons in the cat's lateral geniculate nucleus during tonic and burst response mode. *Vis. Neurosci.* 12:723-741, 1995.

Hardie RC. Phototransduction in *Drosophila melanogaster*. *Jour. of Exp. Biol.* 204:3403-3409, 2001.

Hatsopoulos N, Gabbiani F, and Laurent G. Elementary computation of object approach by a wide-field visual neuron. *Science*. 270:1000-1003, 1995.

Hill RW, Wyse GA, and Anderson M. Animal physiology, 1st edition. Sinauer Associates, Inc. Sunderland, Massachusetts, USA. pp 288-289, 2004.

Hooper SL and Moulins M. Switching of a neuron from one network to another by sensory-induced changes in membrane properties. *Science*. 244:1587-1589, 1989.

Horridge GA. The separation of visual axes in apposition compound eyes. *Philos. Trans.R. Soc. (Lond). [B]*. 285:1-59, 1978.

Israel Z and Burchiel K. Microelectorde recording in movement disorder surgery; Chapter 12 Techniques in data analysis: general principles. *Acta Neurochirurgica.* 147(5):115-117, 2005.

Judge SJ and Rind FC. The locust DCMD, a movement-detecting neurone tightly tuned to collision trajectories. *J. Exp. Biol*. 200:2209-2216, 1997.

Kaneoke Y and Vitek JL. Burst and oscillation as disparate neuronal properties. *J. Neurosci. Methods.* 68(2):211-223, 1996.

Killman F, Gras H, and Schürmann F-W. Types, numbers and distribution of synapses on the dendritic tree of an identified visual interneuron in the brain of the locust. *Cell Tissue Res.* 296:645-665, 1999.

Krahe R and Gabbiani F. Burst firing in sensory systems. *Nat. Rev. Neurosci.* 5:13-23, 2004.

Krapp HG and Gabbiani F. Spatial distribution of inputs and local receptive field properties of a wide-field, looming sensitive neuron. *J. Neurophysiol*. 93:2240-2253, 2005.

Land MF and Nilsson DE. Oxford animal biology series. Oxford University Press, Animal eyes: Fig 7.3, p 128, 2002.

Lesica NA and Stanley GB. Encoding of natural scene movies by tonic and burst spikes in the lateral geniculate nucleus. *J. Neurosci*. 24:10731-10740, 2004.

Li YX, Zhang Y, Lester HA, Schuman EM, and Davidson N. Enhancement of neurotransmitter release induced by brain-derived neurotrophic factor in cultured hippocampal neurons. *J. Neurosci*. 18(24):10231-10240, 1998.

Maier, JX, Neuhoff, JG, Logothetis, NK and Ghazanfar, AA. Multisensory integration of looming signals by rhesus monkeys. *Neuron.* 43:177-181, 2004.

Marsat G. Proville RD, and Maler L. Transient signals trigger synchronous bursts in an identified population of neurons. *J. Neurophysiol.* 102(2):714-723, 2009.

Marsat G and Pollack GS. A behavioral role for feature detection by sensory bursts. *J. Neurosci.* 26(41):10542-10547, 2006.

Matheson T, Rogers SM, and Krapp HG. Plasticity in the visual system is correlated with a change in lifestyle of solitarious and gregarious locusts. *J. Neurophysiol.* 91:1-12, 2004.

Metzner W, Koch C, Wessel R, and Gabbiani F. Feature extraction by burst-like spike patterns in multiple sensory maps. *J. Neurosci.* 18:2283-2300, 1998.

Money TGA, DeCarlo CA, and Robertson RM. Temperature-sensitive gating in a descending visual interneuron, DCMD. *J. Comp. Phys. [A].* 192:915-925, 2006.

Mohr NA and Gray JR. Collision avoidance responses in loosely tethered flying locusts. Program No. 403.20 Abstract viewer/Itinerary planner. Washington, DC: Society for Neuroscience, 2003.

Nolen TG and Hoy RR. Initiation of behavior by single neurons: the role of behavioral context. *Science*. 226:992-994, 1984.

Oliva D, Medan V, and Tomsic D. Escape behavior and neuronal responses to looming stimuli in the crab *Chasmagnathus granulatus* (Decapoda: Grapsidae). *J. Exp. Biol.* 210(5):865-880, 2007.

O'Day P and Bacigalupo J. Current issues in invertebrate phototransduction. *Mol. Neurobiol.* 15:41-63, 1997.

O'Shea M and Rowell CHF. A spike transmitting electrical synapse between visual interneurones in the locust movement detector system. *J. Comp. Physiol.* 97:143-158, 1975a.

O'Shea M and Williams JLD. The anatomy and output connections of a locust visual nterneuron: The lobula giant movement detector (LGMD) Neurone. *J. Comp. Physiol.* 91:257-266, 1974.

Oswald AM, Chacron MJ, Doiron B, Bastian J, and Maler L. Parallel processing of sensory input by bursts and isolated spikes. *J. Neurosci.* 24:4351-4362, 2004.

Oswald AM, Doiron B, and Maler L. Interval coding I. Burst interspike intervals as indicators of stimulus intensity. *J. Neurophysiol.* 97:2731-2743, 2007.

Passaglia C, Dodge F, Herzog E, Jackson S, and Barlow R. Deciphering a neural code for vision. *Proc. Natl. Acad. Sci*. 94(23):12649-12654, 1997.

Pearson KG, Boyan GS, Bastiani M, Goodman CS. Heterogenous properties of segmentally homologous interneurons in the ventral nerve cord of locusts. *J. Comp. Neurol.* 233:133-145, 1985.

Peron SP, Krapp HG, and Gabbiani F. Influence of electrotonic structure and synaptic mapping on the receptive field properties of a collision-detecting neuron. *J. Neurophysiol.* 97(1):159-177, 2007.

Pirenne MH. Some aspects of the sensitivity of the eye. *Ann. NY Acad. Sci.* 74:377-384, 1959.

Reichert H and Rowell CHF. Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. *J. Neurophysiol.* 53:1201-1218, 1985.

Reichert H, Rowell CHF, and Griss C. Course correction circuitry translates feature detection into behavioural actions in locusts. *Nature*. 315:142-144, 1985.

Reichert H and Rowell CHF. Neuronal circuits controlling flight in the locust: how sensory information is processed for motor control. *Trends Neurosci.* 9:281-283, 1986.

Rind FC. A chemical synapse between two motion detecting neurons in the locust brain. *J. Exp. Biol.* 110:143-167, 1984.

Rind FC. Intracellular characterization of neurons in the locust brain signaling impending collision. *J. Neurophysiol.*75:986-995, 1996.

Rind FC and Bramwell DI. Neural network based on the input organization of an identified neuron signaling impending collision. *J. Neurophysiol.* 75:967-985, 1996

Rind FC and Simmons PJ. Orthopteran DCMD neuron: a reevaluation of responses to moving objects. I. Selective responses to approaching objects. *J. Neurophysiol*. 68:1654-1666, 1992.

Rind FC and Simmons PJ. Signaling of object approach by the DCMD neuron of the locust. *J. Neurophysiol*. 77:1029-1033, 1997.

Robertson RM. Modulation of neural circuit operation by prior environmental stress. *Integr. Comp. Biol.* 44(1):21-27, 2004.

Robertson RM and Johnson AG. Retinal image size triggers obstacle avoidance in flying locusts. *Naturwiss*. 80:176-178, 1993.

Robertson RM and Reye DN. Wing movements associated with collision-avoidance manoeuvres during flight in the locust *Locusta migratoria*. *J. Exp. Biol.* 163:231-258, 1992.

Rowat P. Interspike interval statistics in the stochastic hodgkin-huxley model: Coexistence of gamma frequency bursts and highly irregular firing source. *Neural Comp.* 19(5):1215-1250, 2007.

Rowell CHF. Variable responsiveness of a visual interneurone in the free-moving locust, and its relation to behaviour and arousal. *J. Exp. Biol.* 55:727-747, 1971.

Rowell CHF, O'Shea M, and Williams JLD. The neuronal basis of a sensory analyser, the acridid movement detector system. IV. The preference for small field stimuli. *J. Exp. Biol.* 68:157-185, 1977.

Sabourin P and Pollack GS. Behaviorally relevant burst coding in primary sensory neurons. *J. Neurophysiol.* 102:1086-1091, 2009.

Salinas E and Sejnowski TJ. Correlated neuronal activity and the flow of neural information. *Nature Rev. Neurosci*. 2:539-550, 2001.

Santer RD, Simmons PJ, and Rind FC. Gliding behaviour elicited by lateral looming stimuli in flying locusts. *J. Comp. Physiol. [A]*. 191:61-73, 2005.

Santer RD, Rind FC, Stafford R, Simmons PJ. The role of an identified looming-sensitive neuron in triggering a flying locust's escape. *J. Neurophysiol.* 95:3391-3400, 2006.

Santillo S, Orlando P, De Petrocellis L, Cristino L, Guglielmotti V, and Musio C. Evolving visual pigments: Hints from the opsin-based proteins in a phylogenetically old "eyeless" invertebrate. *Biosystems*. 86:3-17, 2006.

Schlotterer GR. Response of the locust descending movement detector neuron to rapidly approaching and withdrawing visual stimuli. *Can. J. Zool*. 55:1372-1376, 1977.

Sherman SM. Tonic and burst firing: dual modes of thalamocortical relay. *Trends Neurosci*. 24:122-126, 2001.

Simmons PJ. Connexions between a movement-detecting visual interneurone and flight motoneurones of a locust. J Exp Biol 86: 87–97, 1980b.

Simmons PJ and Rind FC. Orthopteran DCMD neuron: a reevaluation of responses to moving objects. II. Critical cues for detecting approaching objects. *J. Neurophysiol.* 68:1667-1682, 1992.

Stein RB, Gossen ER, and Jones KE. Neuronal variability: Noise or part of the signal? *Nature Rev. Neurosci.* 6(5):389-397, 2005.

Stern M. Octopamine in the locust brain: Cellular distribution and functional significance in an arousal mechanism. *Microsc. Res. Tech*. 45:135-141, 1999.

Sun H and Frost BJ. Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. *Nat. Neurosci*. 1:296-303, 1998.

Tonkin SP and Pinter RB. Motion processing using asymmetric shunting lateral inhibitory networks. *Comp. Neur. Sys*. 7(2):385-407, 1996.

Tuckwell HC. Computation of spiking activity for a stochastic spatial neuron model: Effects of spatial distribution of input on bimodality and CV of the ISI distribution. *Mathematical biosciences.* 207(2): 246-260, 2007.

Uvarov B. Grasshoppers and locusts. A handbook of general acridology. Volume II. Behaviour, ecology, biogeography, population dynamics. London, UK: Centre for Overseas Pest Research, 1997.

VanRullen R, Guyonneau R, and Thorpe SJ. Spike times make sense. *Trends Neurosci*. 28(1):1-4, 2005.

Waloff Z. Orientation of flying locusts, *Schistocerca gregaria* (Forsk.), in migrating swarms. *Bull. Ent. Res*. 62:1-72, 1972.

Whitington PM. The early development of motor axon pathways in the locust embryo: the establishment of the segmental nerves in the thoracic ganglia. *Development*. 105:715-721, 1989.

Whitlock GG, and Lamb TD. Variability in the time course of single photon responses from toad rods: termination of rhodopsin's activity. *Neuron.* 23:337-351, 1999.

Wicklein M and Strausfeld NJ. Organization and significance of neurons that detect change of visual depth in the hawk moth *Manduca sexta*. *J. Comp. Neurol.* 424:356-376, 2000.

Wine JJ and Krasne FB. The organization of escape behaviour in the crayfish. *J. Exp. Biol.* 56:1-18, 1972.

Wylie DRW and Frost BJ. Responses of neurons in the nucleus of the basal optic root to translational and rotational flowfields. *J. Neurophysiol.* 81:267-276, 1999

Xu XZS, Chien F, Butler A, Salkoff L, and Montell C. TRP gamma, a *Drosophila* TRP- related subunit, forms a regulated cation channel with TRPL. *Neuron*. 26:647-657, 2000.

Yau KW. Phototransduction mechanism in retinal rods and cones. The friedenwald lecture. *Invest. Ophthalmol. Vis. Sci.* 35:9-32, 1994.

Yue S and Rind FC. A collision detection system for a mobile robot inspired by the locust visual system. ICRA 2005. Proceedings of the 2005 IEEE International Conference 1(18-22): 3832-3837, 2005.

Yue S, Rind F, Matthais SK, Cuadri J, and Stafford R. A bio-inspired visual collision detection mechanism for cars: Optimisation of a model of a locust neuron to a novel environment. *Neurocomputing.* 69(13-15):1591-1598, 2006.

Zupanc G. Behavioral Neurobiology: An integrative approach. Oxford University Press. 2004.

Zwislocki JJ. Auditory system: Peripheral nonlinearity and central additivity, as revealed in the human stapedius-muscle reflex. *Proc. Natl. Acad. Sci*. 99(22): 14601-14606, 2002.