Facilitation in Dragonfly Target Motion Detecting Neurons

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Thesis Abstract

Dragonflies are masters of visually guided aerial pursuit. Their visual systems overcome the challenges presented by the environment in which they live and behave. These adaptations come from multiple stages of processing. Perhaps the most important adaptations are those that allow for the detection, identification, pursuit and capture of small moving features in a complex visual environment. Perching Libellulid dragonflies execute prey capture flights with a 97% success rate from a stationary start point (average pursuit time, 184ms, Olberg et al., 2000). The Australian hawking dragonfly, *Hemicordulia tau*, performs similar prey capture flights on the wing. *H. tau* also engages in longer duration territorial and courtship pursuits of conspecifics and other anisopterans, which can last tens of seconds. These chase sequences include changes in velocity and direction, as well as the possibility of target occlusion by background features. The dragonfly brain has been found to contain neurons specialised to respond to small moving target-like objects (O'Carroll, 1993; Olberg, 1986). These small target motion-detecting (STMD) neurons likely play an important role in some or all of these pursuit flights. One of these neurons, the centrifugal STMD 1 (CSTMD1) (Geurten et al., 2007), has recently been shown to respond more robustly following periods of slow continuous target motion (Nordström et al., 2011). This enhancement of response following target motion or self-facilitation is fascinating and was the focus of enquiry throughout this thesis.

I probed this facilitation by quantifying neuronal response to stimulation from intracellular, *in vivo* recordings from dragonfly small target motion detecting neurons, mostly CSTMD1 and the newly identified and characterised neuron BSTMD1 (work from this thesis, published in Dunbier et al., 2012). The key results of the thesis confirmed

that the slow onset time course first observed in CSTMD1 (Nordstrom et al., 2011) was in fact due to a facilitation rather than slow kinetics (using a new computational model). This facilitation effect is most pronounced when targets move at velocities slower than what was previously thought to be optimal in CSTMD1 (Geurten et al., 2007). Further, the facilitation is evident in other wide-field STMD neurons like BSTMD1. The facilitation is disrupted if targets are instantaneously displaced a few degrees from their current location. I investigated this mechanism more fully in the next publication. Presenting single target stimuli that moved along an initial 'priming' path before undergoing spatial, temporal or combined discontinuities in their trajectories, I found that facilitation is initially spatially localized. When larger spatial displacements were combined with a delay in reappearance, however, responses were significantly elevated, even for a 20° displacement with a 500ms delay in reappearance. Backward displacements (i.e. across previously traversed location) yield strongly inhibited responses. This suggests that facilitation is mediated by a process of local gain modulation that actively spreads from the last seen location of a stimulus and in the approximate direction of travel. Such predictive modulation of local target salience may be a key mechanism for selective attention during target tracking.

Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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James Robert Dunbier

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Common Acronyms

Binocular small target motion detector 1 (BSTMD1)

Central nervous system (CNS)

Centrifugal Horizontal (CH)

Centrifugal small target motion detector 1 (CSTMD1)

Chlordimeform (CDM)

Descending contralateral motion detector (DCMD)

Elementary small target motion detector (ESTMD)

Elementary motion detector (EMD)

Facet diameter (D)

Feature detecting (FD)

Horizontal system (HS)

Interommatidial angle ($\Delta\varphi$)

Large monopolar cell (LMC)

Lobula plate tangential cell (LPTC)

Lobula giant motion detector (LGMD)

Rhabdom diameter (d)

Rhabdom length (l)

Region of interest (ROI)

Small field small target motion detector (SF-STMD)

Small target motion detector (STMD)

Target selective descending neuron (TSDN)

Vertical System (VS)

Author's Comments

All publications within this thesis are in the exact form of the original article as published or as prepared for submission in cases where the article is not yet published, with the following exceptions:

Typesetting has been altered so that there is a consistent format throughout the entire thesis.

The figures have been inserted into the text at appropriate places, which may differ from the final published version of the papers.

Figures are referenced throughout the text as they are in the published or prepared versions of the papers, but are captioned based on their chapter and figure number, e.g. Figure 1 in Chapter 2 is captioned as Figure 2-1.

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1 Introduction

1.1 The Dragonfly in Pursuit

Figure 1-1. *Hemicordulia tau* or Tau Emerald. Photo from "Fir0002/Flagstaffotos".

Consider the following scenario: a male Tau Emerald (*Hemicordulia tau)* patrols a section of the riverbank. He approaches one end of his patrol path; he climbs, narrowly avoiding some bowing reeds. As he levels out above the reeds he encounters a whirling swarm of small midges – prey. Selecting one target from amidst the confusion, he darts into the swarm. Inverting his body, he deftly plucks it from the air with his spiny forelegs. Still crunching his latest meal he wheels wide, again avoiding the reeds, and resumes his previous patrol. However, this brief detour to feed has allowed a competitor - another male Emerald - to encroach on his territory. An aerobatic duel follows, this time over many seconds and longer than his predatory flight, which was over in a fraction of a second. During the ensuing duel he keeps the transgressor in sight as he harries him through a series of tight turns and eventually chases him through several other males' territories. This pursuit even excites the interest of a Blue Skimmer

(*Orthetrum caledonicum*), a similarly sized perching dragonfly that shares the same environment. With a burst of speed to lose his pursuers, the Emerald moves to the middle of the river and returns to his territory, which fortunately has not been usurped in his absence.

These short snippets of dragonfly behaviour highlight a number of impressive visual and motor abilities. We can break these down into elements that require the integration of six broad categories of visual stimuli and associated tasks, each with their own subtleties:

1) **Wide field optic flow.** The motion of the background resulting from the insect's self-motion through the world is fundamental to flight control and successful completion of pursuits and avoidance of pursuers (Egelhaaf and Borst, 1993).
2) **Obstacle avoidance**. When patrolling or pursuing it is necessary to avoid intervening objects that provide collision hazards or potential visual occlusions during pursuit (Maimon et al., 2008; Gabbiani et al., 2002).
3) **Target detection**. Even the largest prey or conspecifics occupy only a tiny fraction of the visual field for fast flying animals like dragonflies, whose flights range over tens or even hundreds of metres. This task is complicated by the cluttered, textured and moving backgrounds of natural scenes, which are rife with potential false positives (Nordström et al., 2006, Wiederman & O'Carroll, 2011).
4) **Target discrimination**. Once detected, the dragonfly needs to identify the target. Is it a viable prey item? A conspecific competitor or potential mate? A heterospecific competitor or predator? Or is it an irrelevant distant object like a plane or a leaf falling from a tree? (Olberg et al., 2001).

5) **Visual Attention**. Once a decision to pursue a target is initiated, the dragonfly needs to maintain attention on it whilst ignoring a host of salient distracters including conspecifics and other prey (Corbet, 1999; Wiederman and O'Carroll, 2013).
6) **Closed loop pursuit.** Once all the other conditions have been met the dragonfly must close the distance between itself and the target (Nordström and O'Carroll, 2009).

These topics have been investigated to greater or lesser extents in different insect models. How insects encode wide-field optic flow has been studied extensively since the 1960s, particularly in dipteran flies and much is known about this process (Borst and Haag, 2002). Comparatively, less is known regarding the other outlined points above. In the following pages, I will consider the state of knowledge for the neural mechanisms involved in insect target detection and tracking. In so doing, I will explore the strategies implemented in higher order processing areas of the dragonfly brain to successfully discriminate and pursue prey; mates; and both heterospecific and conspecific competitors. These underlie the central focus for my thesis, which investigates the functional properties of response facilitation, a recently identified property of the target selective neuron CSTMD1.

1.2 Target Detection, Selection and Pursuit

1.2.1 Detection

Behavioural strategies enhancing target discrimination

Insects adopt a number of behaviours that enhance their ability to detect targets in their environment. These behaviours synergise with anatomical and optical adaptations (see Section 1.3) to enhance detection of targets in functionally relevant regions of the visual world. The most basic of these simplifying behaviours – and one adopted by numerous species – is to perch. Initiating predation from a fixed perch provides the advantage that

the motion of a relevant target 'pops out' against the stationary background (Srinivasan, 1998). This 'sit and wait' detection strategy is adopted by several groups of predatory insects including perching dragonflies (Olberg, 2001), tiger beetles (Gilbert, 1997), praying mantises (Rossel, 1986) and robber flies (Shelly, 1985). It is also adopted during sexual behaviour by males of many insects, including dipteran species such as blowflies. These adopt a territorial perch close to food or potential egg-laying sites, sources of interest to conspecifics (Collet and Land, 1975a; Bänzinger and Pape, 2004).

An analogous but more visually challenging mode of detection is adopted by hovering males of several dipteran families, including the hoverflies (Syrphidae) and bee-flies (Bombyliidae). These species hover at a fixed spot within their territory, maintaining position by minimising optic flow. From this position they defend their territory from encroaching males or pursue females who enter the territory (Collet and Land, 1975b; Collett and Land, 1978; Maier and Waldbauer, 1979; Dodson and Yeates, 1990). This strategy provides an advantage over perching that the animal is already in flight (albeit stationary) and thus reduces reaction time for either mate pursuit or predator avoidance.

A more challenging mode of target detection is that adopted by hawking dragonflies such as many species of the Aeshnidae and Corduliidae, which detect and pursue conspecifics and prey whilst patrolling over large areas of land or water (Corbet, 1999; Sherk, 1978). This requires the identification of target motion against a global motion signal generated by the pursuer's ego-motion. A similar task is tackled during sexual encounters by the males of dipteran families such as the Simuliidae and Bibionidae (Kirschfeld & Wenk, 1976; Zeil, 1983a; Zeil, 1983b). In several of these insect groups, the inherent difficulty of target detection against the background is mitigated by two strategies. Firstly, these insects often detect targets in the fronto-dorsal eye region i.e.

against the sky, a relatively untextured, high luminance background (Snyder et al., 1977; Zeil, 1983; van Hateren et al., 1989). Secondly, many species keep the target centred in the frontal eye region, where the expansive ego-motion during their forward translation through the world is minimal (Land, 1981; Land, 1997).

Contrast sensitivity for target detection

Regardless of the behavioural strategies adopted to aid the task of target detection, the ability to discriminate small objects against a moving background is ultimately limited by the resolution and sensitivity of the eye. Indeed, several studies suggest that at the maximum distance for detecting conspecifics or prey, the image of the feature on the retina is as small as (or smaller than) individual photoreceptors in the eye, such that the task is limited by the contrast sensitivity of the eye (Vallett & Coles, 1993; Burton & Laughlin, 2003; O'Carroll & Wiederman, 2013). Contrast is defined by the difference in luminance between two aspects of an image e.g. an object and the background. Contrast sensitivity is the ability to discern between different luminance levels in an image (Land, 1981).

Extensive work has been done using grating patterns to examine neuronal trade-offs between sensitivity and acuity in insect eyes (Land, 1997; Dvorak et al., 1980). Less is known about the influence of contrast sensitivity on detection of small features. Neurons in the wide-field motion pathways are believed to integrate the outputs of many local 'elementary motion detectors' (Dvorak et al., 1980), i.e. to spatially sum local visual motion of many contrasting elements across a large field. The neural pathways that process target motion do not have the luxury of such integration, given that targets are as small as the resolution of the underlying photoreceptor mosaic. The image of these tiny features will only ever be at a single location as it moves across the retina and thus must be processed by comparison of signals generated in neighbouring ommatidia.

A particular problem for insect vision is the influence of optical blur when detecting dark targets against a bright background like another insect silhouetted against the sky. Because of the small diameter of facet lenses in insect compound eyes, optical diffraction produces severe blur which degrades the contrast in the image itself and produces crosstalk between neighbouring photoreceptors (Land, 1981). Due to this blur, contrast at the retina will be lower than the contrast of the actual object against its background (Dvorak et al., 1980; O'Carroll et al., 1996; Nordström and O'Carroll, 2009). When target size is reduced below the nominal limits of resolution supported by the photoreceptor mosaic, the target can no longer occupy the receptive field of a single receptor. Instead, the target is blurred to a low contrast image that resembles the Gaussian acceptance function of the photoreceptors themselves (O'Carroll & Wiederman, 2013). As a result, detection of tiny objects is limited not just by the optical resolution *per se*, but also by the contrast sensitivity of the individual photoreceptors.

1.2.2 Selective Attention

Once a target is detected, a second major challenge for the visual system is to maintain *attention* on it during tracking and pursuit, in the presence of competing or distracting stimuli. Attention refers to the processes by which an organism selects a subset of available information for particular focus, usually enhanced processing (see reviews, Treisman, 1969; Näätänen and Michie, 1979; Driver, 2001). When considering the visual modality it is important for complex biological systems to rapidly detect potential prey, predators, or mates in a cluttered visual world. However, simultaneously identifying any and all interesting targets in the visual field has prohibitive computational complexity even for the most sophisticated biological brains (Tsotsos, 1990). One approach is to restrict complex object recognition process to a small area or a few objects at any one time (Treisman & Gelade, 1980; Crick, 1984; Weichselgartner & Sperling, 1987).

Visual attention is a potential solution to the inability to process all locations or objects in parallel. However, if you are only going to process information from a selected region or object, what determines that 'spotlight' for attention? There are two major theories for the mechanisms by which object selection occurs. The first, 'visual salience' suggests, that brains utilise the early stages of visual processing to make some stimuli stand out from the crowd (Itti and Koch, 2000). Visual salience is a bottom-up, stimulus-driven signal, which determines if a location is sufficiently different from its surroundings to be of interest (Desimone & Duncan, 1995; Itti & Koch, 2001). For example, for an animal with colour vision, a lone red object in an otherwise green field will be salient and attract attention in a bottom-up manner. The other component of attention involves an endogenous, top-down process, by which the organism directs attention to a small subregion of the visual field or subset of the stimuli within it, deliberately suppressing the relative salience of other areas, even if the features there are inherently more salient from a signal detection standpoint (Treisman and Gelade, 1980).

The traditional view of attention-like processes in insects is dominated by the presumption that they lack cognitive ability or consciousness, i.e. that the insect brain is a simple input-output circuit (Giurfa and Menzel, 1997). An external event, a salient stimulus, evokes a fixed motor pattern that could be interpreted as a form of selective discrimination. For example, a male *Drosophila* fruit fly will court and mate with a female fly once pheromonal, visual and mechanosensory cues coalesce in the right pattern, above a certain threshold (Greenspan and Ferveur, 2000).

Insects do not always respond to the same cues in the same way, however, and this is at least partially due to elements of learning and memory. Insects probe their environment in an experience-dependent manner, and react selectively to stimuli according to their behavioural relevance or 'salience', which in turn is modified by memory of their

previous experience (Waddel and Quinn, 2001). However, the fact that experience modifies selection in flies (Tully and Quinn, 1985) cannot necessarily preclude either a role for bottom-up or top-down attention mechanisms. Evidence for simultaneous suppression of competing stimuli has been found in the mushroom bodies of the *Drosophila* brain during an object fixation task (van Swinderen and Greenspan, 2003) and in auditory neurons of the cricket, *Teleogryllus oceanicus*, distinguishing between the direction of sounds (Pollack, 1986; Pollack , 1988). Given the surprising degree of sophistication in task and feature selection shown by honeybees and also demonstrated by other insects such as dragonflies in scenarios like the one which I outlined at the beginning of this thesis, it is tempting to consider that they must employ both bottom-up and top-down mechanisms in target selection.

1.2.3 Pursuit Strategies

Two distinct pursuit strategies have been observed in insects, referred to as *tracking* and *interception* (Collett and Land, 1978). During *tracking*, the pursuer steers to minimize the deviation of the pursued target from the pursuer's visual midline (Figure 1-2). Tracking results in spiralling flights that will result in a successful pursuit if the pursuer is faster than the target. The majority of insects studied to date, both flying and terrestrial, utilise this strategy when pursuing prey, conspecifics and other features (Land and Collett, 1974; Wagner, 1986; Land, 1993a; Land, 1993b; Gilbert, 1997; Zhang et al., 1990).

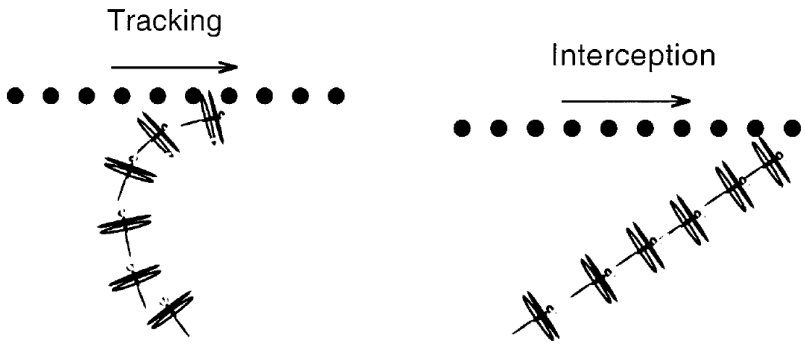

Figure 1-2. Two alternative strategies of Insect Pursuit reproduced from Olberg, Worthington and Venator, 2000.

Less common are examples of insects that utilise the strategy which Collet and Land (1978) described as *interception* (Figure 1-2, right). In this strategy the pursuer maintains the pursued object at a fixed non-centred location within the visual field in order to fly in a relatively straight line that intersects the projected flight path of the target. This behaviour has been observed in male hoverflies (*Eristalis* and *Volucella*) pursuing mates and in dragonflies (Collett and Land, 1978, Olberg et al., 2000). Arguably, interception could be further divided into two sub-strategies. In one group, exemplified by dragonflies, the interception trajectory is updated dynamically through adjustments of their path as they progress towards the target (Olberg et al., 2000). This is in contrast to large male hoverflies such as *Eristalis and Volucella* which compute a single initial intercept trajectory when pursuing potential mates, then transition to a tracking mode when closer together (Collett and Land, 1978).

1.3 Organization of insect eyes for target detection

The visual system is organised into optic lobes (Figure 1-3) comprising four successive neuropils: the lamina; medulla; lobula-plate and lobula; before projecting into central brain regions. The process of target detection begins, of course, with the optical design of the eye itself and the anatomy and physiology of the underlying photoreceptors.

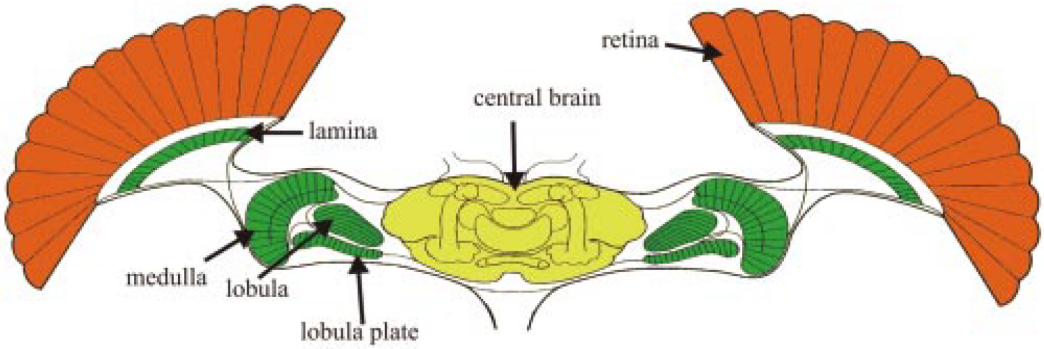

Figure 1-3. Schematic horizontal cross-section through the fly head reproduced from Figure 1 of Borst and Haag, 2002. Dragonflies share a very similar brain structure however they do not possess an anatomically distinct lobula plate.

1.3.1 Optical organisation of apposition eyes

Despite their alien appearance, the compound eyes of insects have been honed over time to fill a number of disparate niches. The most common type of compound eye found in insects, including dragonflies, is the apposition eye (Figure 1-4) (Land, 1981).

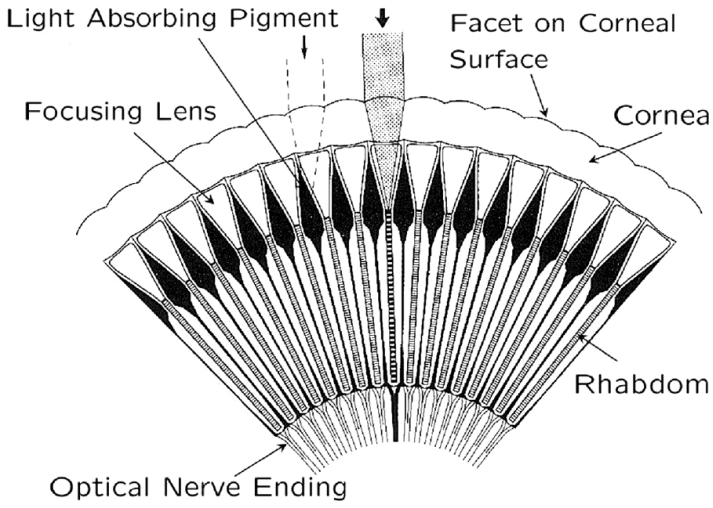

Figure 1-4. Schematic of the Apposition compound eye modified from Figure 2 of Nilsson, 1989.

The apposition eye consists of an array of light sensitive optical units or *ommatidia.* On the outer surface, light is first gathered by corneal lenses (facets) and then directed onto a crystalline cone. Together, these form the dioptric apparatus of the compound eye, focusing light onto the distal tip of a light absorbing rhabdom. Within each ommatidium the rhabdom contains 8 or more photoreceptors comprised of the light-absorbing visual pigments concentrated within the microvilli (rhabdomeres). These rhabdomeres can be

either fused together in a single rod shaped rhabdom (the case in *H. tau*) to form a single effective pixel per ommatidium or they may be separated throughout their length to form an 'open rhabdom' comprising 7 unique pixels, as seen in dipteran flies (Land, 1981). Adjacent ommatidia of such eyes view overlapping angles of visual space, so the same point in space is viewed by a different retinula cell in 6 adjacent ommatidia. This allows the axons projecting from those 6 cells to project to a single cartridge in the underlying lamina, thus providing a 'neural superposition' mechanism that increases the effective photon catch without any cost in terms of acuity (Hardie, 1986). In both fused and open rhabdom eyes, heavily pigmented cells prevent the spread of light between the optical units (review Nilsson, 1989; Strausfeld, 1989).

1.3.2 Specialised Eye Regions for Target Detection

H. tau and many other insect species have what has been described as an 'acute zone', a specialized sub-region of the eye with larger facet lenses and reduced interommatidial angle (Horridge, 1978; Land and Eckert, 1985). These adaptations enhance acuity since larger facet diameter (D) leads to improved lens optical quality (through reduced diffraction), while smaller diameter photoreceptors and reduced angular separation of the optical axes of neighbouring ommatidia, (interommatidial angle, $\Delta\varphi$) allow the retina to exploit the sharper image thus formed and detect smaller features (Kirschfeld, 1976; Land, 1981). The price paid for this increased angular resolution would be a huge potential increase in eye size, if it were maintained across the entire visual field (Kirschfeld, 1976). Hence such specialisations are usually limited to specific 'acute zones' that coincide with localized areas of the visual field (typically frontal or dorsal) where insects attempt to keep their targets during pursuit (Figure 1-5). Despite the high resolution of typical acute zones being limited to a sub-region of the eye, the overall size of many such insect eyes (including dragonflies) are still among the largest of any extant arthropods (Land, 1989; Kirschfeld, 1976). A further potential additional metabolic cost

results from adaptations required in the associated photoreceptors: the smaller the receptive field, the less time an object moving at constant speed stays within it, requiring faster response dynamics.

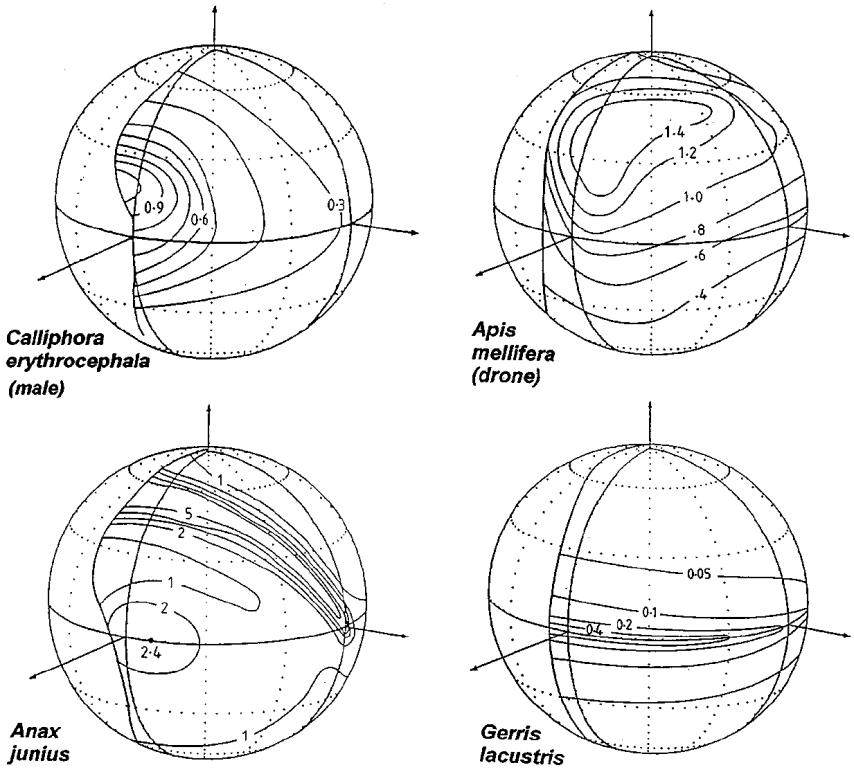

Figure 1-5. Distribution of ommatidial axis densities across the fields of view of four insects reproduced from Figure 7 of Land, 1997. The meaning of axis density: the number of ommatidial axes per unit solid angle plotted onto a sphere around the insect. Numbers are ommatidial axes per square degree.

Similar specialized eye regions have been described in a number of different predatory insect species, and in the males of other species (including potential prey of the dragonfly) as a sexual dimorphism associated with mate detection and territorial behaviour: male *Calliphora vicina* and *Musca domestica* have an 'acute zone' in the fronto-dorsal portion of the visual world where the angle between adjacent ommatidial axes is nearly half that in the lateral eye (Horridge, 1978; Land and Eckert, 1985). Males try to keep the image of females they are pursuing in this region (Boeddeker et al., 2003; Wagner, 1986; Wehrhahn, 1979). It is therefore thought that the male acute zone in these flies is a specialization for detection and tracking of females. As touched on in

Section 1.2.1 this dimorphism can be extreme, as seen in Bibionid flies where the entire dorsal eye of males serves as an acute zone, with a diameter several times that of the female (or the ventral eye) (Zeil, 1983).

Acute zones are much rarer in non-predatory females. However the pipunculid flies in the genus *Chalarus* have greatly enlarged fronto-dorsal ommatidia (Jervis, 1992). These female flies parasitize leafhoppers and need to locate them on the underside of leaves. Males of this species have no equivalent need for keen eyesight and therefore lack an acute zone. However, in predatory species, where both sexes share the need for enhanced acuity, there is no sexual dimorphism: both sexes exhibit a specialized acute zone. This has been observed in most studied dragonfly species (Horridge, 1978; Land, 1997; Land, 1989) and in predatory dipterans such as the robber flies (Asilidae) (Tricca and Trujillo-Cenóz, 1980) and the 'killer fly' *Coenosia*, (Gonzales-Bellido et al., 2011).

An alternative adaptation to improve contrast sensitivity is the 'bright zone' seen in the male blowfly *Chrysomia megacephala* (van Hateren et al., 1989) and male hoverfly *Eristalis tenax* (Straw et al., 2006). Like the acute zones described above, the 'bright zones' exhibit increased facet size (D), which results in increased light capture but this is not accompanied by smaller rhabdoms or large changes in interommatidial angle. As a result, the photoreceptors are more sensitive to light, allowing them to respond to lower contrast features (or operate at dimmer light levels). *Eristalis* displays regional and sexually dimorphic differences in spatial tuning, temporal tuning and contrast sensitivity and this is predicted by the optical variation in facet diameter (Straw et al., 2006).

1.4 Physiological properties of early visual pathways for target detection

Just as the outward features of insects - their behaviour and external anatomy – display significant adaptations to aid target detection and analysis, so too do the underlying physiological properties of the photoreceptors and the higher order neuronal pathways with which they synapse.

1.4.1 Photoreceptor Physiology

Light intensities in the natural environment of an organism vary considerably. These are not simply the long-term changes between day and night (perhaps as much as a 10^8 range of luminance) but also the difference between direct sunlight and the deep shade of a tree. This may easily expose photoreceptors to changes in light level of at least 2-3 log units in a very short time (van Hateren, 1997). Different insects show anatomical adaptations in the size, length and arrangement of the retinula cells and their rhabdomeres (Land, 1981). Functionally, these adaptations affect the range of luminance over which the photoreceptors can effectively code contrast, often coupled with trade-offs in the speed with which the photoreceptors can generate a signal. For example, the more coarse photoreceptor mosaic of some dipteran flies adapted to dim light is associated with 5 times slower response kinetics (Laughlin, 1989; Laughlin and Weckstrom, 1993). While sharper acuity requires smaller photoreceptors, their smaller photon catch works against the requirement for sharper temporal resolution unless compensated for via increase in the total length of the photoreceptors (Land, 1981) thus leading to a larger head volume and mass (Laughlin and Weckstrom, 1993; Gonzales-Bellido et al., 2011)

Within acute zones specialised for target detection in diurnal insects, the concomitant need for higher temporal resolution is met by a number of physiological specialisations. For example, photoreceptors associated with the housefly ‘love spot’ (acute zone) have

superior response dynamics due to speeding up of the biochemical processes involved in phototransduction and by a tuned voltage-activated conductance that boosts the membrane frequency response (Hornstein et al., 2000). This enhanced response is due to the combination of improved image quality from larger diameter lenses and the gain of these specialised male photoreceptors, 3-4 times that seen in the females. These specialised neurons display a pronounced response transient, symptomatic of high-pass filtering in phototransduction (Hornstein et al., 2000). This high-pass filtering acts to deblur the neural image of a target by reducing the spread of response in the direction of motion. Interestingly, high-pass filtering is also thought to contribute to deblurring in the human visual system (Pääkkönen and Morgan, 2001). Burton and Laughlin (2003) further found that the photoreceptors underlying the male *Musca* 'love spot' have a number of nonlinear adaptations that match their response to the high target speeds normally experienced during the pursuit of conspecifics.

These non-linear specialisations to improve photoreceptor performance in the acute zone combine with dynamic non-linear gain controls that have previously been shown to improve contrast coding in response to natural image statistics in blowfly photoreceptors (van Hateren & Snippe, 2001). Such gain controls explain the ability of photoreceptors to adapt rapidly as insects move between direct sunlight and the deep shade of a tree. An interesting consequence of such temporal processing is that it improves the spatial discriminability of targets from background texture by around 70% by photoreceptors alone, even prior to any higher order spatial interactions (Brinkworth et al., 2008). Clearly, the process of enhancing target salience begins at the earliest stages of vision.

1.4.2 Lamina

The second optic ganglion, the lamina, is an important secondary site of redundancy reduction in the insect visual pathway. The neurons of the lamina are arranged

retinotopically forming what are referred to as neuro-ommatidia, each of which corresponds to a sampling point in visual space. This retinotopic organisation is maintained through the subsequent optic ganglia (Laughlin, 1984; Shaw, 1984).

The lamina removes redundant information from correlations in visual scenes through additional filtering in both space and time (Srinivasan et al., 1982). Lamina monopolar cells (LMCs) are directly postsynaptic to the photoreceptors and appear to remove redundancy by high-pass filtering the signal in both space and time (Laughlin et al., 1987). Temporal filtering is believed to result from the transfer properties of the photoreceptor/LMC synapse (Laughlin et al., 1987). These result largely from an unusual neurotransmitter, histamine, which gates chloride conductances in the LMC, leading to a transient sign inversion of the depolarising response of photoreceptors to light (Hardie, 1988) combined with additional feedback mechanisms (Laughlin & Osorio, 1989) and voltage-gated conductances that enhance transient potentials in the photoreceptor axon terminals (Weckström et al., 1992). Spatial filtering in insects is believed to result from spatial lateral inhibition, just as observed in their mammalian counterparts (Srinivasan et al., 1982; Dowling, 1987), although the synapses involved have not been clearly identified. A major ongoing project to identify the 'connectome' in peripheral visual processing of the *Drosophila* lamina and medulla may eventually identify likely candidates, but at this stage has not extensively mapped horizontal synaptic connections between lamina cartridges, including those of amacrine cells that might potentially mediate lateral inhibition (Takemura et al., 2013).

Spatial lateral inhibition in the ventral eye of *Hemicordulia* LMCs (Laughlin, 1974) and their fly counterparts (Srinivasan et al., 1982) operate on the scale of single ommatidia. Surround stimuli exert no inhibitory effect on LMCs beyond three ommatidia from the receptive field center (Srinivasan et al., 1982). One consequence of the spatial

interactions is that the excitatory receptive fields of these second order neurons are both smaller and more sharply defined than those of corresponding photoreceptors (Figure 1-6).

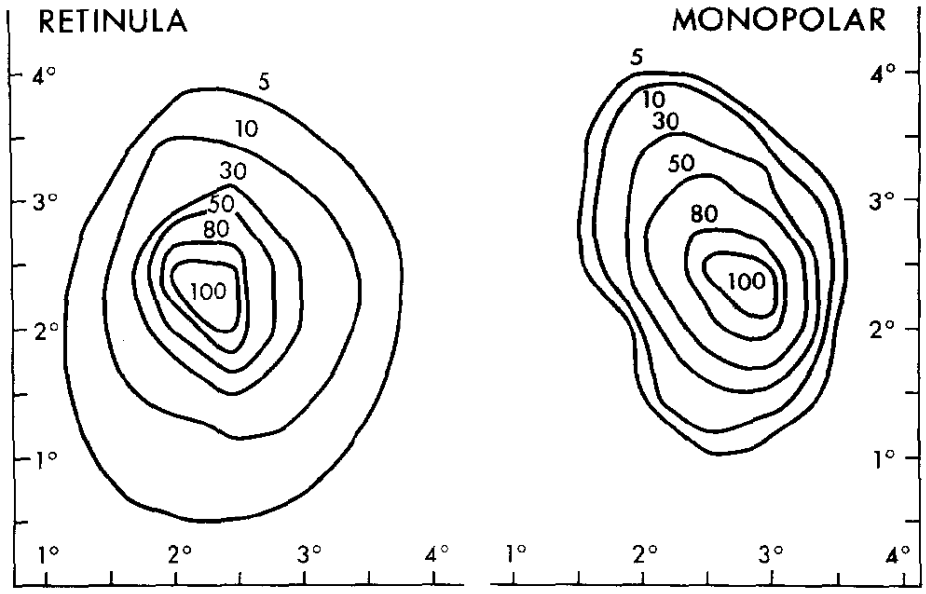

Figure 1-6. The field of view of a dorsal retinula cell compared with that of a LMC receiving its input from the same region of the dark-adapted eye of *Hemicordulia tau* reproduced from Figure 7 of Laughlin, 1973. Contours of iso-percent sensitivity are plotted against the two sets of axes which show the 0.5 ~ grid on which measurements were taken

Further investigation revealed that the degree of high-pass filtering in LMCs in both space and time is dynamically adjusted by the current visual conditions (Laughlin et al., 1987; Juusola et al., 1995; Srinivasan et al., 1990). Under dimmer light conditions (i.e. low signal:noise ratios) the observed receptive fields become more low-pass, consistent with theoretical predictions based on information theory (van Hateren, 1992). These slower adaptive properties are also augmented by rapid adaptation in both the LMCs and so-called rectifying transient cells (RTCs) that have been identified in the locust (Osorio, 1991; O'Carroll et al., 1992), and the blowfly (Jansonius and van Hateren, 1991; Wiederman et al., 2008). These downstream neurons separate transient ON and OFF phases (recently shown to be generated in the outputs of different LMC classes, Joesch et al., 2013) via partial rectification. Each sub-pathway rapidly (and independently)

depolarises to a stimulus but only slowly repolarises, and as such quickly adapts to repeated inputs. As such these RTCs selectively respond to 'novel' stimuli (targets) whilst suppressing response to textural fluctuations (background variance), providing an ideal input signal for downstream target-selective processing (Wiederman et al., 2013).

1.5 Higher Order Pathways

The enhancement of a visual signal simply by modifying the optics and physiological properties of early visual neurons is impressive. True specialization of neurons to address the six fundamental issues of pursuit does not occur until the second and third optic ganglia of the insect brain, the medulla and lobula. Recent work on the dipteran connectome (Raghu et al., 2013; Takemura et al., 2013) has provided anatomical support for proposed theories of neuronal circuits within the medulla (Osorio, 1986; Gilbert et al., 1991). Further specialisation occurs in the subsequent regions of the insect brain: the lobula (and lobula plate) and midbrain centres.

1.5.1 Lobula Plate Tangential Cells and Widefield Motion Detection

Electrophysiological recordings from wide-field motion-sensitive cells in the lobula plate of dipteran fly species have identified roughly 60 LPTCs (Hausen, 1982). These cells are sensitive to directional visual motion in areas of the visual field often correspondent with the three rotational elements (pitch, yaw and roll) as well as with translational, progressive and regressive self-motion (review Borst and Haag, 2002; Krapp and Hengstenberg, 1996). These responses are tuned to the spatial frequency, temporal frequency and contrast of the pattern eliciting the responses (Hausen, 1982; Hausen and Wehrahn, 1989; Hengstenberg, 1982). These LPTCs have been shown to spatially integrate over arrays of elementary motion detecting elements to generate their motion signals (Franceschini et al., 1989). Lobula plate tangential cells are remarkably similar

not just across dipteran species, but also among arthropods generally (Osorio, Averof and Bacon, 1995; Strausfeld, 2005). Less is known about the wide-field sensitive neurons of arthropod species without an anatomically separate lobula plate, including dragonflies.

In dipteran flies, LPTCs can be broadly sub-divided into two systems: the Horizontal System (HS), and the Vertical System (VS) (Figure 1-7). These neuron classes are conserved across taxa, although the number and structure differ from species to species (Buschbeck and Strausfeld, 1997; O'Carroll et al., 1997; Nordström et al., 2008). As their names imply, the systems encode wide-field image motion along the horizontal and vertical axes. The majority of evidence concerning these classes supports their role in encoding rotational motion. The VS neurons are generally considered to encode pitch and roll, whilst the HS cells encode yaw movements (although recent evidence suggests they may preferentially encode side-slip, Tammero et al., 2004; Duistermars et al., 2007). This information is encoded by the LPTCs as graded shifts in membrane potential, often accompanied with superimposed spikelets of irregular amplitude and frequency.

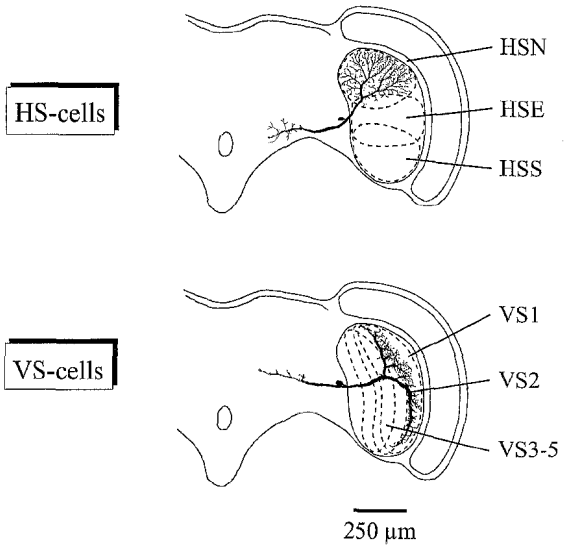

Figure 1-7. Representative cells of each of two cell families of the lobula plate tangential cells reproduced from Borst and Haag, 1996. The dendritic fields of other members of each cell family are also indicated by dashed lines. Note that within each cell family, all members fill the space of the lobula plate by occupying different but overlapping areas with their large dendrites. Per brain hemisphere, there exist three different HS cells (a northern HSN, an equatorial HSE, and a southern HSS cell), and eleven different VS cells (numbered from the lateral VSl to the most proximal VSll consecutively).

1.5.2 Lobula Giant Motion Detector and Descending Contralateral Motion Detector Pathway

Another well-studied pathway for wide-field integration of local motion signals is that of the lobula giant motion detector (LGMD) system in locusts. LGMD1 & LGMD2 are a pair of 'looming' motion selective neurons situated in the lobula of the locust (Strausfeld and Nässel, 1980; Rind and Simmons, 1992; Simmons and Rind, 1997; Gabbiani et al., 2002). Looming neurons are sensitive to the motion of objects growing larger as they approach. The LGMD1 neuron has three large dendritic regions: the largest receiving excitatory local motion inputs, with two providing feed-forward, size dependent inhibition (Rowell et al., 1977; O'Shea and Williams, 1974; Simmons et al., 2010). LGMD1 connects with a post-synaptic neuron - in a one-to-one spiking relationship - that projects directly into the ganglia controlling flight and jumping, the descending contralateral motion detector (DCMD) (Figure 1-8) (O'Shea and Williams, 1974; Pearson et al., 1980). This circuit likely acts to trigger escape/avoidance behaviour when a looming visual stimulus reaches a certain angular size (Hatsopoulos et al., 1995; Santer et al., 2012). When the LGMD/DCMD circuit was first identified it was thought to be selective for small targets (Rowell et al., 1977). The input pathway for these neurons (i.e. in the medulla) is poorly understood, although a horizontally-spreading amacrine cell class described from the proximal medulla of the locust (O'Carroll et al., 1992) was proposed as a possible analogue of the horizontal element in the model proposed by Rowell et al. (1977). The reciprocal local interactions mediated by these cells on columnar medulla units may play a role in shaping the looming response of the LGMD.

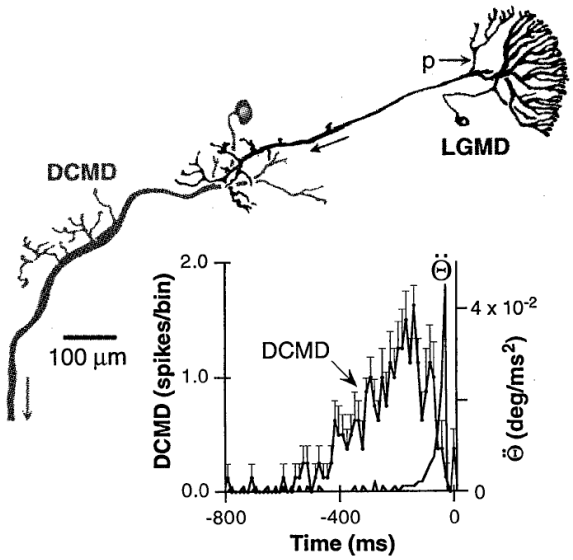

Figure 1-8. The LGMD1/DCMD circuit and DCMD response to a looming target reproduced from Hatsopoulos et al., 1995. Morphology of the LGMD and DCMD neurons. The fan shaped arbor of the LGMD is in the lobula and receives inputs from small-field motion sensitive cells in the medulla. Branch p is thought to collect feed-forward inhibitory inputs from neurons sensitive to wide-field OFF and ON stimuli. The inset graph shows the response of DCMD to a simulated square object (S_{obj} = 6cm) approaching at a constant velocity (v = 2.5m/s). Collision would be at 0ms. DCMD is silent during the first 2s of the 2.75s approach. The stimulus, which was limited to the size of the monitor , topped its expansion at the time indicated by the peak of angular acceleration (Θ). However, DCMD activity started to decline about 150ms before the stimulus peak angular acceleration.

1.5.3 Feature Detecting Neurons of the Lobula

A subset of lobula plate motion sensitive neurons, referred to as feature detecting (FD) neurons, respond to medium size (rather than wide-field) motion (Egelhaaf, 1985). Their existence was proposed following behavioural experiments that showed fly fixation behaviour, with discrimination between the figure and ground of moving random-dot patterns (Reichardt and Poggio, 1979; Reichardt et al., 1983). FD neurons are motion opponent, responding optimally to gratings with limited spatial extent (>10°) travelling in the preferred direction, however being inhibited by grating motion in the antipreferred direction. As the grating stimuli size increases, responses decrease gradually (Egelhaaf, 1985). FD neurons have been suggested to play a part of a simple object classification scheme in insects. *Drosophila* are attracted to elongated vertical objects like environmental vegetative matter and will avoid small features, i.e. potential predators or competitors (Maimon et al, 2008). This kind of basic classification can be elaborated upon when it is behaviourally relevant for the insect. Larger species of dipteran flies display clear behavioural preference for small objects which *Drosophila* would avoid. Male flies of many species, for example, pursue females on the wing during courtship. Such pursuits are seen in, for example, houseflies (*Musca*, *Fannia.* Wagner,

1986*;* Land and Collett, 1974*)*; flesh flies (*Sarchophoga*, *Calliphora.* Collet and Land, 1975); hoverflies (*Syritta*, *Eristalis.* Collett and Land, 1978*)*. Analogous behavior is seen in insects (e.g. long-legged flies, *Dolichopodidae*, robber flies, *Asilidae* and dragonflies) that prey upon small flying insects.

1.5.4 Small Target Motion Detector Neurons

It is likely that the pathways involved in obstacle fixation/avoidance (e.g. the dipteran FD system) are separate from those involved in attraction/pursuit of small objects (Nordström & O'Carroll, 2009). Much progress in recent years has identified a new class of lobula neurons, which respond robustly and specifically to small objects, and thus are likely to provide a basis for the object fixation scheme proposed by Land and Collet (1974) and Maimon et al. (2008). The STMD system likely plays specific roles in resolving the challenges of target detection, target discrimination and potentially also plays a role in visual attention and closed loop pursuit. These STMD neurons are characterised by their ability to give robust, size-tuned response to small targets, which is reduced as object size increases. They give no response to wide-field motion (O'Carroll, 1993).

The selectivity for small targets is the only shared trait of what is an extremely varied family of neurons (Nordström & O'Carroll, 2009). In the species where these neurons have been identified, there is significant variance in the receptive field size, direction selectivity and response modality of the STMDs. Categorisation of receptive field shape using a drifting target of optimal size has revealed a significant variety of receptive fields: some extend across only a few degrees - only marginally larger than the size of the targets they respond to. These 'small-field STMDs' of the hoverfly *Eristalis* were found to be retinotopically organized (Barnett et al., 2007; Figure 1-9) and have been proposed as putative elementary small target motion detectors (ESTMDs) whose outputs are processed by those STMDs with larger, less homogenous receptive fields.

These larger 'collator' STMDs may have receptive fields confined to one hemifield, or extending across one or both hemispheres, often with spatially distinct sub-fields which excite or inhibit the response (Geurten et al., 2007; Nordström & O'Carroll, 2009).

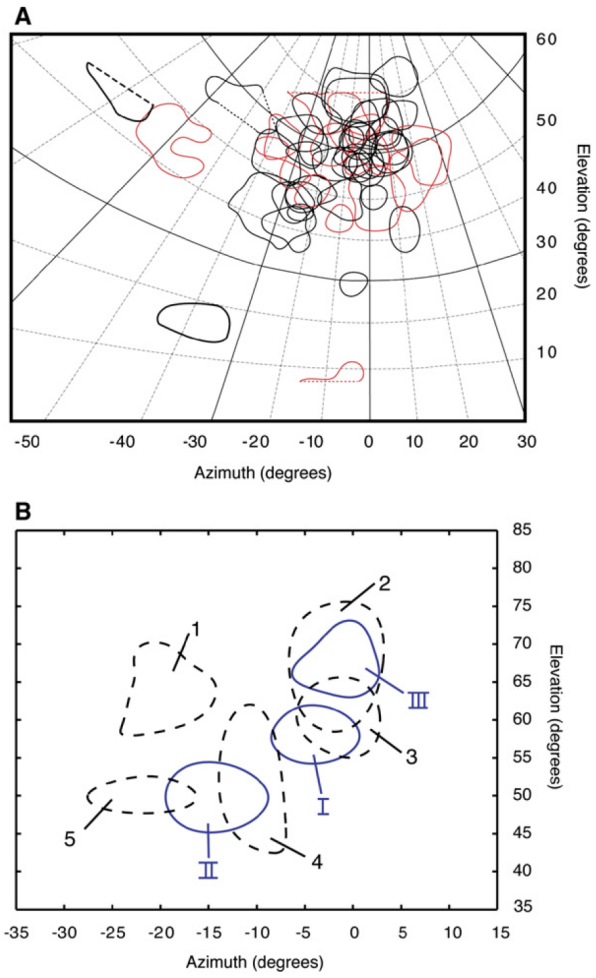

Figure 1-9 Retinotopic Arrangement of Physiological Receptive Fields reproduced from Barnett et al., 2007. (A) 50% maximal response contour of 55 receptive fields from male SF-STMDs. Receptive field boundaries delineated with black lines represent those neurons with a DI < 0.3, and those in red represent neurons with a DI > 0.3. Dashed lines on the plot show regions of uncertainty (e.g., stimulus monitor boundary or discontinuities in the 50% response contour). (B) Consecutively obtained receptive fields from two single electrode tracks (solid blue line and dashed black line) in a male hoverfly. Numeration represents the sequential order in which each receptive field was obtained as the electrode progressed through the neuropil.

The receptive field, as defined by activity in response to a drifting target, is only representative of response to this particular stimulus, due to the complex interactions between each neuron's individual size tuning, velocity tuning, contrast sensitivity and direction selectivity (Nordström and O'Carroll, 2006; Nordström et al., 2006; Barnett et al., 2007; Geurten et al., 2007). In particular, local velocity and spatial tuning varies across the eye (reflecting the optical specialisations described earlier) so receptive fields may vary dependent on the size and velocity of the test target (Wiederman & O'Carroll, 2013).

STMD neurons face similar challenges to the lobula FD neurons when it comes to figure-ground discrimination in so far as they need to segregate the feature motion from that of the background. However, this is amplified since the features that STMDs are tuned to are on the same scale as the spatial sampling resolution of the eye itself, such that optical blur may make the feature contrast very low (Nordström et al., 2006; O'Carroll & Wiederman, 2014). Picking out a single, poorly contrasting pixel moving differently to that of the background is clearly a major challenge. However, this has been clearly met by STMD neurons, many of which respond robustly to targets moving relative to a background, even if the background itself is moving (Nordström et al., 2006).

Intuitively, one would expect that the segregation of the motion of the target from that of the background is a fundamental (if complex) component of any system that can respond as robustly as STMDs. However, a subset of large-field STMDs respond robustly even when the velocity of the target is perfectly matched to the velocity of the background (Nordström et al., 2006). This implies that the spatial statistics of small targets form an important discrimination cue, regardless of any additional role that may be played by background motion cues (Nordström et al., 2006; Wiederman et al., 2008; Wiederman & O'Carroll, 2011).

Target Selective Descending Neurons

Although direct evidence for the post-synaptic targets of lobula STMD neurons is, as yet, lacking, one likely output is the target sensitive descending neurons (TSDNs) described from the ventral nerve cord of dragonflies (Olberg, 1981; Olberg, 1986). The dragonfly CNS contains 16 identified TSDNs, in eight symmetrical pairs, which take their inputs from the deuterocerebrum and protocerebrum and send their outputs to the three thoracic ganglia responsible for flight movements (Olberg, 1986). The axons of the TSDNs are some of the largest in the dragonfly ventral nerve cord (VNC) affording rapid conduction speed for generated behaviours (Figure 1-10). TSDNs are potential

controllers of dragonfly flight during aerial pursuits (Olberg, 2012). Consistent with a role in mediating turning responses towards target motion, electrical stimulation of individual TSDNs has been shown to alter the position and angle of the wings (Olberg, 1978).

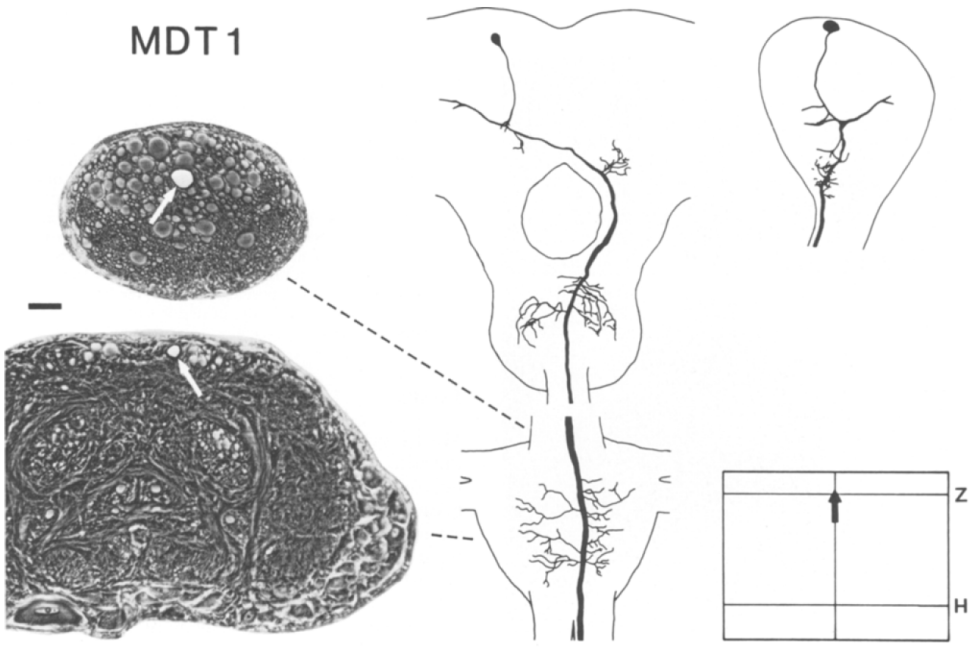

Figure 1-10. Anatomical identification of the giant descending target interneurons reproduced from Figure 3 of Olberg, 1986. Camera lucida drawings (center) of Lucifer Yellow stained neuron profiles in the brain, sub-oesophageal ganglion and prothoracic ganglion viewed from dorsal aspect. Reconstruction of serial sagittal sections (upper right) gives view of the same neuron looking from the right side of the brain (dorsal is left, ventral right). Arrows in cross-sections of the cervical connectives (left top) at the anterior margin of the prothoracic ganglion

All of the TSDNs possess dorso-frontal receptive fields, consistent with the location of the dragonfly acute zone. Within this region, each individual neuron displays unique tuning to target location, direction, and size (Olberg, 1986; Frye and Olberg, 1995). Recent experiments suggest that, together, the TSDNs encode target position as a population vector, also consistent with a role in centring tracked targets in the fronto-dorsal eye field (Gonzalez-Bellido et al., 2013).

1.6 Mechanisms involved in Motion Detection

The ability to determine motion is ubiquitous to all but the most elemental visual systems. It is arguably the next computational progression after the ability to determine differences in light intensity (Land, 1981; Nakayama, 1985). Pursuing a moving object through a dynamic world would be impossible without the ability to detect motion. Above I have introduced the biological systems that address these tasks. How do these visual systems generate these motions signals from information that, at the level of the retina is simply a change of luminance in both space and time? And further how do the visual systems identify a subset of these motion signals as belonging to different features within the scene?

1.6.1 Fundamental Classes of Motion

Much work examining visual physiology or human psychophysics is generated using what are now referred to as 'simple' or 'classical' stimuli: dots, squares, bars and gratings. More recently, largely because technology - both photographic and display - has advanced to represent them accurately, 'natural' stimuli have been put forth as an improved stimulus paradigm for further research (Felson and Dan, 2005). Rust and Movshon (2005) argue for the continued utility of classical visual stimuli.

There are classically two types of motion perception. A classical stimulus, such as a black square moving across a white background, is a luminance-defined object. This creates a Fourier or 'primary' motion signal. Motion detectors can easily extract these signals. However, 'secondary' motion occurs when target movement is defined by changes in texture, flicker, or local contrast that do not result in an increase in luminance or Fourier motion energy. 'Secondary' motion detection requires additional pre- or post-processing by fundamental motion detecting elements (Zanker, 1993; Zanker, 1996a; Zanker, 1996b)

1.6.2 Motion Detection Models in Biology

The computational basis of motion detection in biological systems has long been a focus of enquiry. Across multiple species, one basic scheme with a number of different implementations has been identified. The correlational scheme for an elementary motion detector (EMD) also known as the Hassenstein-Reichardt (H-R) detector (Hassenstein & Reichardt, 1956) was first proposed following experiments on the optomotor turning response of the beetle *Chlorophanus* and will be addressed in more detail by the following section (1.6.3).

Biological motion detecting systems have also been identified that utilize alternative non-linear correlation schemes, e.g. rabbit retina (Barlow and Levick, 1965), turtle retina (Ariel and Adolph, 1985), fly medulla (Mimura, 1972) and locust medulla (Osorio, 1986). All of these systems use some form of asymmetric and non-linear inhibition that has been shown to be fundamentally similar to the correlation detector (Buchner, 1984). Including these variations, motion detectors of this general type have thus been identified in the biological motion detecting systems of a huge range of animals: insects (Hassenstein and Reichardt, 1956), rabbits (Barlow and Levick, 1965), humans (van Santen and Sperling, 1983), wallabies (Ibbotson et al., 1994) and pigeons (Wolf-Oberhollenzer and Kirschfeld, 1994). It is likely that this approach to motion detection has evolved independently a number of times.

During pursuits of conspecifics and prey, the flying insects are faced with two broad categories of motion: movements of the entire visual field, generated by the flies' motion through the world (ego-motion); and the movement of objects within the visual surround. Behavioral experiments first showed that these two types of motion are handled by separate motion detection systems (Palka, 1969; Geiger and Poggio, 1975; Srinivasan and Bernard, 1977; Rowell et al., 1977) followed by subsequent identification of specialized neurons responding to only specific types of motion stimuli.

The lobula plate of flies is a high order motion processing region and recordings from neurons in this region have provided much of the evidence which supports a Hassenstein-Reichardt correlator based motion detection system (review Borst and Egelhaaf, 1989; Borst, 2012). Many of the lobula plate tangential cells (LPTCs) that I described in Section 1.5.1 above are sensitive to directional motion, and seem to get their inputs from local motion detecting elements consistent with this model (review Borst and Haag, 2002). The cells‘ optimal responses are matched to directions of rotation around the flies‘ own cardinal axes (yaw, pitch and roll) (Krapp and Hengstenberg, 1996). The LPTCs have been shown to spatially integrate over arrays of EMDs (Franceschini et al., 1989).

1.6.3 The Hassenstein-Reichardt Detector

The Hassenstein-Reichardt EMD is composed of fundamental sub-units referred to as half-correlators, based on two spatially separated input detectors. The response of one detector is delayed relative to the other before multiplication between the two arms. In biomimetic Modelling efforts, additional processing units are included at various stages of processing to account for factors such as eye optics, photoreceptor kinetics and first order interneurons (for examples of this approach see Reichardt, 1961; van Santen & Sperling, 1983; Wiederman et al., 2008). Each half-correlator is weakly direction selective, however it is very responsive to flicker (i.e. luminance change with only a temporal component). The subtraction of the response from a mirror symmetric half-correlator generates the output of the full correlator unit, with this whole operation defining the elementary motion detector (EMD).

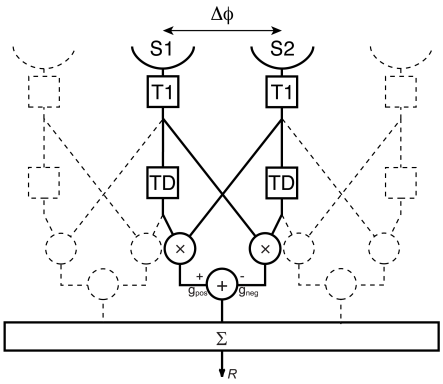

Figure 1–11. The EMD modelled with a Hassenstein-Reichardt correlator. (a) Two sensors (S1 & S2) with separation Δφ receive a luminance signal that is Gaussian blurred to represent the optics of the compound eye. Reproduced from Dunbier at al., 2011.

Correlational type motion detector units do not represent velocity linearly, instead increasing response as velocities tend toward optimum and decreasing once the optimum is exceeded (Buchner, 1984). This optimum varies proportionally to the spatial separation of the receptors and the length of the delay in the half-correlator of the stimulus, maintaining a constant ratio between the two (review Borst and Egelhaaf, 1989). A spatially adjacent array of EMDs can be combined via linear or non-linear integration to generate a motion signal over a wide-field. This has been confirmed experimentally, in fly LPTCs (for examples, Single and Borst, 1998; Haag et al., 2004). Recent work unraveling the connectome of the *Drosophila* optic neuropils has uncovered potential candidates for motion detector circuits in the lamina and medulla (Takemura et al., 2013).

1.6.4 Elementary Small Target Motion Detector

A recent model suggested that, much like the motion detection of LPTCs has been shown to be due to the summation of EMD outputs, STMD neurons with large receptive fields could be the product of summation across a retinotopic array of presynaptic end-stopped elements, putative 'elementary STMDs' (ESTMDs) (Wiederman et al., 2008). The size tuning of these putative ESTMDs and therefore insect STMD neurons strongly resembles that of mammalian hypercomplex neurons (Nordström & O'Carroll, 2009). The Nobel Prize winning work of Hubel and Wiesel (1962) showed that tuning to small objects in the cat cortex was generated by an excitatory centre flanked by spatially

distinct inhibitory end zones. More recent work has shown that reduced response to elongated bars is caused by the combination of active inhibition from end zones together with decreased excitation from presynaptic small-target tuned neurons (Anderson et al., 2001).

These putative ESTMDs are tuned to small targets via a combination of lateral inhibition and fast temporal adaptation similar to that observed in LMC of the lamina. However experimental results from Bolzon et al. (2009) suggested that the spatial scale of these effects is unlikely to be due to LMCs or LMCs alone. Interestingly, whereas the insect lamina and medulla are characterized by 1:1 retinotopic mapping of ommatidia to cartridges, the lobula complex is characterized by a convergence onto larger columns that sum inputs from several medullary inputs (Braitenberg, 1972; Strausfeld, 1976). In a study by Barnett et al. (2007) the receptive fields of 'small-field' (SF)-STMDs in the hoverfly lobula are retinotopically organized. The size of these SF-STMD neurons' receptive fields (45 total ommatidia covering approximately 4.5°-5°) coincides well with the proposed extent of inhibition observed by Bolzon et al. (2009). Hence an intriguing possibility is that spatial tuning in putative ESTMDs occurs as a result of powerful lateral inhibition between lobula columnar neurons similar to the fly SF-STMDs. This could result either from direct inter-column inhibitory synapses or be mediated via local feedback within the receptive fields of a lateral inhibitory element (e.g., an amacrine cell) as previously proposed for locust neurons including LGMD (Rowell et al., 1977; O'Carroll et al., 1992).

1.7 High-order interactions and their effect on target detection and pursuit

Tasks such as attention and closed loop pursuit are complex phenomena that are solved by highly specialised neurons or neural circuits. In this section I describe recent findings

in insects regarding response modulation due to arousal and attention and introduce the concept of facilitation.

1.7.1 Arousal and Attention

During the course of my thesis research a series of exciting new findings have been made regarding the behavioural state of invertebrates during electrophysiological recordings. Advances in technology have allowed research groups around the world to investigate neuronal activity via patch clamping from a behaving animal - something that had previously been only possible using extracellular recording techniques. One of the first of these papers to emerge was from the California Institute of Technology (Maimon et al., 2010) and found that gain in VS cells of *Drosophila* was significantly modulated by tethered flight (See Figure 1-12).

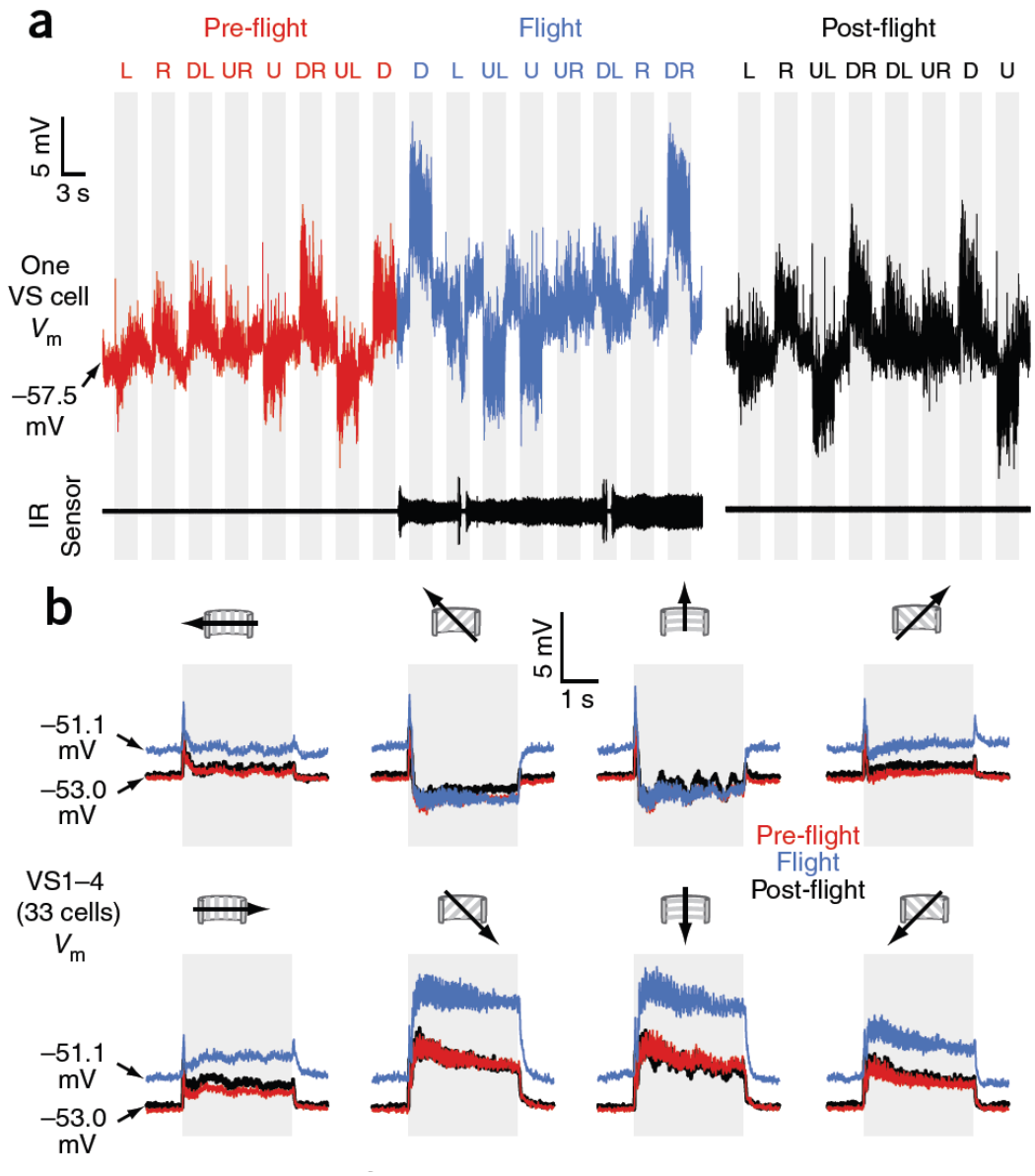

Figure 1-12. Visual responses of VS cells are boosted and the resting potential depolarises during flight reproduced from Maimon et al.,2010. (a) Membrane voltage of a VS cell before, during and after flight. This fly stopped flying twice during the flight epoch shown (breaks in the infrared sensor trace), but immediately restarted each time following delivery of an air puff. The post-flight trace shares the same y axis scale and offset as the pre-flight/flight trace. D, down; DL, down-left; DR, down-right; L, left; R, right; U, up; UL, up-left; UR, up-right. (b) Mean responses of 33 VS neurons from the right lobula plate to the eight directions of grating motion. Note that the membrane potential before (red) and after (black) flight was quite similar, even though these data were collected ≥8 min apart. This stability of the membrane potential was typical of our recordings.

Also in 2010, Chiappe et al. used two-photon microscopy to monitor intracellular calcium activity and showed that *Drosophila* LPTCs showed stronger calcium transients to the same visual stimulus while walking than at rest. Further, Chiappe et al. (2010) also showed that active flight changes the velocity tuning of the LPTC H1 in the blowfly

Lucilia and found that the same change can be approximated by application of the octopamine agonist chlordimeform (CDM). Further work has provided strong confirmation that these activity dependent effects can be attributed to insect octopamine secreting neurons (Longden and Krapp, 2010; Haan et al., 2012; Suver et al., 2012).

Less work has been published on the effects of motor activity on the gain of dragonfly neurons, although CDM has been shown to increase the firing rate of the TSDNs (Frye and Olberg, 1995). Considering this thesis reports recordings from restrained dragonflies (a necessary limitation given the technical difficulty of recording), we therefore need to be cautious in interpreting such data as necessarily indicative of the natural flying state of the animal.

I introduced attention as a concept in Section 1.2.2, particularly with reference to salience and bottom-up attention theories. Salience results from the interaction of a stimulus with other stimuli, as well as with the specific 'filters' that comprise the visual system. Nevertheless, because visual salience arises from fairly low-level and stereotypical computations in the early stages of visual processing, the factors contributing to it are generally quite comparable from one observer to the next. This leads to similar experiences across a range of observers and behavioural conditions. We have ample behavioural evidence of dragonflies successfully detecting, identifying and pursuing until capture tiny prey animals (Corbet, 1999; Olberg et al, 2000). We have also identified a likely neural substrate for these behaviours in the STMD class of neurons found in the lobula and midbrain, even as far as the likely flight muscle controllers of this class, the TSDNs (Olberg, 1981; Frye and Olberg, 1995; O'Caroll, 1993). However, aspects of the mechanics of this system remain unexplained. How do STMD neurons respond so robustly to small/low contrast targets? How do dragonflies

pursue one target out of an array of potential targets? Could either of these properties potentially be explained by the newly identified gradual response onset?

1.7.2 Facilitation

Facilitation in its broadest definition can be thought of as the enhancement of response of a neuron to a stimulus following prior stimulation. The term has been used to describe several phenomena in neuroscience, perhaps most widely for the cellular level process of short-term pre-synaptic plasticity, e.g. paired pulse facilitation (PPF). However in vision research it often simply refers to a situation where a measured response is enhanced by an earlier or, in some cases, additional stimulus.

The Hassenstein-Reichardt EMD itself can be considered a specific example of local facilitation. To generate direction-selective outputs, a non-linear operation is required. Conceptually, the simplest possible non-linear process is a direct facilitatory interaction such as a multiplication like that proposed in the Hassenstein-Reichardt detector. No direct evidence has been found physiologically to suggest that a multiplication can occur at a single synapse (Egelhaaf and Borst, 1992), however non-linear facilitation may arise through linear combination of signals (Watson and Ahumada, 1985) followed by subsequent non-linear operations that have been identified at the synaptic or cellular level (Adelson and Bergen, 1985; Mizunami, 1990; Jagadeesh et al., 1997).

1.7.3 Facilitation in Biological and Synthetic Visual Systems

As I pointed out in the opening of Section 1.7.2, facilitation in its broadest definition can be applied to multiple processes in neuroscience. However in this section I will limit myself to discussing biological and synthetic visual systems. Other species have been shown to display similar modulations in sensitivity and responsiveness following the presentation of target-like visual stimuli. Perhaps the closest comparison can be drawn from extracellular electrophysiology, EEG and telencephalic EEG in the visual tectum of

different toad species (Genus *Bufo*). Following presentation of prey-like stimuli, a sustained negative potential shift occurs in the extracellular surroundings. This kind of sustained shift was proposed to partially depolarise the local neurons, reducing the spiking threshold for excitatory visual stimuli (Laming and Ewert, 1984). The toad T5 class neurons preferentially respond to target like elements and discharge whilst the toad orients towards the object whilst freely behaving, but no longer fires during snapping behaviour. However the discharge peak frequencies depend on the animal's motivational and attentional state (Schürg-Pfeiffer, 1989).

Facilitation has been extensively studied in human visual psychophysical experiments. It has been associated with several high-order properties of human vision including orientation and search behaviour as 'inhibition of return'. Immediately following visual stimulation at a peripheral location, processing of other stimuli in the neighbourhood is enhanced. However once this enhancement is shifted to another region of the visual field the response in the previously facilitated region is delayed (Posner and Cohen, 1984; review Klein, 2000). Many studies associate facilitation with priming and attention mechanisms enhancing search results or discrimination of complex multi-modal objects (Giard and Peronnet, 1999). Additionally, there is an extensive body of knowledge surrounding the influence of arousal states in humans including emotional states and motivational states (ie. thirst and hunger) on task performance, much of which has been replicated in other animals including non-human primates (humans: Keil et al., 2003; Paus, 2000; Schupp et al., 2003; Stoffels et al., 1985. cat: Livingstone and Hubel, 1981. Primates: Witte et al., 1996; Wilson and Rolls., 1990) .

Engineering and computer sciences also seek to solve the same problems as biology. The fundamental building block of any of these systems, which extend to tracking moving targets, is a filter for recursive target state estimation. These are broadly categorised as particle filters. The most widely known of these is the Kalman Filter (Kalman, 1960). The

Kalman Filter predates the origin of the term 'Particle Filter'. However because the Kalman Filter is limited to linear Gaussian problems, this has led to a surge of modified and extended Kalman Filters or entirely new particle filters to handle non-linear and non-Gaussian situations (Chan et al., 1979). The Kalman Filter and many of its modifications utilise a series of measurements over time. These noisy measurements are used to generate estimates of unknown variables, with greater weight in the calculations given to those variables with greater certainty. Work done in our lab (Halupka et al., 2013) exploring the implementation of facilitation in a bio-inspired target tracking system using an ESTMD-EMD motion detection system took an approach with an updating centre of facilitation equivalent to a single state, high-gain Kalman filter with saturation (Ristic et al, 2004).

1.7.4 CSTMD1: an emergent model system for studying response facilitation

An STMD neuron from the lateral mid-brain of the dragonfly known as 'centrifugal small target motion detector 1' (CSTMD1) is emerging as a useful model system for investigating neuronal facilitation and several other high-order properties. Because my thesis primarily focuses on recordings from this neuron, I will provide an in-depth description here of its anatomical and functional properties, followed by a brief review of recent evidence for facilitation within its receptive field by targets moving across large visual angles.

CSTMD1 was initially identified and categorized in *H. tau* (Geurten et al., 2007). However a close homologue has also been recorded from the closely related *H. australiae* (Bolzon et al., 2009). Physiologically CSTMD1 is identifiable by a unique, large receptive field with a distinctive shape (Figure 1-13). This includes a sharp delineation in the excitatory receptive field along the animal's frontal midline (i.e. 0° azimuth). The excitatory receptive field has a prominent hotspot – a region of enhanced response - at approximately 60° elevation, which corresponds with the dorsal acute zone of *H. tau* in

the right visual hemisphere (Horridge, 1978). The left visual hemisphere contains an inhibitory receptive field where the already low spontaneous firing rate of the cell (approx. 20 Hz) is suppressed. This unique receptive field structure taken in combination with the neuron's characteristic spike shape – large (60-80mV in a healthy recording), fast (<3ms) biphasic action potentials - allows us to confidently identify this neuron in successive recordings from different animals, confirmed by intracellular labelling in earlier studies (Geurten et al., 2007).

CSTMD1 neuron responds optimally to targets between 1-3° but still gives a robust response to targets as small as 0.16° within the frontal hot-spot. The selectivity for small sized objects is position invariant within the large receptive field (Geurten et al., 2007) but the neuron is tuned maximally to smaller and slower moving targets frontally and somewhat larger targets in the periphery (Wiederman & O'Carroll, 2013).

Anatomically, CSTMD1 is a heterolateral neuron, with a large axon traversing the brain to outputs in the contralateral protocerebrum and lobula complex. Its ispilateral inputs (with respect to the location of the excitatory receptive field) are a small group of spiny processes (inset III, Figure 1-13C) adjacent to the cell body in the lateral mid-brain. CSTMD1 shows a mass of dense, heavily beaded arborisations (likely output aborisations) across the entire contralateral lobula (inset I, Figure 1-13C). A second putative output arborisation arises in the contralateral mid-brain (insets II, Figure 1-13C).

Because the mid-brain inputs correspond well with the expected location of the outputs of its contralateral counterpart, Geurten et al. (2007) proposed that the symmetrical pair of CSTMD1s may mediate mutually inhibitory interactions. This is supported by the relatively similar shape of the inhibitory receptive field in the contralateral hemisphere, as well as the weak direction selectivity in this region, which again is a mirror image of

that in the recorded neuron (Bolzon et al., 2010). It is also supported by the size tuning of the inhibition itself, which is similar to that within the excitatory receptive field so that small targets produce the most potent inhibition (Wiederman et al., 2011b).

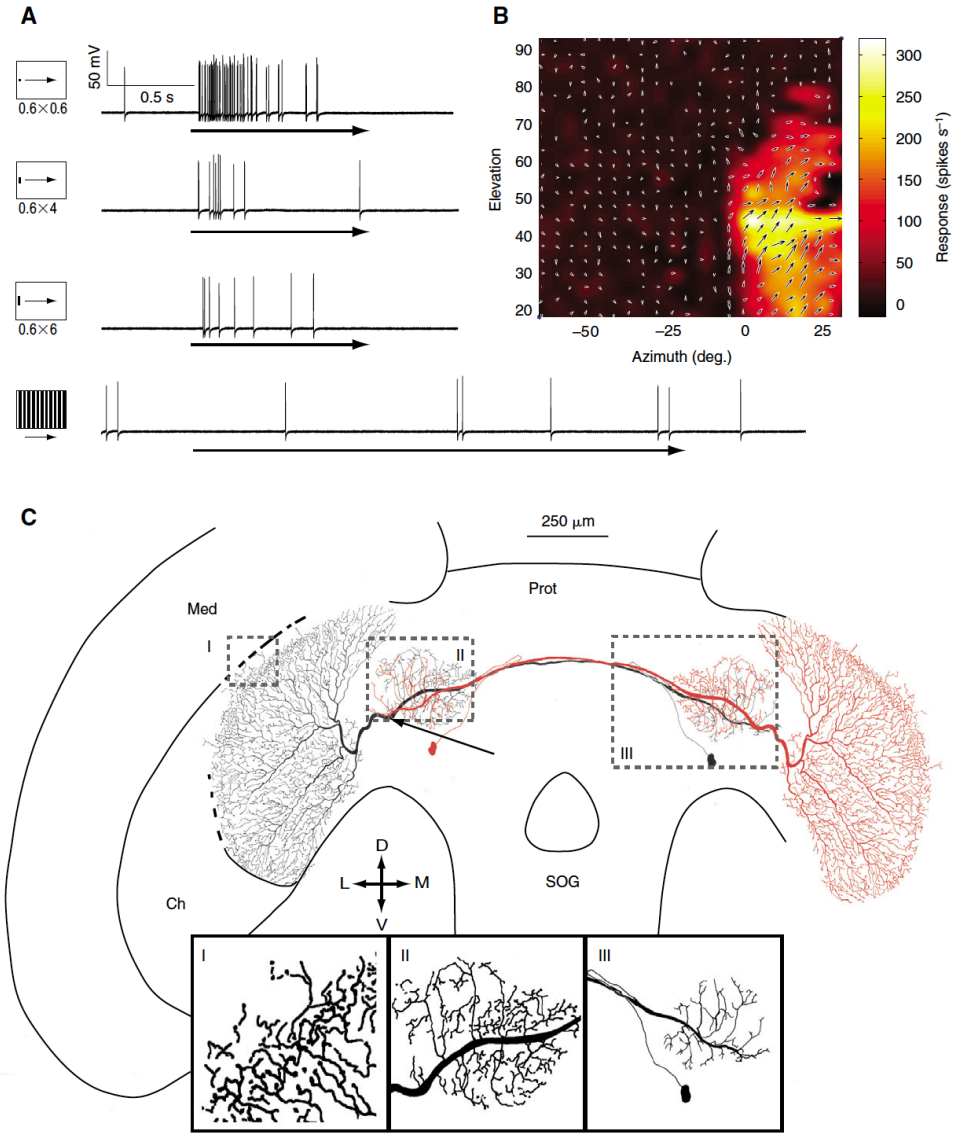

Figure 1-13. Characterization of CSTMD1 reproduced from Figure1 Geurten et al. 2007. (A) Intracellular responses to targets of different sizes illustrate the extreme size selectivity of CSTMD1. While a 0.6x0.6° high-contrast target traversing the receptive field evokes a strong response (top trace), larger targets elicit lower spike frequencies (middle traces), and full screen gratings (bottom trace) give no response above spontaneous firing rates. The targets scanned the center of the receptive field at 26·deg.·s–1, as indicated by arrows under each trace. (B) A physiologically recorded (from the left hemisphere) CSTMD1 receptive field shows excitation in the opposite hemisphere only. The false color plot was generated by scanning the entire monitor horizontally with a high contrast 0.8x0.8° target 21 equidistant locations at 50·deg.·s–1. Arrows indicate the strength of the directionality at each location as constructed by drifting targets in four directions across the stimulus display (see Materials and methods). Elevation values are positive above the equator, and azimuths negative to the left of the midline. (C) A reconstructed Lucifer Yellow fill of CSTMD1 shows massive arborizations (black) in the left hemisphere (recording side). As the soma is located in the opposite hemisphere, and the dendrites of this hemisphere are not beaded (inset III) unlike the arborizations on the left side (insets I and II), the right side most probably provides the input. A displayed mirror image projection of the neuron (red) shows how output arborizations (II) from one hemisphere co-localize with input dendrites from the other hemisphere (III), thus providing the opportunity for synaptic control of responses. Arrow indicates the recording site. Med, medulla; Ch, inner optic chiasm; Prot, protocerebrum; SOG, sub-oesophageal ganglion; L, lateral; D, dorsal; M, medial; V, ventral.

1.7.5 Facilitation in CSTMD1

CSTMD1 has a short absolute latency of response (approx. 30ms). This may result from synaptic delays on its complex input pathway: its inputs in the lateral mid-brain suggest that it is unlikely to receive its input directly from elementary STMDs, but rather indirectly through other STMDs projecting from the ipsilateral lobula (Bolzon et al., 2009). However, these synaptic delays are unlikely to account for a subsequent prolonged response build-up observed in a recent study when objects moved continuously through the receptive field (Figure 1-14). This slower build-up reaches a steady state over a time course of hundreds of milliseconds (Nordström et al., 2011).

A key finding of this recent work was that at cessation of target motion, response offset is dramatically faster than the build-up at response onset. Nordström et al. (2011) argued that this was strong evidence against a parsimonious explanation for the slow onset: simple low-pass filter mechanism in higher order neurons that integrate local motion detectors. This would impart sluggishness to both onset and offset independent of the contrast of the feature (Figure 1-14).

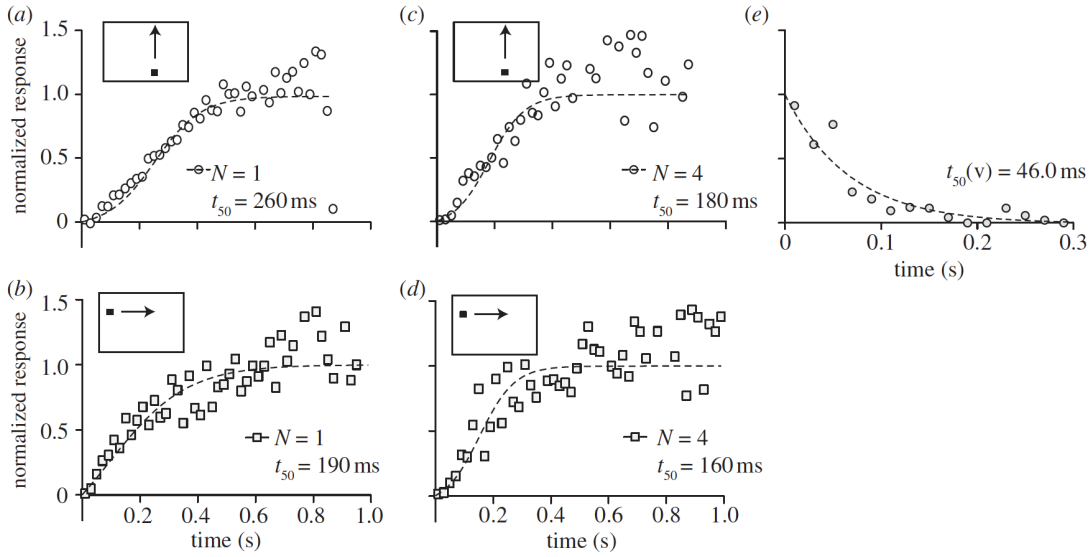

Figure 1-14. CSTMD1 Response onset and offset half-times reproduced from Figure 2 of Nordström et al., 2011. (a) Normalized response time course averaged across all start positions. (b) Normalized response onset from the same neuron, to targets drifting horizontally. (c) Response to vertical drifts, pooled across four neurons. (d) Response to horizontal target drifts, pooled across four neurons. (e) The normalized response decay when targets disappeared close to the hotspot (three different receptive field locations, N = 1, n = 39).

Having rejected a simple 'sluggish' low pass mechanism, and given the observation that the target moved across large numbers of underlying ommatidial axes during the build-up in response, Nordström et al. (2011) concluded that it must result from an alternative non-linear mechanism. They proposed an alternative possibility: the STMD pathway uses a second order motion detector network (e.g. Zanker, 1994). In such a scheme, the first layer would mediate initial target detection as in the elementary small target motion detection scheme proposed by Wiederman et al. (2008). These local target signals would then be processed by a second layer of motion detectors, operating on a larger spatial scale and with correspondingly longer delays. As a second order system, this mechanism would potentially be sensitive to second order (non-Fourier) motion stimuli. While responses to such stimuli have not been demonstrated directly in insect STMDs, behavioural evidence for the tracking of second order feature motion has been

found in *Drosophila*, with long response delays (several hundred milliseconds Theobald et al., 2008, Aptekar et al., 2012).

Why would CSTMD1 employ a higher order facilitation mechanism of this kind? One benefit of a second-order facilitation mechanism such as that proposed is that it would potentially reject noise in local motion detector outputs (as this would not correlate in space and time), permitting the very high amplification required to respond to very small or low contrast targets. Yet, in doing so, the system would maintain the small size selectivity of its local motion detector input elements, as well as their sensitivity to targets travelling at biologically relevant velocities (i.e. high angular speeds of prey or conspecifics during prolonged pursuit).

This leads me to three hypotheses for the functional role of this facilitation:

1) We know that target sensitivity is limited by the contrast sensitivity of early visual elements. Facilitation may thus serve as a higher order mechanism to improve the reliability of target signaling.
2) An alternative hypothesis is that facilitation is unrelated to the emergent properties of the underlying spatio-temporal interactions of the motion detector network and might instead be an artifact of a saliency enhancement, e.g. a simple bottom-up arousal from a resting state where gain is regulated to save energy.
3) Facilitation may be mediated by a higher-order ('cognitive') function such as either a top-down or bottom-up attention mechanism.

Although each of these hypotheses had some potential patency at the commencement of my thesis, I aim to primarily address hypotheses 1 and 2 for several reasons. Firstly the work of Nordström et al. (2011) did not test several alternate hypotheses in relation to temporal filtering by motion detectors. These are sufficiently simple that they are worthy of thoroughly testing and refuting. Secondly, the prior work had only speculated

that the observed facilitation relies upon continuous target motion. We cannot reject hypothesis 2 without directly testing the degree to which facilitation relies upon continuous target motion using discontinuities in the path.

Furthermore, before we can speculate as to models for the underlying mechanisms (including hypothesis 3 above), we need more fundamental information about the spread of facilitation in space at any instant, and the time course over which it evolves. Providing this information was the second major aim for my thesis.

Addressing these aims was not an easy task as the small target motion detecting system is extremely prone to habituation. Minimising this requires extremely lengthy recordings to allow a 'rest' period between stimuli. There is only a limited set of STMD neurons identified to date that reliably provide such recordings. For this reason, I primarily focused on experiments from CSTMD1. With its large axon supporting the required long recordings and its relatively high-order position within the hierarchy of the STMD system it is an ideal subject to test these hypotheses. Nevertheless, in the process of recording from CSTMD1 I did also identify and describe (Chapter 5) a new neuron from which occasional long recordings can also be obtained.

Despite CSTMD1 being an ideal target for these experiments, my thesis involved attempted recordings from 488 dragonflies, from which I penetrated CSTMD1 214 times. Of those, only 48 recordings were of sufficient length to run a subset of the experiments. 12 of those had to be discarded because they did not meet our strict criteria for recording health and stability to obtain the results presented in this thesis. Hence, addressing hypotheses 1 and 2 was already a technically demanding project, leaving plenty of questions remaining for those who follow in my path, as well as some definitive answers and insight as to how this system works. Despite hypothesis 3 ending up beyond the scope of my work, a concurrent project in our lab by Steve Wiederman

led to the somewhat serendipitous discovery that CSTMD1 indeed displays a form of selective attention (Wiederman and O'Carroll, 2013). I will discuss this parallel finding in Chapter 7 (Conclusions).

1.8 Thesis Aims and Scope

In this thesis I investigate this facilitation in CSTMD1 and its potential functions, by using computational modelling and measuring the electrophysiological response of the neuron to target motion. In Chapter 3 we model a potential inhibitory interaction between multiple targets presented simultaneously in a STMD neuron's receptive field. Work following from this publication leads to an important finding of selective attention in CSTMD1 (Wiederman and O'Carroll, 2013) that provides an important interpretation point for the findings of the subsequent chapters. In Chapter 4 we rule out a parsimonious explanation for this gradual onset time course utilising a computational model of the fundamental elements of biological motion detection. In Chapter 5 we determine that facilitation requires continuous local motion and has a more potent effect on relatively slower moving targets. And finally in Chapter 6 we investigate the spatial extent of this facilitation, and its temporal longevity and this provides a fascinating insight into the potential underlying mechanics of this facilitation and the role that it plays in the dragonfly's pursuit.

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2 Methods

2.1 Electrophysiology

Electrophysiological experiments were carried out on Tau Emerald dragonflies (*Hemicordulia tau*) caught in the wild from Adelaide's public gardens. The dragonflies were subsequently stored in a refrigerator (5-8°C) for no more than 4 days to lower the dragonflies' metabolism and reduce their activity.

In preparation for recordings, the dragonflies were immobilized with a wax-rosin (1:1) mixture and fixed to an articulated stand. The animal's head was fixed tilted forward to allow access to the posterior head surface. A small hole was dissected over the area of the left lobula with enough surrounding chitin removed to allow electrode access to the target region and to permit visual inspection of landmarks for electrode placement.

Aluminium silicate electrodes were pulled on a Sutter Instruments P-97 and filled with 2M KCl. When using dye-filling techniques to examine the underlying neuroanatomy, electrodes were tip filled with Lucifer Yellow and further back filled with LiCl. Electrodes typically had a tip resistance between 60 and 110 MΩ.

The animal was placed at a fixed distance in front of the display monitor. The frontal midline of the dragonfly was aligned to the horizontal centre of the monitor and the screen centre was approximately 40° above the animal's visual horizon. Additionally, to calibrate for individual dragonflies, I measured the position of their head relative to the stimulus display.

2.2 Visual Stimuli

Visual stimuli were presented to the animals on a high-resolution LCD computer monitor at a frame rate of 120Hz. Stimulus scripts were written and generated using

MATLAB's Psychophysics Toolbox (psychtoolbox.org). The display subtended approximately 110° x 80° (width by height) at the animal's eye, with a screen resolution of 1920 x 1080 pixels (12 pixels/° at the screen centre) and a white-screen luminance of 300 $Cd.m^{-2}$. The electrode was advanced into the brain with a piezo-electric stepper (Marzhauser-Wetzlar PM-10).

The electrophysiological responses of penetrated neurons were examined with probe stimuli (e.g. varying size features; drifting gratings) to determine the size selectivity of the neuron's response. If the neuron exhibited robust responses to optimal targets (1-3°), the neuron's receptive field was mapped by presenting a series of drifting targets along both horizontal and vertical paths. I identified CSTMD1 by its unique receptive field structure as well as its characteristic large, biphasic action potentials (Figure 1-13B, Geurten et al., 2007). Once identified as CSTMD1, I measured the neurons spiking activity in response to small black targets (~1-2°) drifting vertically up the stimulus display at a fixed velocity of $33°.s^{-1}$. Black targets were presented moving against a white background within the neuron's excitatory receptive field.

Intracellular responses were digitized at 5 kHz using a 16-bit A/D converter (National Instruments, Austin, TX, USA) and analysed off-line with MATLAB (www.mathworks.com).

2.3 Neural Coding

An action potential is an 'all or nothing' event and an individual spike does not convey magnitude, unlike graded changes in membrane potential (e.g. photoreceptors or some dipteran LPTCs) (review LPTCs, Borst and Haag, 2002; review Photoreceptors, Weckström and Laughlin, 1995). Thus, information in a spike code is contained within the timing of spike events, either relative to the previous activity of a neuron or the activity of its neighbours (Butts et al., 2007).

Previous literature has shown that neurons can encode information using various forms of spike timing. Thus the resultant structure of a spike train is determined both by the dynamics of the stimulus and the encoding mechanism employed. For example, in a 'rate code' the number of spikes in a period of time encodes the intensity of the stimulus presented. One of the earliest examples of such a code was in muscle stretch receptors, where the force applied to a muscle produces a specific extent of stretch that in turn translates non-linearly to a specific firing rate in the sensory neuron (Adrian and Zotterman, 1926).

In contrast to a rate code, a temporal code is one where information is represented by the precise timing of individual spikes, rather than absolute firing rate. Such spike timing frameworks include: time to first spike (Van Rullen et al., 2005); variance, skewness or kurtosis of the inter-spike interval probability distribution (Perkel et al., 1967; Schwalger et al., 2010); and patterns of spiking such as bursts and coincident single spikes (review Kostal et al., 2007). Temporal codes may underlie the high temporal resolution of some neuronal systems that can function within millisecond time scales (mammalian visual cortex: Bair and Koch, 1996; Buracas et al, 1998; mammalian lateral geniculate nucleus: Liu et al, 2001; mammalian retinal ganglion cells: Uzzel and Chichilnisky, 2004).

Current evidence supports CSTMD1 as a rate coding system. The spike rate increases as target contrast increases, and the neuron's response is tuned to both target size and velocity (Geurten at al., 2007; Nordstrom et al., 2011). The spiking responses to any given target is strongest near the frontal ''hot spot''. This "hot spot" coincides with the region of maximal optical acuity in the frontal-dorsal visual field in the *H. tau* eye (<0.5°), but is notably reduced in the peripheral eye (Horridge, 1978). Given this optical specialisation, it is not surprising that CSTMD1 exhibits greater sensitivity to small targets frontally and larger targets in the periphery (Wiederman and O'Carroll, 2013).

CSTMD1 responds to low contrast objects (below 25%). However the excitatory receptive field is less extensive than for the same sized target at higher contrast.

2.4 Spike Rate Analysis

Additional experimental design and analytical techniques were required to quantify CSTMD1's change in spike rate at the onset of target motion. Previous researchers recording from CSTMD1 (Geurten et al., 2007; Nordstrom et al., 2011) quantified neuronal activity by counting spikes that occur within a time 'bin'. To meaningfully quantify spike rate in this manner, the size of the bin must contain enough spikes for a reliable estimate of the response (Brown et al., 2004). A large bin (50-100ms) is required to quantify CSTMD1's low spontaneous spike rate (2-20 spikes per second). However, such large bin sizes introduce uncertainty when rapid changes in spike rate occur, as observed when a small moving object traverses the receptive field of the neuron. In this case, responses may be more accurately represented with a smaller bin size (<20ms).

In contrast to binning, the duration of time between individual spikes, i.e. the inter-spike interval (ISI), does not discretise data in the time domain. Instead, an inverse ISI produces an instantaneous measure of spike-rate, from the time elapsing between the new spike and the preceding spike(Mainen & Sejnowski, 1995; Passaglia et al., 1997; Martinez-Conde et al., 2000). This technique has often been applied with mammalian visual neurons (Bair & Koch, 1996; Berry & Meister, 1998; Lewen et al., 2001; Reinagel and Reid, 2000). I compared these two approaches (spike histogram and inverse ISI) on a subset of my data to examine which representation better encapsulated the change in spike rate in response to the onset of target motion.

An advantage of the spike histogram is the ease in averaging across the individual trials in response to the onset of target motion. This is calculated by simply taking the mean

values for each time bin over all trials. With instantaneous spike rates, calculating the average is more challenging, however can be achieved by interpolating over a finely discretized time axis (Figure 2-1).

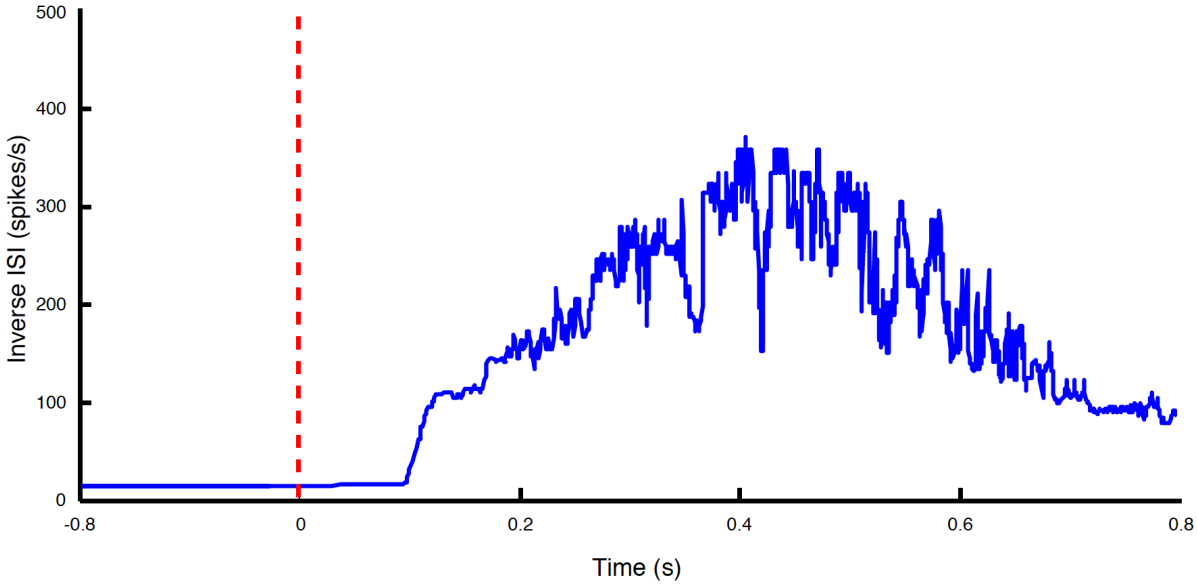

Figure 2-1. A). The mean pooled instantaneous spike rate response from 40 repetitions of a target commencing motion within CSTMD1's receptive field. Target motion commences at time 0 (the vertical red line).

CSTMD1 responses to the onset and duration of an optimal target include: (1) a short latency period (2) an initial rise and then near linear increase (either slow or rapid, depending on the state of facilitation) (3) a final plateau as the neuronal response saturates. I represented this time course by curve-fitting various mathematical forms with the data. I used three functions: tanh ($y = \tanh(x)$)and log ($y = log_b(x)$), and the Weibull cumulative distribution function ($y = 1 - e^{-(x/\alpha)^{\beta}}$). In a test sample of response onset data the Weibull cumulative distribution function best represented the time course properties (r^2 : Weibull = 0.69; tanh = 0.58; log = 0.56).

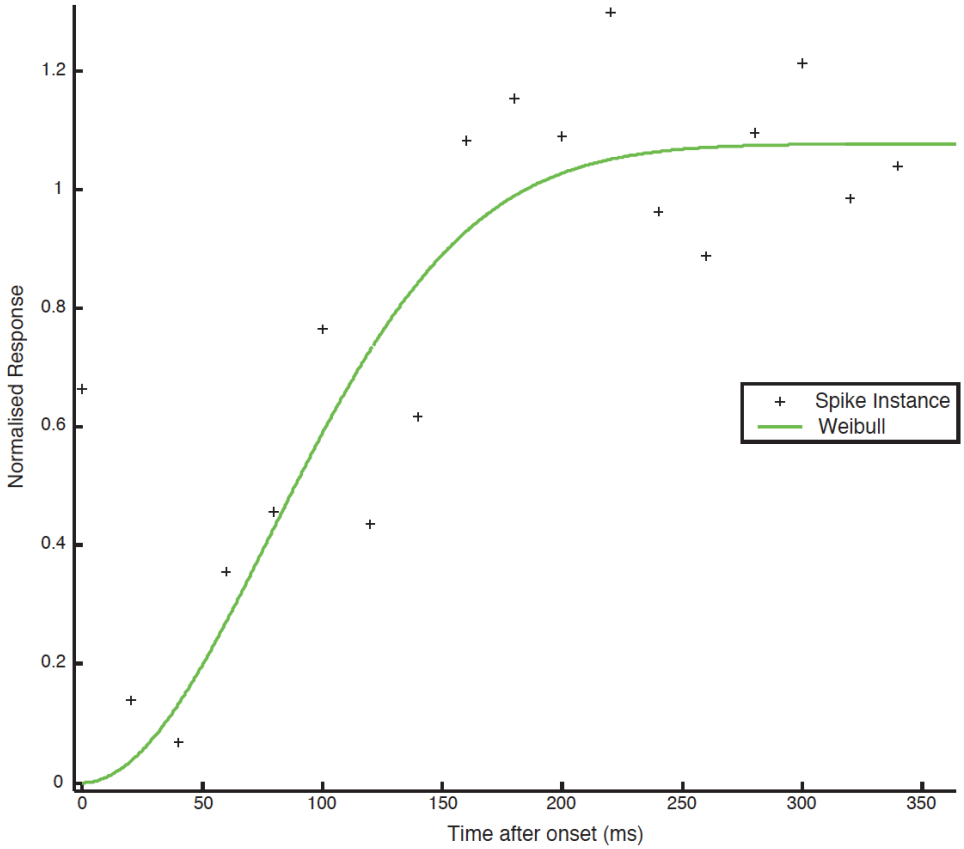

Figure 2-2. Example of a Weibull curve fit to the normalised response onset time course.

A Weibull curve fit ($y = 1 - e^{-(x/\alpha)^{\beta}}$) is defined by two parameters:

1) The α value or scale parameter is the value for *x* where *y* is 2/3 of the function's plateau value.
2) The β value or shape parameter influences the curve's shape (see examples in Figure 2-3).

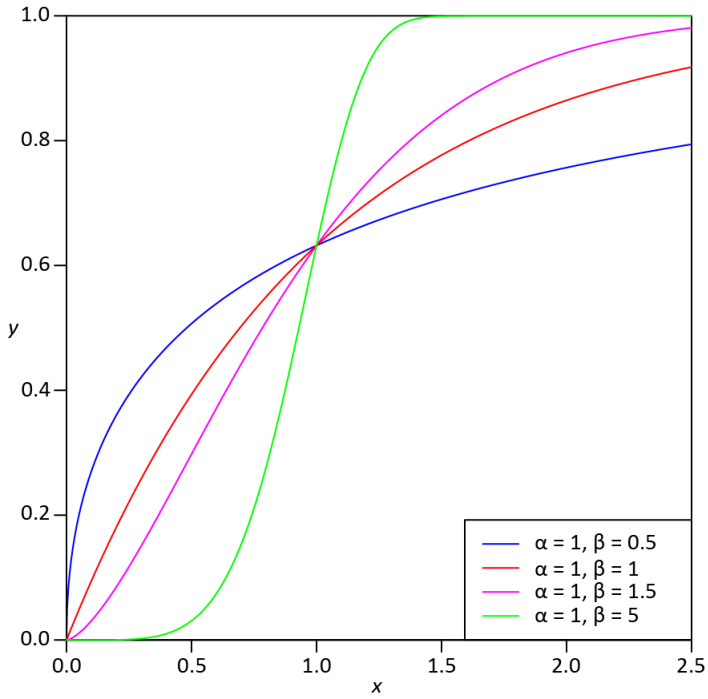

Figure 2-3. Examples of effect of shape parameter on Weibull cumulative distribution function shape.

The α value provides a reliable metric of the rate of increase i.e. the time it took for a given response to reach 2/3 of its maximum (T_{66}). I made the decision to instead represent each onset time course as its T_{50} (or time to reach 50% of maximal). This is both a more common metric for expressing rise times of functions and more intuitively understandable for those without specific knowledge of the parameters of a Weibull curve.

A final question remained: did this T_{50} metric of individual onsets derived from inverse ISI curve fits provide any additional information when compared to similar metrics obtained from binned spike histograms? Rather than calculate a T_{50} from the binned spike histogram, which would have lower precision, I instead compared the inverse ISI T_{50} to the spike rate in a window representative of response onset. In this metric, a lower value represents a slower rise time with more time spent closer to spontaneous rate and a higher value is indicative of a faster rise to plateau levels. This simpler metric was in near perfect agreement with the T_{50} metric generated from the inverse ISI curve

fits. This simpler analysis did not change the statistical significance of our results in further experiments.

Therefore for the sake of consistency and ease of computation I presented results in Chapter 6 as spike rate histograms and the windowed spike rate metric. In this particular neuron this approach offered no noticeable advantage over binned spike data. However, this approach may have applications in neurons with different response dynamics or coding strategies.

2.5 Receptive Field Normalisation

CSTMD1 has a large receptive field, which makes it ideal for experiments that involve stimulating the neuron with targets travelling long, continuous paths. However, the receptive field is not spatially homogenous i.e. CSTMD1 does not respond in the same way to the presentation of an identical target in various parts of its receptive field. When considering temporal aspects of the neuron's response, like its onset time course, it becomes necessary to account for this inhomogeneity. I used the technique Nordström et al. (2011) utilised when they first quantified the response onset time course in CSTMD1. To remove the effects of spatial inhomogeneity, I normalised responses by a control scan: a target moving along the entire length of the display (i.e. CSTMD1 was in a facilitated state). Normalization by division of target scans with the 'spatially equivalent' region of the control scan, accounts for spatial inhomogeneity. The resulting neuronal response is thus purely representative of the developing time course (see Figure 1-14).

Accounting for Local Habituation

In vivo electrophysiological recordings from CSTMD1 can be held for extended periods of time (over several hours). However, CSTMD1 will habituate if a stimulus is repeated in relatively short succession within a local area (1°). This presents a challenge that can be overcome with considered experimental design. To avoid such local habituation, I

repeated target presentations at horizontally separated (2°) locations within CSTMD1's large excitatory receptive field (see Figure 1-13B). By accounting for CSTMD1's heterogeneous receptive field structure (as explained above) I could average the response onsets of trials presented on these separated paths. Each target presentation was followed by a period (5-15 seconds) with no stimulus. Presenting scans along these adjacent paths allowed for frequent stimulation of the neuron whilst maximising the time before direct stimulation of any individual path (See Figure 2-4).

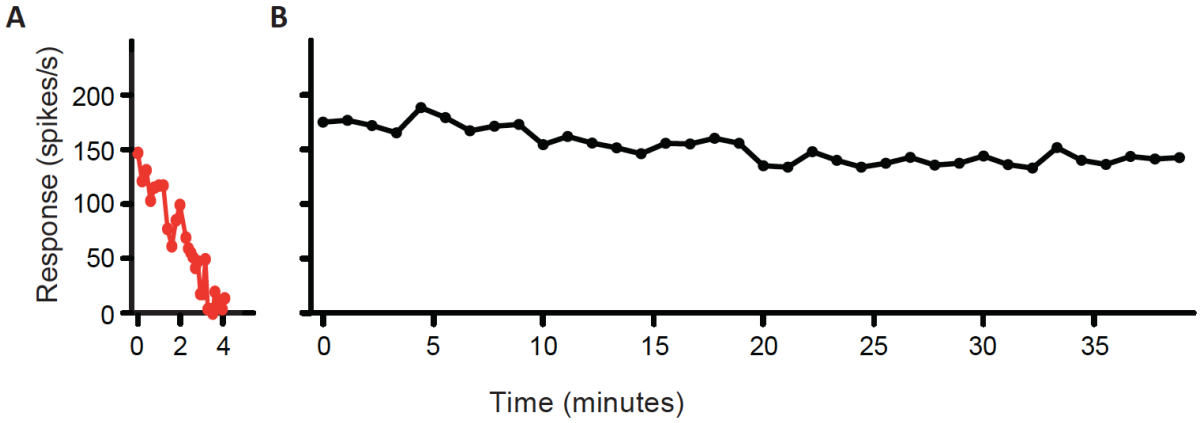

Figure 2-4. Minimised habituation in CSTMD1. A. Response (spike rate in a 500ms window from 0.5s-1s during the target trajectory) habituation in CSTMD1 provoked by 25 repetitions of the stimulus on the same path over 4 minutes. B. The spiking response of CSTMD1 (spike rate in a 500ms window from 0.5s-1s during the target trajectory) to a target drifting along a single path regularly interspersed during a 40 min long recording.

Therefore to quantify the facilitation time course of CSTMD1 I could simply count spikes within a 200ms interval, after implementing techniques to account for local habituation and the inhomogeneous receptive field.

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3 Modelling Inhibitory Interactions Underlying Neural Responses to Multiple Features

Context

In order to develop a more complete model for STMD neurons (including phenomena such as facilitation that involved interactions not just within local motion detectors, but *between* them), we wanted to see whether or not the inhibitory interactions operating on different spatial scales observed in earlier experiments (i.e. Bolzon et al., 2009) were subserved by low-level inhibition (e.g. between local flicker detectors) or higher level inhibition (e.g. between units that were already selective for small features). This required an extension of experiments by Bolzon et al. (2009) that had used only paired stimuli with a single target size. We executed experiments involving a single (optimal) test target and distracters with different sizes and varying distance from the stimulus target. This allowed us to characterise the selectivity of units responsible for inhibition and develop simple models to explain these inhibitory interactions.

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Signature		Date	22/7/2013

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Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Provided significant editing support.		
Signature		Date	19/7/2013

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4 Modelling the Temporal Response Properties of an Insect Small Target Motion Detector

Context

The dragonfly neuron, CSTMD1 had been identified as having a form of spatial facilitation of response (Nordstrom et al., 2011). We initially hypothesised that the facilitation acted to enhance neuronal response to relevant targets that either due to distance from the dragonfly or small absolute size were sub-optimal, that is did not entirely occupy an ommatidium's receptive field.

Could slow kinetics have been mistaken for facilitation? This possibility was raised by Nordström et al. (2011), but dismissed because the response offset was an order of magnitude faster than the response onset time course identified, indicating that there was no simple low-pass filter operating in the integration of local motion signals. However, such an asymmetry in the kinetics of response onset versus offset could still potentially result from slow kinetics of filters at an earlier stage of processing, in the motion detectors themselves. This possibility was neither discussed nor considered in the earlier work (Nordström et al., 2011).

In this chapter we made a new computational model to test this hypothesis, i.e. can a slow delay in cross-arms of an array of Hassenstein-Reichardt detectors (see Figure 1-11 or Figure 4–3a) - the front-end of CSTMD1's motion detection system – replicate the highly asymmetrical onset–offset relationship to a moving discrete object? We then moved a discrete object past these inputs at a number of speeds, with the only adjusted variable in the model being the time constant (τ) of the exponential delay filter of the Hassenstein-Reichardt detectors. Finally, we compared the predictions of this family of

models (i.e. with different delay time constants) with the observed time course of CSTMD1 responses and their velocity tuning.

Our results clearly refute the hypothesis. We found that a sufficiently long value for τ would in fact produce a convincingly similar time course for response onset while still retaining a short offset, but such a model leads to very little sensitivity to fast moving targets and thus a massive mismatch to the observed CSTMD1 velocity tuning. Our data and modelling thus support the major thrust for my thesis, i.e. that facilitation in response is a high-order modulatory phenomenon that cannot be explained by simple linear filtering in early stages of visual processing

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Signature		Date	23/7/2013.

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Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Provided significant editing contribution.		
Signature		Date	22/7/2013

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Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Provided editing contribution.		
Signature		Date	7/8/2013

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Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Lead construction of the model. Wrote the paper with James Dunbier. Contributed all figure components with model output		
Signature		Date	19/7/2013

Dunbier, J.R., Wiederman, S.D., Shoemaker, P.A. & O'Carroll, D.C. (2011) Modelling the temporal response properties of an insect small target motion detector.
Presented at: 7th International Conference on Intelligent Sensors, Sensor Networks and Information Processing, pp. 125-130

5 Facilitation of dragonfly target-detecting neurons by slow moving features on continuous paths

Context

I next investigated the scale on which facilitation acts. CSTMD1 is an example of an STMD neuron with a very large receptive field, relative to the targets to which it responds optimally. Does the facilitation enhance responsiveness across the entire receptive field? Or does the facilitation operate on a more local scale, enhancing response in regions near the last position of the target?

Also in this chapter I test whether facilitation is unique to CSTMD1? As a side-goal, I aimed to record from any non-CSTMD1 large-field STMD neurons and test for facilitation. In addition to recording and characterising these neurons physiologically, I wanted to characterise these neurons anatomically. In this chapter I present my characterisation of the binocular STMD 1, which also displays local facilitation of response to target motion.

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Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Conducted Electrophysiological Experiments. Performed all electrophysiological data analysis. Assisted in construction of the model. Primarily wrote the paper with significant contributions from the other authors listed below. Made all figures with modelling elements provided by David O'Carroll.		
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Facilitation of dragonfly target-detecting neurons by slow moving features on continuous paths

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Abbreviations: Binocular small target motion detector 1 (BSTMD1); centrifugal small target motion detector 1 (CSTMD1); target selective descending neuron (TSDN); small target motion detector (STMD); elementary small target motion detector (ESTMD); elementary motion detector (EMD); lobula plate tangential cell (LPTC); region of interest (ROI)

5.1 Abstract

Dragonflies detect and pursue targets such as other insects for feeding and conspecific interaction. They have a class of neurons highly specialised for this task in their lobula, the 'small target motion detecting' (STMD) neurons. One such neuron, CSTMD1, reaches maximum response slowly over hundreds of milliseconds of target motion. Recording the intracellular response from CSTMD1 and a second neuron in this system, BSTMD1, we determined that for the neurons to reach maximum response levels, target motion must produce sequential local activation of elementary motion detecting elements. This facilitation effect is most pronounced when targets move at velocities slower than what was previously thought to be optimal. It is completely disrupted if targets are instantaneously displaced a few degrees from their current location. Additionally, we utilise a simple computational model to discount the parsimonious hypothesis that CSTMD1's slow build-up to maximum response is due to it incorporating a sluggish neural delay filter. Whilst the observed facilitation may be too slow to play a role in prey pursuit flights, which are typically rapidly resolved, we hypothesise that it helps maintain elevated sensitivity during prolonged, aerobatically intricate conspecific pursuits. Since the effect seems to be localized, it most likely enhances the relative.

5.2 Introduction

Detecting and tracking small moving targets within a visual scene is a complex task, yet one of great importance to many animals that have evolved sophisticated anatomical, behavioural and neural mechanisms for target analysis (Collett and Land, 1978; Frye and Dickinson, 2007; Land, 1993; Land and Collett, 1974; Olberg et al., 2007; Wehrhahn et al., 1982; Zeil, 1973). Flying insects such as the larger dipteran flies and dragonflies display a spectacular ability to track and intercept prey or conspecifics that move against visually cluttered backgrounds (Collett and Land, 1975; Nordström, 2006; Nordström & O'Carroll, 2009). Dragonflies capture prey with success rates of 97% (Olberg et al., 2000) yet do so even in the presence of distracters such as conspecifics or swarms of prey (Corbet, 1999).

Target selective descending neurons (TSDNs) likely to be involved in this impressive behaviour were first described from the dragonfly ventral nerve cord (Olberg, 1986). Optic lobe interneurons that are inputs to such pre-motor pathways were more recently characterised in both dragonflies (O'Carroll, 1993; Geurten et al., 2007) and hoverflies (Nordström et al., 2006; Barnett et al., 2007; Nordström and O'Carroll, 2009). These 'small target motion detector' (STMD) neurons display an impressive selectivity for small moving objects, yet give very robust responses even against complex backgrounds (Nordström et al., 2006; Nordström and O'Carroll, 2009).

An interesting problem that STMD neurons must deal with is that the tiny stimuli they respond to only occupy the receptive fields of photoreceptors in one or two adjacent ommatidia at a given moment. By contrast, the neurons involved in insect optic flow analysis, lobula plate tangential cells (LPTCs), can sum local motion across large arrays of detectors (Krapp & Hengstenberg, 1996). This allows them to generate a reliable global motion response to wide-field stimuli down to very low stimulus contrasts

(below 3%) - extraordinary contrast sensitivity that aids coding of a wide range of image speeds (O'Carroll et al., 1996; Harris et al., 2000; Straw et al., 2006). This spatial integration also allows LPTCs to smooth out local variance (pattern noise) due to the inhomogeneous structure of the surrounding scene, further improving motion coding (Borst et al., 1995; Meyer et al., 2011; Barnett et al., 2010; O'Carroll et al., 2011). STMD neurons do not have this luxury: Some certainly have receptive fields as large as their LPTC counterparts, presumably via summation across arrays of putative local 'elementary small target motion detectors' (ESTMDs) (Geurten et al., 2007; Wiederman et al., 2008). However, given the spatially circumscribed nature of the stimulus, simple spatial summation cannot improve reliability for target discrimination by averaging out local noise as the feature moves across different parts of the background.

How then does the STMD pathway respond so robustly and selectively to moving targets that occupy only a fraction of their receptive field? An interesting hypothesis emerges from our recent analysis of the response time course in the large-field dragonfly STMD neuron CSTMD1 (Nordström et al., 2011). Despite a short initial latency, CSTMD1's response to continuous target motion builds to its maximum over several hundred milliseconds. Nordström et al. (2011) hypothesised that the STMD pathway might utilise a second-order motion detector network (Zanker, 1994). This might enhance target detection by some form of additional non-linear integration of adjacent ESTMD outputs, such as the delay and correlate mechanism intrinsic to direction selective motion detectors. Such a mechanism could take advantage of a distinguishing characteristic feature of *natural* target motion: true targets tend to move along continuous paths, even if they change direction or vary in contrast as they move across the background. A response in one local motion detector ought to be well correlated with an appropriately delayed response in neighbouring detectors (i.e. matching the target velocity). Noise, on the other hand (including spurious feature motion of the background caused by events

such as foliage moving with wind), would be local and variable and thus less likely to persist along continuous trajectories. This second-order system would thus allow rejection of feature motion not correlated across multiple local adjacent input detectors, permitting amplification to enhance robustness whilst maintaining selectivity to stimuli on the spatial scale of single ommatidia of the eye.

In this paper we test this hypothesis by determining if the facilitation identified by Nordström et al. (2011) is propagated globally by motion throughout CSTMD1's receptive field, or requires sequential local activation (i.e. continuous motion along a trajectory). Our findings strongly support a higher-order integration mechanism, since disruption of stimulus trajectories into discontinuous paths dramatically reduces the effectiveness of stimuli that sweep the same total area of the receptive field. We also combine computational modelling with further analysis of the velocity tuning and time course of the CSTMD1 response, to rule out a parsimonious explanation that the slow facilitation time course simply reflects long delay time-constants in the underlying motion detectors.

5.3 Materials and Methods

5.3.1 Electrophysiological Methods

Experiments were carried out on 12 male, wild-caught dragonflies (*Hemicordulia tau*). The dragonflies were immobilized with a wax-rosin (1:1) mixture, and the head was tilted forward to gain access to the posterior head surface. A small hole was cut over the left lobula. Neurons were recorded intracellularly using aluminium silicate micropipettes pulled on a Sutter Instruments P-97 puller and filled with 2M KCl. Electrodes typically had a tip resistance between 60 and 110 MΩ. We identified CSTMD1 by its characteristic large, biphasic action potentials and distinctive receptive field shape in the frontal dorsal visual field, mapped with a drifting target stimulus as described by

Geurten et al. (2007). Visual stimuli were presented to the animals on a high resolution LCD computer monitor (Samsung 2233RZ) at 120Hz frame rate, using VisionEgg software (Straw, 2008). The animal was placed on an adjustable stand and aligned at a fixed distance from the display, using a calibration frame fitted to the front of the display. The small access hole allowed visualization of surface landmarks on the brain only over a very limited range of angles, such that individual dragonflies were always oriented in similar positions, with the frontal midline corresponding to the horizontal centre of the monitor lower edge, such that the screen centre was approximately 40° above the horizon. Small individual differences in elevation alignment were further accounted for by measuring the angle of inclination of the dragonfly's head relative to the vertical. Azimuth alignment with the mid-point on the screen was subsequently confirmed by scanning the receptive field with horizontally drifted targets, since the CSTMD1 receptive field cuts off sharply at the frontal midline (see Geurten et al., 2007 & Figure 1 of Bolzon et al., 2009). The display subtended approximately 110° x 82° (width by height) at the animal's eye, with a resolution of 1680 x 1050 pixels (corresponding to 10 pixels/° at the screen centre) and a background luminance of 280 $Cd.m^{-2}$. Data were digitized at 5 kHz using a 16-bit A/D converter (National Instruments, Austin, TX, USA) and analysed off-line with MATLAB (www.mathworks.com).

5.3.2 Stimuli

We defined a sub-region of the CSTMD1's receptive field, 56° vertical and 15° horizontal extent, in which we tested the response to long trajectories for a moving target. This region of interest (ROI) was close to the central excitatory region, but terminated slightly (4-5°) below the centre of the prominent receptive field hotspot (a sub-region of the receptive field with a higher spiking response to targets) and just inside the medial boundary corresponding to the midline separating the visual hemifields (0° azimuth). Five vertical paths (56° total height, spaced at 3° intervals) were defined within this ROI

(Figure 5-1). During each trial, a small target (~1.1° square) drifted upwards from the bottom of the trial region to the top. Targets either moved along the full 56° length of one of the 5 paths (single segment, figure 5-2a) or 'jumped' laterally to shorter segments of other paths, with either 2, 4 or 6 segments per traversal (28°, 14° and 9° segment length respectively, Figure 5-1A). Each vertical traversal took the same amount of time, regardless of how many segments it was broken into. A set of five such trials was completed during each experimental run. The order in which segments were traversed ensured that each segment in a sequence was separated by at least 6° laterally (twice the path separation) from the last segment travelled, so that directly neighbouring paths were never travelled consecutively (see Fig 5-1A). For each of the four different segment lengths, five such traversals were completed during each stimulus set, such that the entire length of each of the 5 paths was traversed only once. Hence, the complete stimulus set effectively measures the response from the same parts of the receptive field, for each segment length.

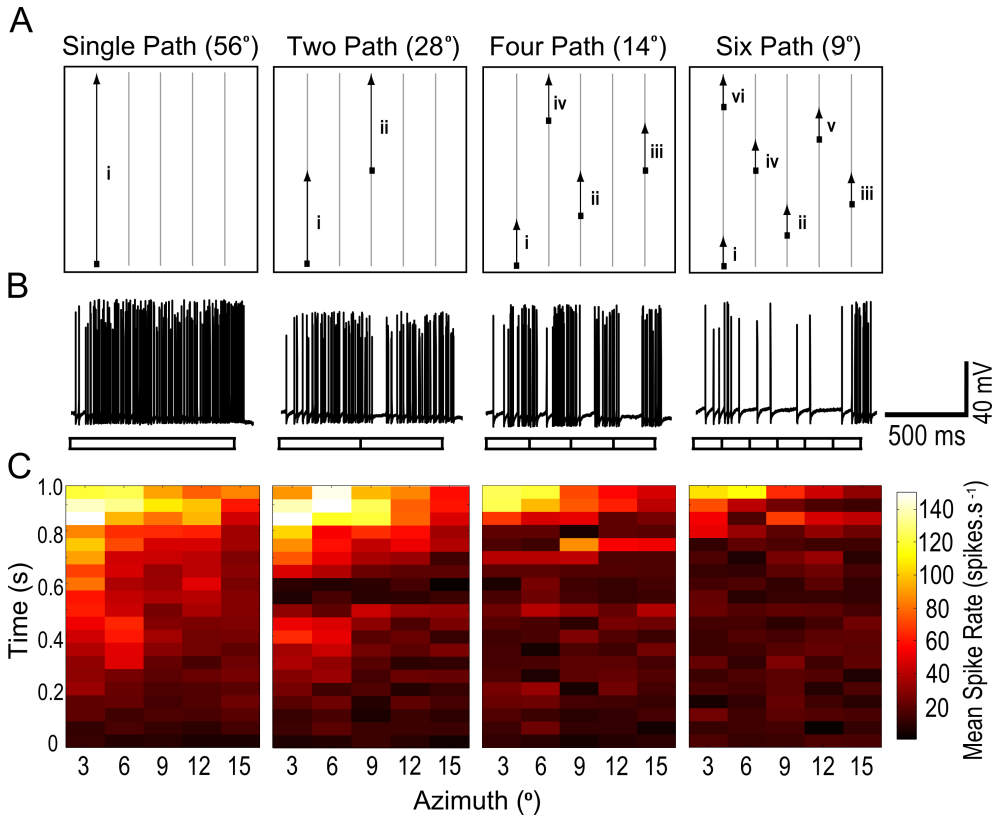

Figure 5-1. (A) A moving target with its path segmented into varying lengths, is presented within a region of interest (ROI) of CSTMD1's receptive field. For the single path variant, the target moves vertically to the top of the ROI. For two paths, the target traverses midway before jumping to a new horizontal location. Similarly, shorter segment lengths result in multiple jumps. A stimulus set is composed of five such traversals, each started from the bottom of the ROI, at varying horizontal locations. Thus for any segment length variant, an individual path is traversed only once and the entire ROI is covered. (B) Raw responses to 1 of the 5 traversals, for each of the 4 segment length variants (56°, 28°, 14° and 9°). The neuronal response to a single continuous path (i) builds to a strongly facilitated state. In the second raw trace (two paths), CSTMD1's response resets at the single spatial discontinuity (i to ii), with responses slowly re-building to their facilitated level. Additional discontinuities reset CSTMD1's response more often thus decreasing activity as segment length decreases. (C) From the five traversals, responses during a segment are excised and concatenated corresponding to the spatial location of the target. These reconstructed receptive fields are then averaged (n=7 neurons). From left to right, as segment length is decreased, the receptive field shows a decrease in neuronal activity across the entire ROI.

5.3.3 Computational Modelling

We simulated a one-dimensional linear array of correlation-type Elementary Motion Detectors (EMDs) in Matlab. Input stimuli were defined as animations of dark targets (nominal luminance 0) moving against a bright background (nominal luminance 1.0) with a temporal sample rate of 1000Hz. Two input sensors (S1 and S2, sensor separation ($\Delta\varphi$) 1°) received a Gaussian blurred ($\Delta\rho$ 1.4°) luminance signal to account for the optics of the ommatidia. This was a wider $\Delta\varphi$ than the minimum separation

measured in the dragonfly acute zone (Horridge, 1978), but it was more representative of the average Δφ across CSTMD1's large receptive field, as traversed by our target stimuli. Each signal arm was convolved with a lognormal temporal filter that mimics the temporal low-pass properties of the insect photoreceptor (T1) (T_p = 10.1ms, σ = 0.197). The output of S1 was then further convolved with a linear first-order delay filter (TD) with a variable time constant. This delayed signal was multiplied with the undelayed signal from S2. This process was repeated in a mirror symmetrical fashion and the two outputs subtracted from each other to give an individual EMD's response. Since discrete features stimulate individual EMDs at different times relative to stimulus onset, model outputs were then summed linearly across an EMD array limited in size (for computational efficiency) to that traversed at the velocity of a given stimulus. Simulations were run at a sample rate of 1KHz and 1000 samples per degree of visual space. For further details of the computational model see Dunbier et al. (2011)

We used a simplex search method to optimize the delay filter time constant of our model to provide the best fit to the observed response onset of CSTMD1 at 56°.s^{-1} averaged across 20 trials in a particularly healthy individual CSTMD1 recording. This time course was normalized to take account of receptive field inhomogeneity using the method described by Nordstrom et al. (2011). Briefly, this involved dividing the binned spike rate (bin size 20ms) for targets commencing motion within the receptive field by the spline-smoothed and fully facilitated response along the same trajectory on a longer path commencing at the screen base (i.e. for a minimum of 500ms before entering the same location within the receptive field).

5.3.4 Velocity Tuning

We determined velocity tuning using a test target (~1.1° square) that commenced upwards motion within the receptive field in the excitatory region just outside receptive field 'hotspot' at one of twelve velocities logarithmically spaced between 5°.s^{-1} and 200°.s^{-1}. These test responses were analysed within a short window (100 ms) commencing 50ms after target onset to account for the absolute latency of the response. To partially account for inhomogenous receptive field shape (given that high velocity stimuli moving over a different range of locations in the same time analysis period) we varied test location across 5 different vertical trajectories (each horizontally separated by 3°) and two test trajectory origins (vertically spaced 5° apart). Test stimuli were either preceded by: (1) an adapting blank screen of mean luminance, allowing us to determine the 'un-facilitated' velocity tuning in a after stimulus onset; or (2) a facilitating stimulus consisting of a relatively low velocity (33°.s^{-1}) target that drifted upwards from the bottom of the screen to the test location before accelerating. This permitted determination of velocity tuning in the facilitated state.

5.4 Results

5.4.1 Response reduction to stimuli on discontinuous paths

Targets drifted along prolonged single trajectories within the receptive field of CSTMD1 elicit a response that slowly builds to a 'facilitated' level (Nordström et al., 2011). Does this facilitation build by successive stimulation of local regions along a continuous trajectory or is it established and maintained by global activity of CSTMD1, irrespective of the locality of the target within the receptive field? If the mechanism of facilitation is global, it ought to transfer to a new location. We tested this by designing a stimulus protocol where targets moved upwards though a sub-region of the receptive field, with 4 different degrees of spatial discontinuity (Figure 5-1). In each case targets moved at 56°.s^{-1}, matched to the velocity optimum observed in our previous experiments

(Geurten et al., 2007). Figure 5-1A illustrates one of the 5 sets of upward scans. Each of the 4 stimulus conditions had an equivalent amount of total motion energy, so varied only in the local distribution of this motion over time. As can be seen from the raw responses (Figure 5-1B), disruption of the path into smaller (shorter duration) segments leads to a strong reduction in the response, despite even the smallest segments still being 7° and thus representing local trajectories that must cross numerous underlying local motion detectors.

The reduction in response for short paths evident in Figure 5-1B is biased by the inhomogeneity of the receptive field, since the longest path illustrated (left) passes through the most sensitive part of the receptive field. However, after repetition of all 5 sets of segmented trajectories, the target has traversed the same total area of this inhomogeneous receptive field. We therefore reconstructed receptive fields (Figure 5-1C) by excising and concatenating the spiking responses that correspond to target traversal of a particular spatial region of the visual field. These receptive fields were then averaged over several neurons (n=7). As observed in Nordström et al. (2011), single segment paths reveal time courses building to a facilitated level over several hundred milliseconds (Figure 5-1C). Following this, CSTMD1's response then reflects the spatial structure of the receptive field, including a 'hotspot' in the upper left corner of the region of interest. However, as target paths are split into smaller and smaller segments (left to right), the reconstructed receptive fields exhibit consistently weaker activity at all locations. Thus local spatial discontinuities cause responses to reset and begin to re-facilitate. The shortest path lengths are still long in comparison to the distribution of photoreceptors, given an interommatidial angle of just 0.5 – 1° within this part of the visual field in *Hemicordulia* (Horridge, 1978). This decrease in activity cannot, therefore, simply be attributed to the interruption of stimuli within the receptive

field of single underlying ESTMDs. We conclude that the facilitated response state does not transfer to a new location and is unlikely to be due to a simple global mechanism.

5.4.2 Quantitative analysis of the influence of path discontinuity

To quantify the reduction in activity due to local discontinuities, we calculated mean spike rate over each of the reconstructed receptive fields for both BSTMD1 and CSTMD1 and plotted this against segment length (Figure 5-2). Data for CTSMD1 (n=7, mean ± SEM) are well fitted by a saturating exponential curve (r^2=0.86) showing that responses are not fully saturated even at a path length of 56°. Hence, targets must traverse many dozens of ommatidia in order to produce a maximal response.

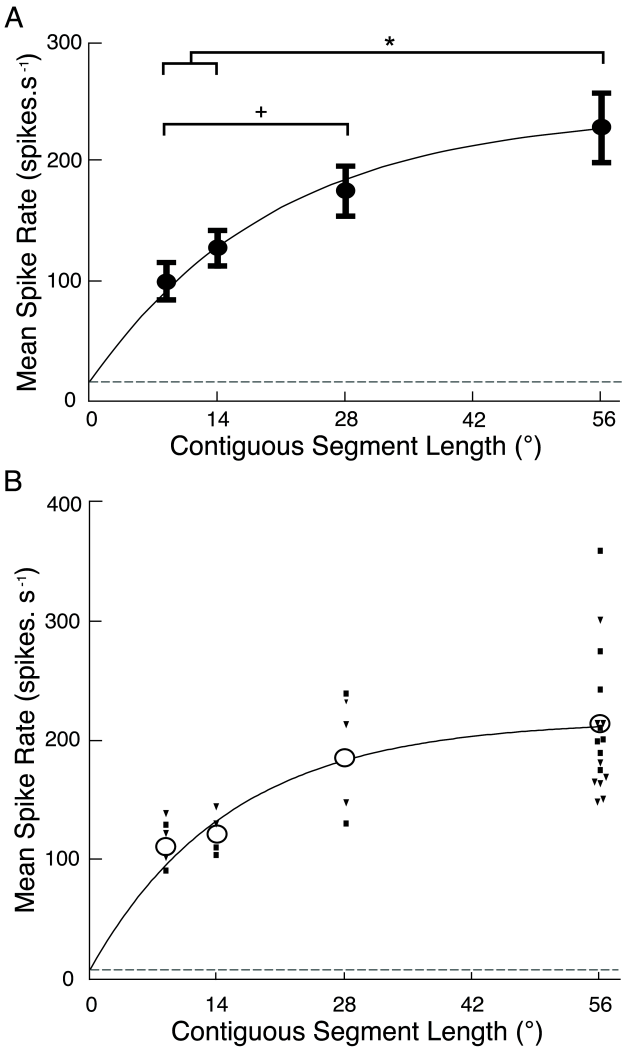

Figure 5-2. (A) CSTMD1 spike rate averaged over each reconstructed ROI (Figure 5-1C) from the segment length variants (mean ± SEM, n=6 neurons). As segment length increases, overall activity increases at a decreasing rate (grey line, exponential curve fit). Though the target crosses many ommatidia at all segment lengths, the 56° single path produces significantly stronger CSTMD1 activity than either the 14° or 9° segment lengths (* P<0.05). Additionally, responses to the 28° two paths is significantly higher than the 9° six paths (+ P<0.05). The dashed line indicates levels of spontaneous activity. (B) Mean BSTMD1 spike rate averaged over the reconstructed ROI for each of the segment length variants (unfilled circles, mean, n= 2). Individual replicates for each cell are represented as filled squares and triangles. Neuronal activity increases as segment length increases, in a similar manner to CSTMD1 (Figure 5-2A).

While our primary aim was to record from CSTMD1, we also recorded several times from a previously unidentified size-selective STMD neuron (1°-2°), which we hereafter refer to as the 'binocular small target motion detector 1', BSTMD1. This provided an opportunity to repeat these experiments and thus test whether local facilitation was unique to CSTMD1 or also seen in other STMDs (and thus likely to be due to a mechanism expressed in the local inputs to these neurons). Because this neuron was previously unidentified, we subsequently characterized its receptive field and reconstructed its morphology following intracellular Lucifer Yellow injection (see *BSTMD1 Physiology and Neuroanatomy*, below). Local facilitation is not unique to CSTMD1, as BSTMD1 produces a similar curve (n=2, Fig 5-2B). Thus, either local facilitation is a property of underlying processing elements common to the two neurons, or is simply a characteristic shared by the larger receptive field STMD neurons.

5.4.3 Does facilitation depend on path length, path duration or velocity?

To test whether facilitation depends on path length (i.e. the number of ommatidia traversed by a target) or the duration of the trajectory, we repeated the discontinuous path experiment at half and double the original velocity (i.e. at 28°.s^{-1} and 112°.s^{-1}). These speeds lie either side of CSTMD1's optimal velocity as suggested by our earlier work (Geurten et al., 2007). The different velocities stimulate the same spatial region with the same path lengths, but for different periods of activation. We found that mean spike rate over each of the reconstructed receptive fields was substantially stronger at 28°.s^{-1} than at the velocity tuning peak of 56°.s^{-1} expected from the Geurten et al. (2007) data (Figure 5-3A). We re-plotted this same data, but now as a function of the time for segment traversal, rather than the segment length (Figure 5-3B). Response dependence on segment duration are similar at both 28°.s^{-1} and 56°.s^{-1} although the initial response (in the first 200ms) would appear to be strongest for 56°.s^{-1}.

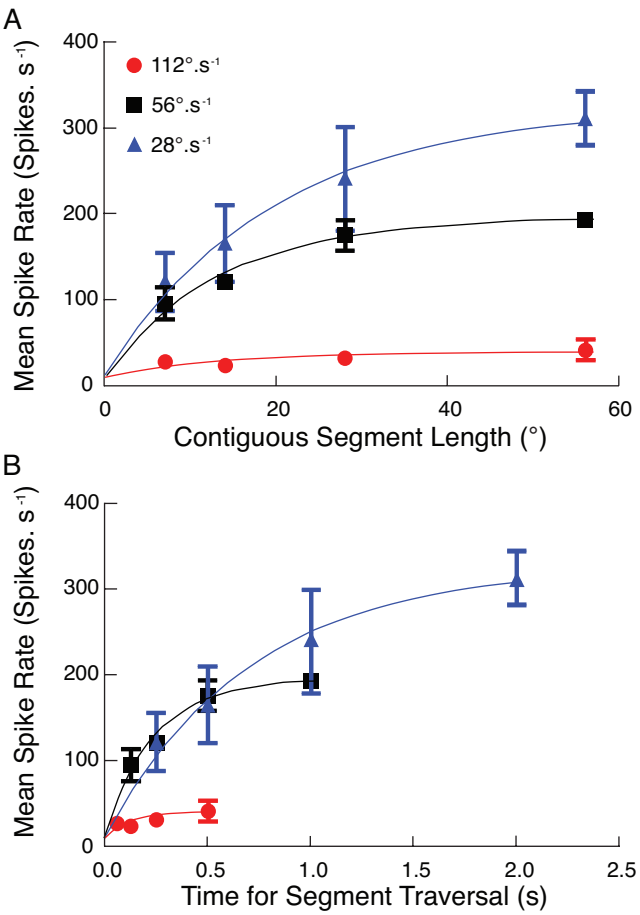

Figure 5-3. (A) The segmented path experiment (Figure 5-2) at target speeds of 56°.s^{-1} is repeated at two additional velocities (28°.s^{-1}, 112°.s^{-1}). At all three velocities, CSTMD1 activity increases as segment length increases, with strongest responses at the slowest speed of 28°.s^{-1} (mean ± SEM, n=6, 3 trials in 2 neurons). (B) As velocity increases, targets traverse the same number of ommatidia but for a shorter time. The effect of this on CSTMD1 activity is revealed by re-plotting data in (A) as a function of segment duration, rather than segment length. For a particular duration, CSTMD1 activity is similar whether the target moves at 28°.s^{-1} or 56°.s^{-1}. For example, a target moving at 28°.s^{-1} over 14° (0.5s) results in the same mean CSTMD1 spike rate as a target moving at 56°.s^{-1} over 28° (0.5s).

The difference in velocity tuning highlighted by our experiments compared with Geurten et al. (2007) illustrates an inherent problem in defining the velocity tuning for a neuron that responds only to discrete features, yet has an inhomogeneous receptive field. Geurten et al. (2007) evaluated their responses in an 'early' time window within a few hundred milliseconds after target motion commenced within the receptive field. This allowed them to estimate responses up to relatively high speeds without the target moving out of the receptive field. However it is clear from our data that such a stimulus does not allow facilitation to fully build for slower moving targets. Our data show that a target travelling at lower speeds over a long path may eventually reach a higher firing rate.

It is possible that our new data are influenced strongly by the inhomogeneity of the receptive field, since they always drift upwards through it (i.e. towards the more sensitive receptive field centre). Hence the raw time course of the response at any

velocity (Figure 5-4A) reflects both the receptive field structure and the underlying response kinetics. To account for this we normalised the second half of the 2-segment paths (Figure 5-1C) by dividing it through by the single path data. The assumption here is that the response is fully facilitated after the first 500ms in the longest path (at 56°.s^{-1}) such that subsequent response modulation is due primarily to the receptive field shape. The resulting time course can then be normalized by its own maximum to allow comparison of the response time course at different velocities, whilst ignoring the underlying spatial inhomogeneity (Figure 5-4B). This analysis reveals a clear dependence of time-course on target speed, with the slowest (28°.s^{-1}) target producing a much slower roll-on in response than at 56°.s^{-1} or 112°.s^{-1}. We should note that the final normalization may underestimate the speed of saturation in the fastest case, because the target never reaches a steady state before it leaves the receptive field (and indeed our stimulus display).

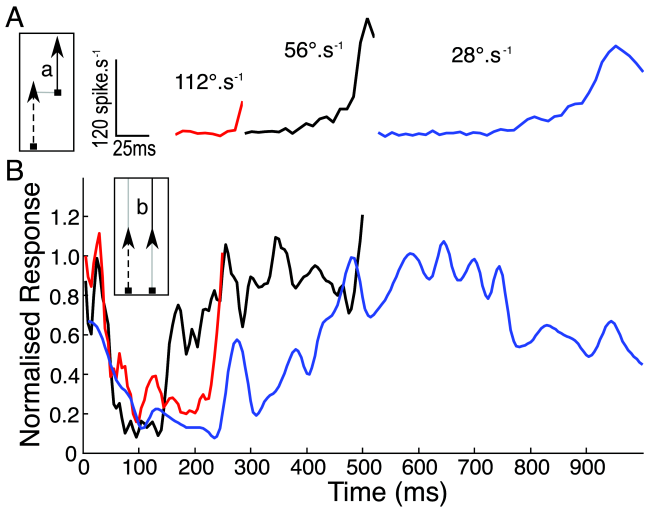

Figure 5-4. (A) Mean CSTMD1 response during the second segment (a) of the 28° two path stimulus, illustrates the onset time course at each of the three velocities (n=30, 3 trials of 5 traversals in 2 neurons). (B) To account for spatial inhomogeneity of the receptive field, time courses in (A) are normalised by response during the second half (b) of the corresponding single path experiment (i.e. in the facilitated state). Normalised responses initially decrease as 'left over' excitation from the first path decays. At 28°.s^{-1}, response builds over hundreds of milliseconds more slowly than the 56°.s^{-1} and 112°.s^{-1} time courses.

5.4.4 Mechanisms underlying slow response roll-on

A simple explanation for the slow response onset at very low target speeds would be a long time constant in any filters either on the inputs to motion detectors or on their outputs. Such sluggish response kinetics might enable responses to accumulate as targets traverse successive local motion detectors. Nordström et al. (2011) discounted

simple sluggish filter kinetics as an explanation for slow facilitation in CSTMD1. They showed that when targets stop within the receptive field, the responses decay back to resting levels in 1/10 of the time that they take to facilitate. However, such asymmetry between response onset and response decay could be explained if it was the delay filter intrinsic to local motion detection itself that had the long time constant (Hassenstein and Reichardt, 1956).

Is it possible to reproduce a slow onset time course (as seen in CSTMD1) with slow delay time constants in primary motion detecting sub-units? To test this hypothesis we created a computational model of a 1-dimensional array of motion detectors based around known properties of the insect eye (Figure 5-5A). We avoided our predictions being confounded by speculation over the complex adaptive filters and additional nonlinearity required to explain the selectivity for small targets (Wiederman et al., 2008) by modelling only elementary motion detectors (EMDs) of the correlation type. Although not an STMD computational model *per se*, we previously showed that responses summed across such EMDs arrays provide a good explanation for velocity tuning to features seen in CSTMD1 and may even explain the dependence of the latter on target dimensions along the direction of travel (Geurten et al., 2007). We used a simplex search method to optimize the delay filter time constant of our model to provide the best fit to the observed response of CSTMD1 at 56°.s^{-1} (redline in Fig 5-5B). We repeated this optimization for a number of different target speeds, from 1°.s^{-1} to 100°.s^{-1}.

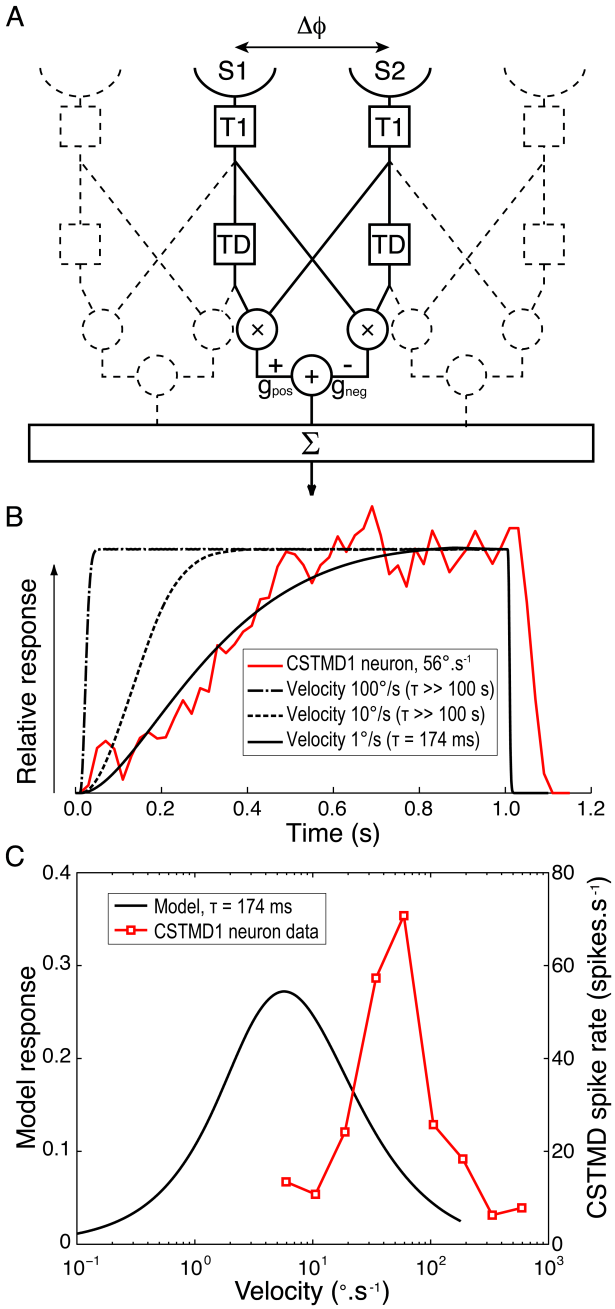

Figure 5-5. (A) A block diagram for our computational model for an EMD array. For each EMD in the array two adjacent sensors (S1 & S2) with separation Δφ receive a luminance signal that is Gaussian blurred to represent the optics of the compound eye. Each signal arm is then convolved with a lognormal temporal filter (T1) that mimics the temporal properties of the insect photoreceptor. For a target moving left to right the output of S1 is then further convolved with an exponential delay filter (TD). The undelayed signal from S2 was multiplied by the delayed signal from the neighbouring arm. This process was repeated in a mirror symmetrical fashion and the two outputs subtracted from each other with equal gain (G) to give an individual EMD's response. Responses were then summed across the array traversed by the target. (B). Spatially normalised CSTMD1 response to a target commencing motion within the receptive field at at 56°.s^{-1} reveals a time course that builds over hundreds of milliseconds (red line). The model EMD array was then fitted to this data with a delay time constant optimized to match this physiological response. An excellent fit (black line) is possible when modelling the response to a low target speed (1 °.s^{-1}) with a long time constant (τ=174ms) for the delay filter (TD). However at faster target velocities (dashed lines) the model cannot fit the data and the model response rolls on too fast for even the largest time constants fitted (>100s) (C) Velocity tuning of the EMD model with a time constant of 174ms (back line) has a peak response at a speed 1/10th that we previously observed from CSTMD1 (red line, Geurten et al., 2007).

At very low speeds (e.g. 1°.s^{-1}) our EMD model can provide an excellent fit to the observed CSTMD1 time course with a long delay constant (τ = 174ms, Figure 5-5B, solid black line). Consistent with the above hypothesis, the EMD response is also strongly asymmetric, showing slow onset and rapid offset once target motion ceases. By even 10°.s^{-1}, however, the model time course is far too short to explain the observed data, even if we increase the delay time constant to the maximum constraint imposed by our optimization (τ >100 seconds). By speeds comparable to those used in the actual

experiment, the model response onset is very rapid, dominated primarily by the kinetics of the early visual processing rather than that of the long time constant delay filter. This is because a target moves into and out of the receptive field of the EMD inputs in a much shorter time than the time constant of the delay filter. It would thus appear that the response of an array of EMDs to targets moving at biologically relevant velocities cannot reproduce the slow onset in CSTMD1s response under any conditions.

Furthermore, given the long time constant delay required to fit the observed responses for even very low speed targets, such a motion detector could not explain the relatively high sensitivity of CSTMD1 to higher speed motion, since it would be tuned to correspondingly slow speeds (Borst and Bahde, 1986). This is illustrated in Figure 5-5C, which compares the velocity tuning (in the steady state) for the EMD model with a slow delay time constant (τ = 174ms) with that of CSTMD1 (redrawn from Geurten et al. 2007). The model velocity tuning peaks at velocities less than 1/10th of those shown to be the best drivers for CSTMD1, either in the earlier work (Geurten et al 2007) or as revealed by our analysis above (Figure 5-4).

5.4.5 Could facilitation boost responses to low velocity features?

From our analysis above, we conclude that the characteristic features of response facilitation cannot be explained by asymmetries in response time course that result from the basic mechanisms involved in local motion detection. But we also nevertheless observe dependence in the response time course on the velocity of target motion. Could this be due to facilitation primarily operating to boost responses to lower velocities over a prolonged time course? To test this we examined the influence of facilitation to a relatively low velocity target on subsequent velocity tuning across a range of target speeds using a modification of the stimulus protocol of Geurten et al (2007). Our stimulus used a test path commencing within the receptive field. In two sets of stimuli this was either preceded by an adapting blank screen of mean luminance, allowing us to

determine the 'un-facilitated' velocity tuning in a short time window after stimulus onset, or by a relatively low velocity (33°.s^{-1}) target that drifted upwards to the same location to allow us to measure the velocity tuning in the same time window but in the facilitated state.

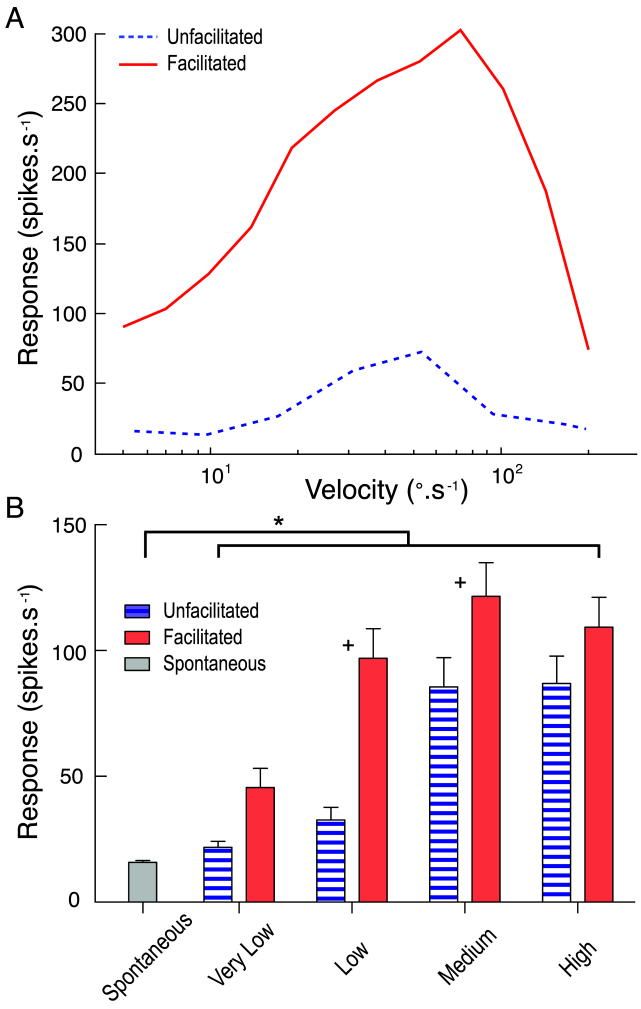

Figure 5-6. (A). The unfacilitated velocity tuning curve (blue dotted line) measures CSTMD1's response to targets commencing movement within the receptive field. The facilitated velocity tuning curve (red line) is measured at the equivalent receptive field location, however follows a facilitating stimulus (target moving at 33°.s^{-1}). Facilitated responses are much stronger across the velocity range. (B) Results pooled by grouping velocity ranges (very low, < 10°.s^{-1}; low, between 10°.s^{-1} and 30°.s^{-1}; medium, between 30°.s^{-1} and 100°.s^{-1}; and high, > 100°.s^{-1}; 10 trials per velocity in 3 neurons) show facilitation has the largest effect at low and medium range velocities (p < 0.05 and < 0.0001 respectively).

The results are shown in Figure 5-6. Following the slow facilitating stimulus, CSTMD1 is much more responsive to targets for a range of subsequent velocities (Figure 5-6A). We should note that due to the confounding influence of receptive field shape (and high velocity stimuli moving over a different range of locations in the same time period) the specific shape of such curves is quite variable, depending on the site selected for the test stimulus (data for a single neuron at 1 location shown in Figure 5-6A). We therefore varied test location across several positions within the receptive field and pooled data from 4 velocity ranges (very low, < 10°.s^{-1}; low, between 10°.s^{-1} and 30°.s^{-1}; medium,

between 30°.s^{-1} and 100°.s^{-1}; and high, > 100°.s^{-1}) across 3 CSTMD1 recordings (Figure 5-6B). The largest and most significant boost to subsequent responses by the facilitating stimulus is for slower target velocities. Indeed, these results show that responses to velocities below the un-facilitated optimum (particularly between 10°.s^{-1} to 30°.s^{-1}) are the most enhanced by facilitation.

5.4.6 BSTMD1 Physiology and Neuroanatomy

The second neuron included in this study, BSTMD1, has not previously been described in the literature. To assist future researchers in identifying their recordings should they encounter similar neurons, we therefore reconstructed its receptive fields in detail, and its morphology following intracellular injection of Lucifer Yellow, using standard methods (as described in Geurten et al., 2007).

BSTMD1 is a compact, multipolar mid-brain intrinsic neuron, with a putative input arborization on the proximal (output) side of the lobula and with several other regions of inputs or outputs in the lateral midbrain (Figure 5-7A,B). BSTMD1 has a pronounced binocular receptive field and gives mixed mode responses, with both action potentials and large graded components (Figure 5-7C-F). Responses are not direction selective. Figure 5-7E, F shows raw responses to targets drifted upwards through the receptive field, a few degrees either side of the frontal midline (along the stimulus tracks shown in Figure 5-7C,D). BSTMD1 spikes in response to targets presented in either visual hemifield, but intriguingly exhibits graded depolarising responses only to stimuli presented in the ipsilateral hemifield (Figure 5-7E,F). Such stimuli elicit large graded depolarisations (up to 10mV), suggesting that our recording site (indicated approximately by the * in figure 5-7A) is very close to the ipsilateral excitatory synaptic input. Contralateral stimuli elicit more biphasic spikes (Figure 5-7F) of larger amplitude. These ride upon a pronounced hyperpolarization at our recording site, suggesting that

the contralateral excitatory inputs are actually more remote (presumably in the dendrites located in the lateral mid-brain) and that action potentials may be initiated at more than 1 location.

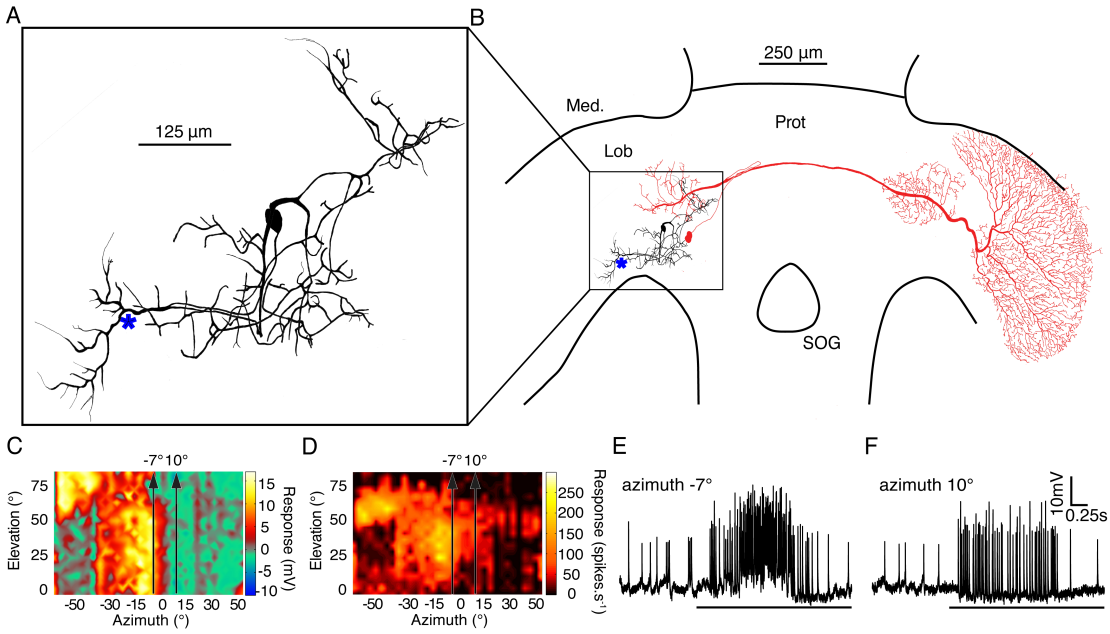

Figure 5-7. (A) A Lucifer Yellow CH fill of the compact mid-brain small target motion detector neuron, BSTMD1. (* indicates approximate recording site) (B) BSTMD1 (black) in context of the dragonfly brain showing a likely input arborisation in the proximal lobula and a second region of inputs/outputs in the lateral midbrain. CSTMD1s (red) inputs are in a similar region of the lateral midbrain. (C) The graded (generator potential) receptive field of BSTMD1 mapped by upward motion of a 1.2° square target. The depolarisation response is monocular and ceases at the midline whereas the contralateral visual hemifield is hyperpolarised. However, the spiking receptive field (D) is prominently binocular, with spikes on both graded responses. (E and F) Example traces from targets moving upward through the receptive field at the two positions indicated by arrows in receptive field plots (C and D) at -7° and 10° azimuth.

The midbrain dendritic region of BSTMD1 corresponds well with the position of the midbrain arborisations of CSTMD1 (illustrated in red in Figure 5-7B). In our earlier work on CSTMD1 (Geurten et al., 2007) we noted that these two mid-brain arborisations are mirror symmetric. Hence it is possible that CSTMD1 is pre-synaptic to BSTMD1 and is responsible for its contralateral excitatory input. It is also possible that BSTMD1 in turn makes bi-directional synaptic contact with CSTMD1 and is responsible for its ipsilateral input. This arrangement is further suggested by the very sharp boundary between the depolarisation and hypolarisation sub-regions of the receptive field (Figure 5-7C), which closely correspond to an equally sharp boundary in the CSTMD1 excitatory

field. This boundary is surprising for either neuron when we consider that at elevations of 40-50° this represents not only the dorsal acute zone (an area of unusually large ommatidial facets) but also to a region of approximately 15° of binocular overlap in the underlying ommatidial input (Horridge 1978). An obvious potential role for such binocularity would be the summation of local motion detector responses to improve reliability for discriminating small features in this highly salient part of the visual world, and this is certainly supported by the characteristic hotspot in the CSTMD1 receptive field (evident at the upper left corner of receptive fields in Figure 5-1C; see also Geurten et al., 2007). The physiology of BSTMD1, however, suggests that ipsilateral excitation only comes from local inputs up to the midline itself. Local motion detectors from the binocular zone of the contralateral eye could still contribute to the sensitive excitatory 'hotspot'. This would, however, require additional local neurons to cross the brain and connect directly with the neurons at the corresponding receptive field location in the contralateral hemisphere of the lobula or brain. Such neurons have yet to be identified.

5.5 Discussion

We have established that the slow build up in response that characterizes facilitation of CSTMD1 (and most likely other higher-order wide-field STMD neurons such as our newly described BSTMD1) is re-set to a 'naive' time course after relatively small lateral displacements (6°) in the target path. This reduces the overall activity of the neuron in response to discontinuous target motion. We further ruled out the possibility that the slow response time course is simply a by-product of a long neural delay in correlation mechanism underlying local motion detection. Together, our findings discount global properties of the neuron, such as axonal integration of its inputs or active conductances within the axon, as potential mechanisms for facilitation. The lack of transfer to new locations for discontinuous motion, combined with our observation that prior

facilitation by targets moving slowly along a prolonged continuous path exert the most potent effect on subsequent stimuli that also move at low velocity (Figure 5-7), suggests that facilitation does not spread instantaneously to new locations within the receptive field, but rather spreads slowly away from the current target location.

The next challenge for future work will be to quantify the extent of spread of facilitation in space, time and direction away from the current location of the target. Full characterisation of these parameters should provide better clues as to the underlying mechanisms and possible pharmacological targets for experimental testing. In approaching these experiments, it is also worth considering the degree to which facilitation is a bottom-up process, i.e. an emergent property of the underlying network of neurons, versus the possibility that it is also recruited by a top-down modulation of stimulus salience. Recent findings have suggested that responses of visual neurons are strongly modulated by the behavioural state of the animal during the recording (Maimon et al., 2010; Chiappe et al., 2010; Jung et al., 2011). It is worth remembering that in our experiments the animal is restrained with wax and subjected to long periods of repetitive stimulation – hardly a natural condition. Certainly we observe a degree of pronounced habitation in response to repeated stimulation of these neurons by identical stimuli (Geurten et al., 2008; Bolzon et al., 2010). We control against this via randomization of experiment order and with long rest breaks between trials. While at this stage it is unrealistic to propose recording from CSTMD1 in unrestrained or tethered flight, it ought to at least be possible to test for similar modulation as shown in other insect visual neurons by exogenous application of neuromodulator agonists. In particular, the Octopamine agonist chlordimeform (CDM) has recently been shown to mimic the effect of free flight in altering the responses of wide-field motion sensitive neurons in flies (Haan et al., 2012; Longden and Krapp, 2010).

Whatever the underlying mechanism, the facilitation we observe must to some degree represent a form of second order motion processing. STMDs respond to relatively high velocities (indicating short neural delays in underlying local motion detectors) and show very sharp tuning to very small features on the scale of single ommatidia. This tells us that the primary motion detectors must be operating on short time scales and at the resolution limits of the eye. Facilitation, on the other hand, is a non-linearity that apparently operates across spans of tens of ommatidia even for optimum speed targets, and over time-courses of hundreds of milliseconds. A possible consequence of such a second-order non-linearity, cascaded with the (already highly nonlinear) operation of local target motion detection (Wiederman et al., 2008; Wiederman and O'Carroll, 2011) would be a potential sensitivity to non-Fourier motion stimuli such as theta motion (Zanker, 1994). While we have not yet subjected CSTMD1 to non-Fourier stimuli, their application to freely flying *Drosophila* reveals sensitivity to second order motion (Theobald et al., 2008). This sensitivity also develops over a prolonged time course (several hundred milliseconds) compared with the response to Fourier motion (Theobald et al., 2008; Lee & Nordström 2012).

While our future work may test these hypotheses and reveal the underlying mechanisms in more detail, the properties that we have revealed to date beg the question as to what role facilitation plays in the behaviour of the animals? The relatively slow build up, in some cases over half a second or more, seems an eternity compared with the minimum response delays observed in dragonflies to target stimuli during prey pursuit, which are only 25-30 ms (Olberg et al., 2000). Given this short latency, it seems unlikely that facilitation is necessary for target detection per se – at least if targets are viewed under ideal conditions (i.e. optimal size, speed and high contrast against the background). One

possibility is that once a target is initially detected by the underlying network of local motion detectors, localized facilitation helps maintain an elevated sensitivity in the neurons adjacent to the most recently 'seen' location, providing a form of robustness against possible future occlusions (e.g. as the target passes in front of a luminance matched feature of the background scene). While flights for pursuit and capture of prey can be very brief in total duration in dragonflies (Olberg et al., 2000; Olberg et al., 2005), we have frequently observed males of *Hemicordulia* and a number of similar perching and hawking dragonfly species engage in prolonged pursuit flights of conspecifics, lasting several seconds or longer. The tight turns so characteristic of insect territorial pursuit flights (Collet and Land, 1975; Collet and Land, 1978) would frequently place their target against complex background texture. Furthermore, while retinal velocities of the background scene would be extremely high (Voss and Zeil, 1998) if such pursuit flights are to be effective, the relative slip speed for the target would be inherently much slower. Hence even a slow mechanism such as we observe could serve a useful role in boosting the relative 'salience' of the current target location, and aid in target re-acquisition following either temporary occlusion by foreground features or loss against the background.

Acknowledgements

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6 Facilitation to continuous target motion causes dynamic changes in apparent receptive field structure.

Context

Having established that facilitation is a local phenomenon; I next wanted to test the spatial extent over which facilitation operates, as well as its persistence and spread over time. This information will play an important role in determining the neuronal processing that produces CSTMD1's facilitated response.

Upon reviewing the draft manuscript of this chapter in early 2013, we elected to hold back the submission of this chapter for publication. We felt that results of this chapter regarding the spatial extent and temporal persistence of facilitation could be combined with concurrent work from the laboratory on selective attention to create a manuscript that could be submitted to a high impact publication. However, this has required waiting for additional data on selective attention to complete that manuscript which will not to be included in this thesis.

Statement of Authorship

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Name of Principal Author (Candidate)	James Dunbier		
Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas.. Conducted all Electrophysiological Experiments. Performed all electrophysiological data analysis. Wrote the paper with editing contributions from the other authors. Made all figures.		
Signature		Date	23/7/2013

Name of Co-Author	Steven Wiederman		
Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Provided significant editing contribution.		
Signature		Date	22/7/2013

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Contribution to the Paper	Participated in the initial conceptualisation and experimental design and refinement and development of ideas. Provided significant editing contribution.		
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Contribution to the Paper			
Signature		Date	

Facilitation to continuous target motion causes dynamic changes in apparent receptive field structure

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6.1 Abstract

The ’small target motion detecting ‘ (STMD) neurons of the dragonfly lobula likely play an essential role in the animal’s aerobatic pursuit behaviours. One of these neurons CSTMD1, exhibits two fascinating properties: facilitation - a slow increase to maximum firing rate over hundreds of milliseconds of continuous target motion - and selective attention. Intracellular recordings from CSTMD1 showed the facilitated area changes over time. The facilitation has a small instantaneous spread away from the target. This facilitation persists for at least 0.5 seconds at the target’s last location following disappearance and the extent of the facilitated region increases during this period. We also present evidence of states (attentional/motivational) in the same neuron and dynamic and reversible changes between such states. These findings suggest that the ‘receptive field’ of insect large field STMD neurons changes with attention state, much like effects noted in primates.

6.2 Introduction

Dragonflies engage in aerobatically complex conspecific pursuits that can last tens of seconds (Corbet, 1999). Prey pursuits by Libellulid dragonflies, however, take a second or less even in the presence of potential distracters and are successful 97% of the time (Olberg et al., 2000; Olberg et al., 2007). This ability of dragonflies to detect and track small moving targets within a visual scene is a complex task that requires sophisticated adaptations of anatomy, behaviour and neural mechanisms for target analysis (Collett and Land, 1978; Frye and Dickinson, 2007; Land, 1993; Land and Collett, 1974; Olberg et al., 2007; Wehrhahn et al., 1982; Zeil, 1973).

Optic lobe interneurons that respond specifically to small targets, the small target motion detectors (STMDs), have been characterised in both dragonflies (O'Carroll, 1993; Geurten et al., 2007) and hoverflies (Nordström et al., 2006; Barnett et al., 2007; Nordström and O'Carroll, 2009). These STMD neurons display an impressive selectivity for small moving objects. STMDs in the hoverfly, *Eristalis tenax*, respond robustly to very small targets of 0.18° with a neural contrast of <2% (Nordström et al., 2006; Nordström and O'Carroll, 2009). How does the system give such robust responses to sub-optimal targets?

The firing response of large field STMDs – including the centrifugal STMD 1 (CSTMD1) - has a short initial latency but builds to its maximum level over hundreds of milliseconds of continuous target motion, which we describe as response facilitation to continuous motion (Nordström et al., 2011; Dunbier et al., 2012). CSTMD1 has recently been found to exhibit the first neuronal correlate of selective visual attention in an invertebrate (Wiederman and O'Carroll, 2013). The neuron has been recorded responding selectively to the motion of one of two targets moving simultaneously within the receptive field of the neuron as if the other were not present.

Given these findings, what is the function of this facilitation? The discovery of selective attention supports a hypothesis raised in the discussion of Dunbier et al. (2012), that this facilitation helps maintain an elevated sensitivity in elementary motion detecting elements whose receptive fields are proximal to the most recently 'seen' location of the target. This could increase the chance of reacquiring a target lost due to occlusion or some other disruption. This is an example of a dynamic saliency map (Ohayon et al., 2008; Einhäuser & Koènig, 2003; Parkhurst and Niebur, 2003) that would be useful during both short distance prey pursuits and the longer more complicated conspecific interactions (Corbet, 1999; Olberg et al., 2007).

We have proposed that this facilitation could be explained by a second order - or higher - motion detection mechanism, integrating spatially adjacent sampling areas (Nordstrom et al., 2011; Dunbier et al., 2011; Dunbier et al., 2012). Such a system would be a boon, as it would reject non-target noise in primary motion detector outputs. This would also provide sensitivity to second order motion. Evidence for sensitivity to non-Fourier motion has been observed in behavioural studies in *Drosophila* (Theobald et al., 2008).

In this paper we test the properties of the facilitation observed in CSTMD1. We probe the spatial and temporal persistence of the facilitation state and how this modulates the classical representation of a STMD receptive field, by using combinations of spatially separated and temporally separated facilitating paths and their effect on the response time course evoked by a target travelling a test path. Our results strongly support a higher-order integration mechanism that works on a scale that suggests integration may occur over the retinotopically arranged small field STMDs (Barnett et al., 2007).

6.3 Results

6.3.1 CSTMD1's Classical Receptive Field

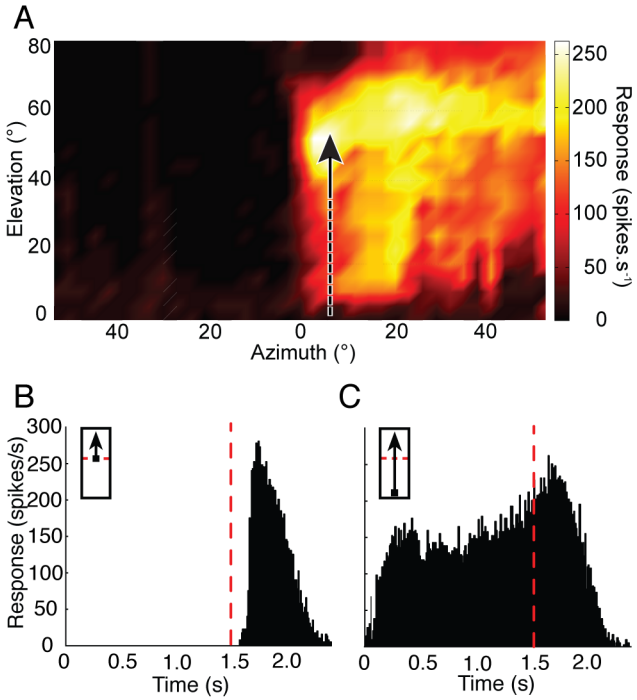

Figure 6-1. The spiking receptive field of CSTMD1 (A) mapped by upward motion of a 1.2° square target. The spiking response is monocular and ceases at the midline whereas target motion in the contralateral visual hemi-field suppresses the spontaneous spiking response. An arrow represents the central path of nine trial paths. The entire path length describes the 'long' condition/control. The dashed portion represents the trajectory of the 'short' condition/control. (B) The mean spiking response over the 'short' condition path. Expressed as spike rate (spikes/s) in 10ms bins. The red line indicates the onset of target motion. (C) The mean spiking response to target traversal of the entire screen. The red line indicates the equivalent screen position to the start of target motion in the 'short' condition path.

CSTMD1 responds robustly to targets significantly smaller than its size-tuning optimum (Geurten et al., 2008). We routinely define the receptive field of STMD neurons by continuous motion along long paths (the height and width of our display) and associate the activity at a given time with the target's position on the screen (Figure 6-1A). Note the distinct narrow black bar at the base of the receptive field (approx. elevation 0-3° above visual horizon). This is not a property of the receptive field itself but rather the neuron's response dynamics, facilitating gradually to targets commencing motion at the bottom of the screen. This is also true when targets commence motion higher on the display (Nordström et al., 2011). A target commencing motion in the upper third of the display generates the response histogram seen in Figure 6-1B. Compare this to the response histogram for a traversal of the entire height of the display. It takes more than 100ms of target motion before response levels reach those achieved during continuous target motion.

6.3.2 CSTMD1's short response latency and slow facilitation

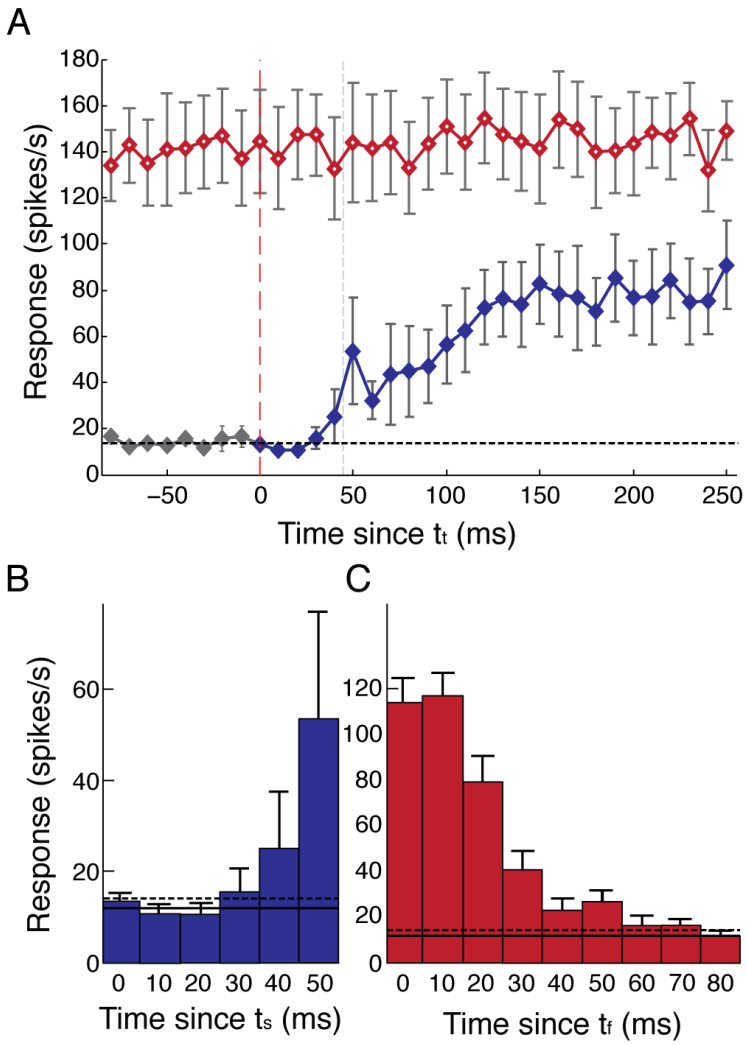

Figure 6-2. The mean response onset time course of CSTMD1 (± standard deviation n =8, blue diamonds, pre-stimulus period = grey diamonds) (A) generated by dividing the mean spiking response to target motion over the 'short' control path in each (like that in Figure 6-1B) by the response to target motion over the same path but preceded by at least a second of motion (like that in Figure 6-1C). Compared to the mean response level of CSTMD1 to target motion in the same region preceded by a second of facilitating motion (± standard deviation, n =8, red diamonds). Vertical red dashed line marks the start of target motion in the unfacilitated trials, or the beginning of traversal of the equivalent spatial region in the facilitated trials. Light grey dotted line marks the absolute response latency. Horizontal dotted line is the upper 95% confidence interval of the spontaneous spike rate. The absolute mean response onset latency ± standard deviation (B) the dotted line is the upper 95% confidence interval of the spontaneous spike rate and the solid line is the mean spontaneous spike rate. The absolute mean response offset latency ± standard deviation (C) the dotted line is the upper 95% confidence interval of the spontaneous spike rate and the solid line is the mean spontaneous spike rate.

We calculated the mean onset time-course for CSTMD1 by comparing two responses. The 'short' condition is the response to a target commencing motion in the middle of the receptive field and travelling 20° (~1.2° square target at 38°.s^{-1}). This 'short' condition is divided by the response in the same region preceded by a second of continuous target motion (the 'long' condition). We calculated the mean response onset time course for 8 healthy neurons, positively identified as CSTMD1 (Figure 6-2A). 250ms following the onset of target motion during the 'short' condition', response is not yet at levels seen in the 'long' condition. This result is consistent with previous findings of the facilitation time course (Nordstrom et al., 2011; Dunbier et al., 2011; Dunbier et al., 2012). We also quantified the absolute onset response, and decay response. Unlike the long time course of facilitation, it takes only 40ms for mean response level to be above the upper 95% confidence level for spontaneous activity (Figure 6-2B). Further, when target motion ceases in the receptive field the response decays to spontaneous rate with a similar time course (Figure 6-2C). A short time course in this range was predicted in modelling of CSTMD1's underlying motion detection before the identification of the facilitation (Geurten et al., 2007).

6.3.3 Facilitation and Discontinuities

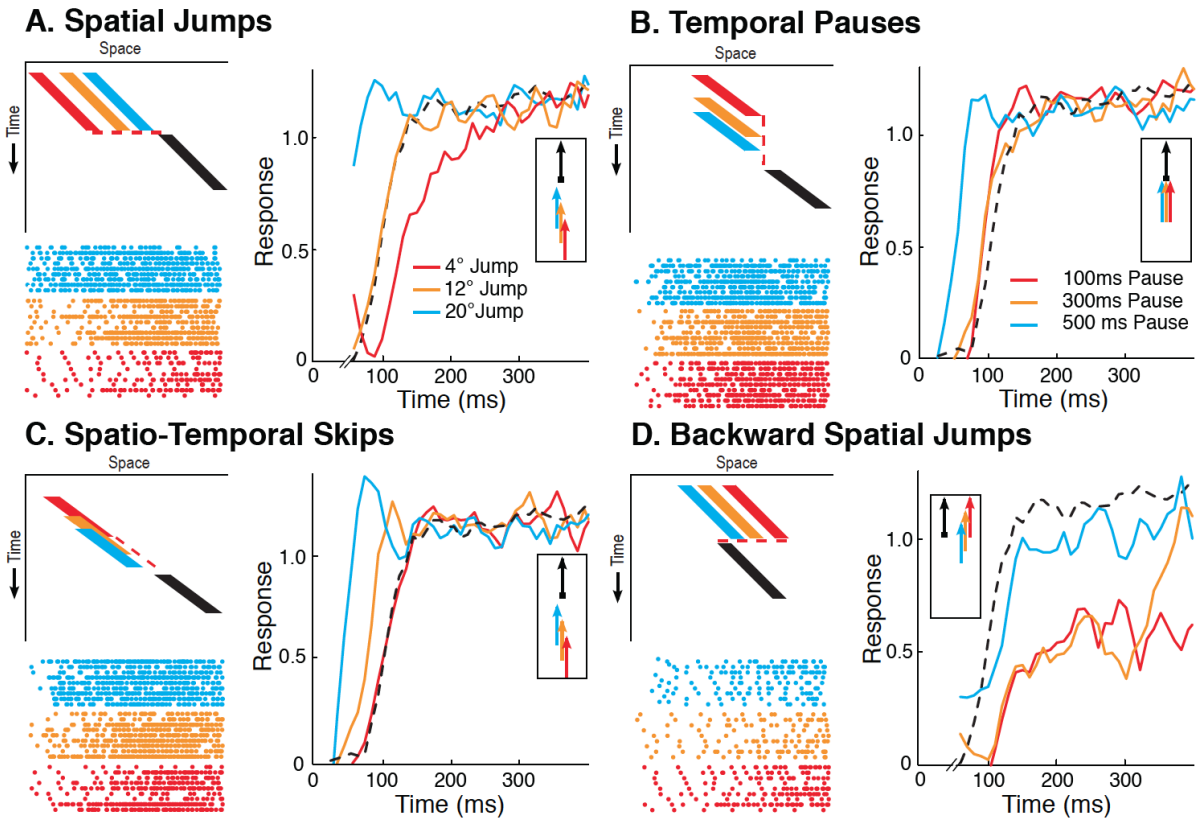

Figure 6-3. Summary of the discontinuities and the effect on response. A) The instantaneous jumps in the direction of travel or 'forward jumps'. Space-time plot indicating the 3 sizes of 'jump' (Red = 'long jump' 20°; Orange = 'medium jump' 12°; Blue = 'small jump' 4°). Nine spike rasters for each condition from an example neuron. Mean onset time courses in the same example neuron. B) The temporal pause during travel or 'temporal pauses'. Space-time plot indicating the 3 sizes of 'pause' (Red = 'long pause' 500ms; Orange = 'medium pause' 300ms; Blue = 'small pause' 100ms). Nine spike rasters for each condition from an example neuron. Mean onset time courses in the same neuron. C) The 'forward jumps' matched with 'temporal pauses' to produce 'spatio-temporal skips'. Space-time plot indicating the sizes and durations of the 'skips' (Red = 'long skip' 20° and 500ms; Orange = 'medium skip' 12° and 300ms; Blue = 'small skip' 4° and 100ms). Nine spike rasters for each condition from an example neuron. Mean onset time courses following skips in the same neuron. D) The instantaneous jumps in the opposite direction to travel or 'backward jumps'. Space-time plot indicating the 3 sizes of 'jump' (Red = 'long jump' 20°; Orange = 'medium jump' 12°; Blue = 'small jump' 4°). Nine spike rasters for each condition from an example neuron. Mean onset time courses in the same example neuron.

We have established that CSTMD1 facilitates to continuous target motion (Nordström et al., 2011; Dunbier et al., 2012). We have also shown that a horizontal displacement during otherwise continuous target motion resets the facilitation state (Dunbier et al., 2012). What is the spatial extent of this facilitation in the direction of travel and how long does it persist in time? We tested this by analysing the neuron's response to target motion in a fixed test region (identical to the 'short' condition outlined above). Motion in

this test region was preceded by facilitating target motion that was either: spatially separated from the start of the test region; directly spatially preceding the test path but separated by a period of time with no target; matched combinations of the two preceding conditions; or targets that displaced backwards, against the direction of facilitating motion. These responses were all compared against the 'short' condition mentioned in the experiments described in Figures 6-1 and 6-2 (Figure 6-4).

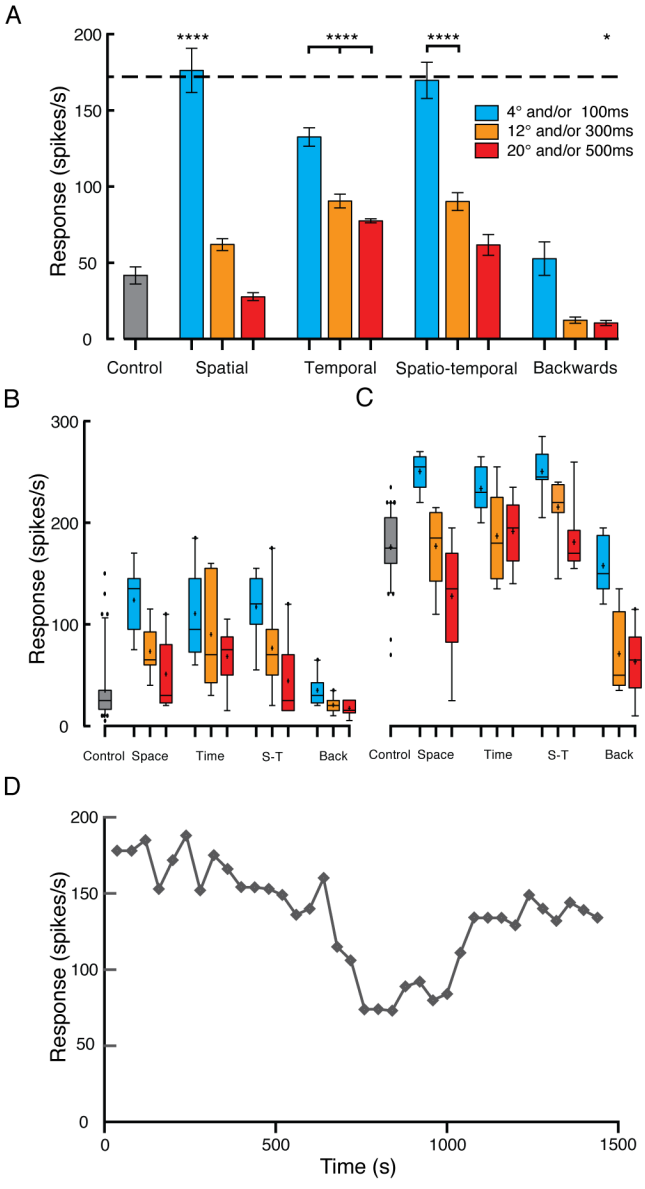

Figure 6-4. Mean response rate in a window 30ms-230ms post-discontinuity. A) Changes in this response illustrate the effect of facilitation on the early period of response following a discontinuity. This is considered relative to two points 1) the same period of activity in a target commencing motion preceded by no motion (control, grey bar) and 2) the upper 95% confidence interval of response in the same region when preceded by a second of continuous target motion (dashed black line). * = $p < 0.05$ compared to control & **** = $p < 0.0001$ compared to control following a Dunnet's multiple comparison test. B) and C) show the distribution of values from individual trials from two of the neurons. Note the different overall response levels in the analysis window. D) An example of reversible state change seen in a neuron, measuring neuronal response during the long path condition in a window from 1030ms to 1430ms plotted at the time the scan took place relative to the start of the experimental protocol. Between 600s and 1200s the spike rate to an identical target is reduced with no change in spike size and no significant change in spontaneous spike rate before returning to a comparable level after 1200s.

6.3.4 Instantaneous Forward Jumps

We tested instantaneous displacements in the direction of travel to establish the spatial scale over which facilitation works natively (small - 4°, medium - 12° or large - 20°, Figure 6-3A). The small jump, 4°, passes over several (6-10) ommatidia in the region of the eye sampled, so any first-order motion processing requiring successive neighbour or next nearest neighbour activation would be unable to generate a motion signal, yet the latency of response is essentially zero. Compare this with the measured onset and offset latency quantified in Figure 6-2B and C. Despite having motion continuously in the neuron's receptive field both the medium and large spatial discontinuities provoke a return to spontaneous or lower firing rates followed by a return to maximal firing rates (Figure 6-4A).

6.3.5 Temporal Pauses

We tested the persistence of the facilitated state at the last seen location of the target (small - 100ms, medium - 300ms and large - 500ms, Figure 6-3B). All the temporal conditions return to facilitated response levels before the control condition. Facilitation persists at the last seen location of the target for at least 500ms if no other competing stimuli are presented. This rapid onset time course follows the neuron's return to spontaneous response levels during even the 100ms pause (Figure 6-4A).

6.3.6 Spatio-temporal Skips

Does the spatial extent of facilitation change over time? We tested combinations of the previous two stimuli to establish whether facilitation moves away from the last seen location of the target (small - 100ms and 4°, medium - 300ms and 12°, large - 500ms and 20°, Figure 6-3C). We found that regions which when tested immediately after a facilitating target ('the forward jumps') showed no significant facilitation were now significantly facilitated after a pause (Figure 6-4A).

6.3.7 Instantaneous Backward Jumps

Finally, we tested 'backwards jumps'. These were instantaneous spatial displacements against the direction of facilitating motion (small - 4°, medium - 12° or large - 20°) (Figure 6-3D). These facilitating paths travelled a portion of the test path before the test target appears. The shortest backward jump is not significantly different from control. However, the two larger 'backward jumps' provoke inhibition. Facilitation is maintained at the last seen target location but the facilitating path itself is inhibited (Figure 6-4A).

We calculated the mean spiking response rate in an early window following the discontinuity (30-230ms post- discontinuity). Following a 1-way ANOVA we performed a Dunnet's multiple comparison test against the unfacilitated control response in the same window (Figure 6-4A).

6.3.8 Variability of response within and between neurons

CSTMD1s display some variability in this onset time course. In Figure 6-4B and C we present two examples of CSTMD1s. Neuron 1 (Figure 6-4B) is in a slower rising state, note the low firing activity seen in the control. Whereas, Neuron 2 (Figure 6-4C) has a higher firing rate in the control but this does not dramatically change the effect of discontinuities. The most notable difference in these faster activating neurons is the extent of inhibition to the larger 'backward jumps', and an emergent inhibition to the large 'forward spatial jump'. We also include an example of CSTMD1 switching these 'states' dynamically. Figure 6-4D shows the spike rate in a 200ms window during the 'long condition' paths. These were regularly interspersed during the experimental sequence (for details on stimulus structure and length see Section 6.5). This trace shows a reversible downward modulation of neuronal response. We determined that it was not pathological because this was not accompanied by any change in spike size or shape or significant modulation in spontaneous rate before, during or after the modulation.

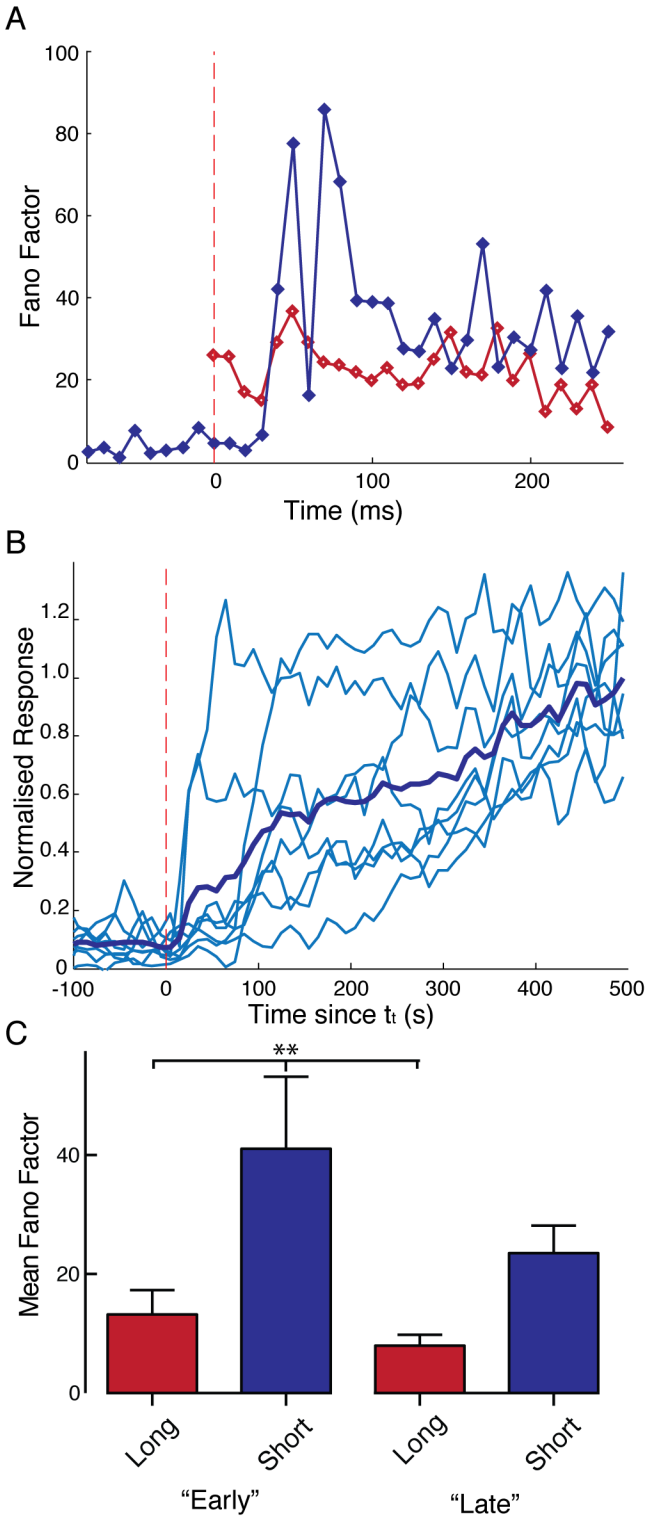

Figure 6-5. Fano factor of the mean response onset time course (A) (blue line) and Fano factor of the 'long' control path over the equivalent region (red line). The dashed red line indicates the onset of target motion. The normalised mean onset time courses of the eight cells (B) plotted as light blue lines plotted behind the mean response onset (dark blue line). The eight cell mean Fano factor ± SEM in two 200ms windows (C) (Early window = 30ms - 230ms in the 'short' condition (blue) & 1030ms – 1230ms in the 'long' condition (red); Late window = 300 – 500ms in the 'short' condition & 1300 – 1500ms in the 'long' condition; * = p < 0.05 compared to 'short' early window)

We found it unusual in Figure 6-5A that the standard deviation of the spike rate in any given 10ms period does not increase proportionally to the spike rate (Tolhurst et al.,1981; Warzecaha and Egelhaaf ,1999). We confirmed this by calculating the Fano Factor, the variance of spike rate as a proportion of the mean firing rate. We did this for both the 'early' window (30-230ms) and the 'late' window (300-500ms) (Figure 6-5A). Apart from a noticeable peak coincident with the absolute latency of the motion response, the Fano Factor plateaus despite an increasing mean spiking response throughout both time periods.

However, this dataset consists of 8 neurons. Could this early peak in mean Fano Factor be due to differences between cells? All 8 cells were positively identified as CSTMD1 by receptive field shape, size, position, and spike size and shape. Plotting each individual cell's mean time course we notice two 'modes' of CSTMD1 onset response (Figure 6-5B). 2 cells show a noticeably faster rise to peak firing rates within 150ms of the target commencing motion, whereas others display a more typical gradual increase over several hundred milliseconds to fully facilitated response levels.

We quantified Fano factor within cells to rule out this source of variance. We calculated the Fano Factor in an 'early' window (30-230ms) and a 'late' window (300-500ms) in each of the 36 'short' condition trials in each cell, and compared that to the Fano Factor in the period when the target was travelling the equivalent region in the 36 'long' condition trials i.e. 1030-1230ms and 1300ms-1500ms. The Fano Factor of the early window of the 'short' condition was significantly different from the Fano Factor in both time windows for the 'long' condition. This rules out CSMTD1's modal response as a source of early variance.

6.3.9 Dynamic Receptive Field Modulation

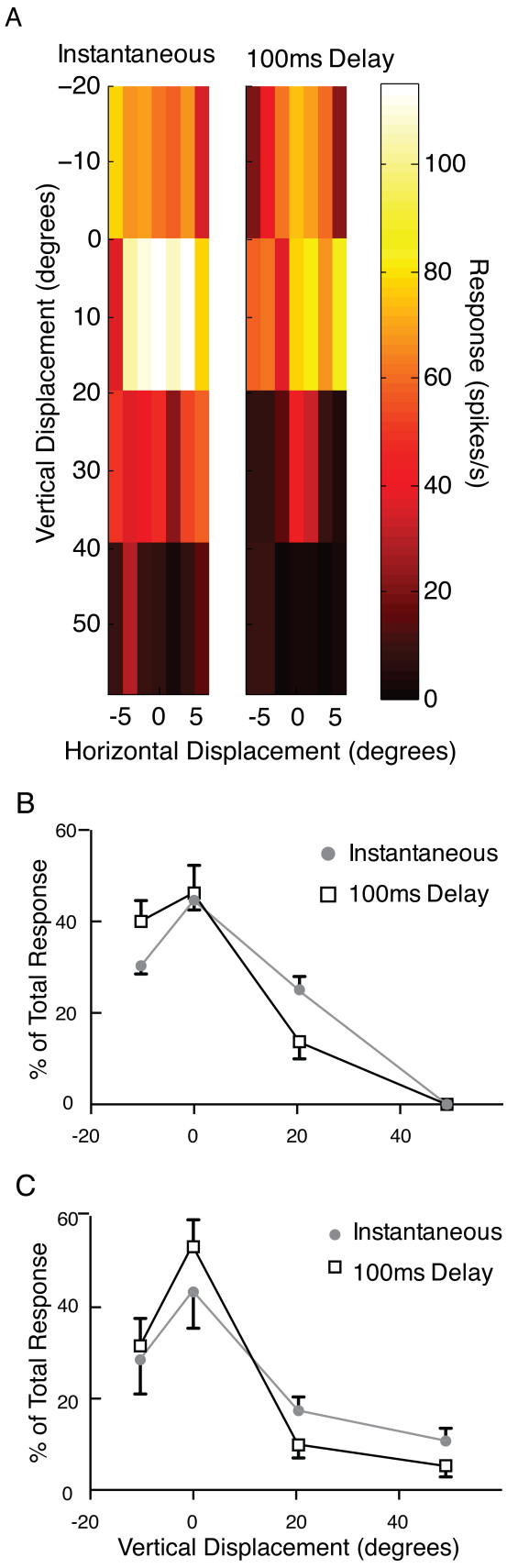

Figure 6-6. The 2-dimensional structure of the facilitated state in two neurons. A) We tested vertical displacements similar to those outlined in Figures 6-3 and 6-4 except they were combined with horizontal displacements to try and ascertain the 2-dimensional structure of the receptive field. In addition to instantaneous jumps (left panel),we tested a fixed temporal delay of 100ms (right panel). Jumps behind the facilitating target, the equivalent of the earlier backward jumps, were further inhibited following a 100ms pause. B) and C) are summaries of the pooled horizontal displacements showing the dynamic change in receptive field sensitivity following the 100ms pause.

Finally we tested whether facilitation spreads perpendicular to the direction of facilitating motion. This involved a similar protocol to that used in the single path facilitation experiments, except in this case some of the facilitators were also horizontally displaced from the test path. We tested this with instantaneous displacements (Figure 6-6A, left panel) and those with a delay of 100ms between the end of the facilitating trajectory and the beginning of the test (Figure 6-6A, right panel). We tested a relatively narrow horizontal extent due to our previous findings (Dunbier et al., 2012) that a 7° horizontal displacement was sufficient to prompt a return to spontaneous firing rates. Narrowing of the receptive field was evident following the 100ms delay. Following the 100ms delay the suppression of response behind the facilitator is exaggerated while response ahead of the facilitator's position is enhanced.

6.4 Discussion

CSTMD1 is a high order neuron in the dragonfly brain that displays several fascinating properties. We found that CSTMD1's facilitated state instantaneously spreads a short distance away from the last seen location of a target. This facilitated state persists at the target location for at least 500ms. During this 500ms, distant regions of the receptive field (up to 20° away) begin to demonstrate facilitation. This spread away from the last seen location of the target is not symmetrical; motion along the path of the facilitating target is inhibited. Facilitation enhances response to the facilitating target. However, if the motion of the facilitating target is disrupted, responses to targets travelling along its likely trajectory will reach maximal response faster.

It was recently found that CSTMD1 displays selective attention when two targets are presented simultaneously (Wiederman and O'Carroll, 2013). Facilitation potentially plays an important role in this competitive selection. Wiederman and O'Carroll (2013) observed that the attended target changes between trials or even during individual

trials. The degree to which each target facilitates or whether one target commences motion within a facilitated region may prove to be key factors in determining the attended target.

Recent findings have suggested that responses of visual neurons are strongly modulated by the behavioural state of the animal during the recording (Maimon et al., 2010; Chiappe et al., 2010; Jung et al., 2011). This is a potential explanation of our observations of variability in CSTMD1's response. We observed variability in the response onset time course of individual CSTMD1s (Figure 6-5B) and a reversible modulation during a recording where response to identical stimuli is reduced for ~10 minutes and then returns to its previous level (Figure 6-4D). These were all healthy, responsive neurons yet they exhibited differences in response onset. It is worth remembering that in our experiments the animal is restrained with wax – an unnatural condition - that prevents changes of behavior. This suggests that modulations to neuronal response need not accompany behaviour but may also be associated with motivational state.

Whatever the underlying mechanism, this facilitation must represent a form of second order motion processing. The velocity tuning and size selectivity of STMDs tell us that primary motion detectors operate on short time scales and at the resolution limits of the eye. Our results show that facilitation operates across tens of ommatidia and hundreds of milliseconds. Given the spatial spread of facilitation it now seems likely that any second order motion detector likely integrates the signal of lobula STMD neurons with smaller receptive fields, the small field STMD neurons (Barnett et al., 2007; O'Carroll, 1993). Another potential consequence of second order motion processing is the rejection of noise (as this would not correlate in space and time). CSTMD1 is unlike previously examined sensory neurons where Fano Factor increases as firing rate increases

These findings suggest that the dragonfly displays a dynamically shifting centre of salience, and possibly attention, that is dependent on previous stimulation. This shows invertebrates have similar attentional, motivational and behavioural modulation to that observed in humans and primates (Vuilleumier and Driver, 2007; Moratti et al., 2003)

6.5 Methods

Electrophysiological Methods

Experiments were carried out on 10 male, wild-caught dragonflies (*Hemicordulia tau*). The dragonflies were immobilized with a wax-rosin (1:1) mixture, and the head was tilted forward to gain access to the posterior head surface. A small hole was cut over the left lobula. Neurons were recorded intracellularly using aluminium silicate micropipettes pulled on a Sutter Instruments P-97 puller and filled with 2M KCl. Electrodes typically had a tip resistance between 60 and 110 MΩ. We identified CSTMD1 by its characteristic large, biphasic action potentials and distinctive receptive field shape in the frontal dorsal visual field, mapped with a drifting target stimulus as described by Geurten et al. (2007). Visual stimuli were presented to the animals on a high resolution LCD computer monitor (Alienware) at 120Hz frame rate, using Matlab's Psychophysics Toolbox (pyschtoolbox.org). The animal was placed on an adjustable stand and aligned at a fixed distance from the display, using a calibration frame fitted to the front of the display. The small access hole allowed visualization of surface landmarks on the brain only over a very limited range of angles, such that individual dragonflies were always oriented in similar positions, with the frontal midline corresponding to the horizontal centre of the monitor lower edge, such that the screen centre was approximately 40° above the horizon. Small individual differences in elevation alignment were further accounted for by measuring the angle of inclination of the dragonfly's head relative to the vertical. Azimuth alignment with the mid-point on the screen was subsequently confirmed by scanning the receptive field with horizontally drifted targets, since the CSTMD1 receptive field cuts off sharply at the frontal midline (see Geurten et al., 2007 & Figure 6-1 of Bolzon et al., 2009). The display subtended approximately 110° x 82° (width by height) at the animal's eye, with a resolution of 1920 x 1080 pixels (corresponding to 12 pixels/° at the screen centre) and a background luminance of 300

Cd.m^{-2}. Data were digitized at 5 kHz using a 16-bit A/D converter (National Instruments, Austin, TX, USA) and analysed off-line with MATLAB (www.mathworks.com).

Stimuli

Discontinuities

We defined a stimulus region of each CSTMD1's receptive field, spanning the monitor's height and just inside the medial boundary corresponding to the midline separating the visual hemifields (0° azimuth). A small target (~1.1° square) drifted upwards from the bottom of the trial region to the top along one of nine vertical paths (spaced at 3° intervals) these traversals were used to determine the facilitated response level of the neuron. A section of the stimulus region was defined as the test region. This was defined such that at least 500ms of target motion would occur before the target reached the centre of the receptive field hotspot (a sub-region of the receptive field with the highest spiking response to targets) and all facilitating paths would have their full 500ms of target motion on the screen. The response to a naïve traversal of this test region (Figure 6-1B) was divided by the facilitated response over the same region (Figure 6-1C) to give the onset time course. In the test conditions, traversal of the test region was preceded by one of twelve different facilitating paths. These were divided into 4 categories each with 3 magnitudes (Figure 6-3): instantaneous jumps in the direction of target motion (zero latency; spatial displacements of 20°, 12° and 4°); pauses, where the target disappears for a period of time at the end of the facilitating path and then recommences motion from the point of disappearance which is also the start of the test path (latencies of 100ms, 300ms and 500ms); spatio-temporal skips, matched combinations of the previous two conditions to produce the effect of the target passing behind an opaque occlusion before the test region (latency 100ms paired with 4° displacement, 300ms and 12° and 500ms paired with 20°); finally, instantaneous jumps against the direction of target motion (zero latency; spatial displacements of -4°, -12° and -20°). These

facilitated tests were compared with traversal of the test region that was never directly preceded by target motion. These were also interspersed with full traversals of the 9 test paths to serve as a normaliser and an index of overall activity to determine the presence and extent of habituation/adaptation in the ROI.

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7 Conclusions

7.1 Facilitation and its potential roles

Over the course of this thesis project the understanding of CSTMD1's function has increased through both my work and my supervisors' related research. The results presented here include CSTMD1's response to multiple objects presented in the inhibitory and excitatory regions of its complex receptive field (Chapter 3). These results suggest an underlying logic useful in the pursuit of a single target that would be unstable in multiple feature environments. However, this conclusion was drawn prior to the discovery of selective attention in the neuron (Wiederman and O'Carroll, 2013).

I confirmed that the slow response onset first described in Nordstrom et al. (2011) is definitely not caused by slow response kinetics but, rather, facilitation to continuous target motion within the receptive fields of large field STMD neurons (Chapter 4). With this confirmed I went on to determine that this facilitation does require locally continuous motion and that the enhancement of response due to facilitation was larger for slower moving targets (Chapter 5). This preferential enhancement of response to slow moving targets could functionally enhance responses to those features travelling within a range of biologically relevant velocities. However, a significant factor that determines the upper limit of the velocity tuning of an object detector is determined by how long a discrete object remains within its receptive field. Therefore, it would be of little use to enhance the most highly transient signals.

The instantaneous spatial extent of this facilitation is limited but perhaps my most exciting finding is that given time the facilitation will spread away from the last seen location of the target. This means locations that are unfacilitated - or in some neurons inhibited - immediately following a target's presentation demonstrate facilitation (Chapter 6). But what are the potential roles this facilitation play in the dragonfly brain?

7.1.1 Attention and Salience

Wiederman and O'Carroll (2013) made a compelling case that CSTMD1 reflects competitive selection of one target. Competition is key because the target attended changes between trials or even within individual trials. They also suggest that when the response to two targets lags behind the response to either of the two individual targets is suggestive of the underlying conflict in the neural network to determine the attended target. They posit that the variability they observed in the attended target strongly implies either modulation of the underlying salience of targets over trials (the facilitation being an interesting possibility for this role) or a higher-order mechanism of bias (Desimone, 1998). This kind of accurate encoding of an attended target independent of distracters would play a valuable role in a control system for target pursuit. This independence from distracters would enable tracking of individual targets amidst swarms, without changing the gain in the control loop already in existence for the simpler instance of tracking a sole salient target.

Despite these advances in knowledge we lack direct evidence for where CSTMD1 sits within such a target pursuit control system or the underlying hierarchy of mechanisms of competitive selection. Our knowledge of CSTMD1's morphology (Geurten et al., 2007) and the inhibition by targets presented in the contralateral visual field (Bolzon, 2009; Chapter 3), suggests a form of inter-hemispheric gating. CSTMD1 could reflect the output of a bottom-up attention mechanism emerging from a competitive process occurring at a lower level in the STMD pathway. However, there is no evidence which rules out a top-down, endogenous attention process.

7.1.2 Enhancement and Hyper-acuity

Despite the exciting finding that facilitation plays a role in salience and attention, this does not exclude my initial hypothesis that facilitation enhances detection of very small targets. STMD neurons respond to targets below the nominal resolution limit of the

compound eye and would thus be blurred by the optics to a very low contrast image (effective contrast below 2% *Eristalis tenax*; Nordstrom et al., 2006). Similarly, dragonfly STMDs also display high gain to low contrast targets (Geurten et al., 2012).

In a second-order correlator, the noise (false positive target motion) would not correlate and this rejection would allow for high gain, which is necessary to enhance the weak signals generated in photoreceptors by small targets. I found that facilitation requires spatially continuous motion or the enhancement resets (Chapter 5). Further, in Chapter 6 I showed that the Fano Factor does not increase during long target traversals of CSTMD1's receptive field. This is unlike previously examined sensory neurons that have shown increased Fano Factor as firing rate increases (Figure 6-3). This plateaued Fano Factor whilst firing rate increases may be suggestive of the postulated high gain but with some mechanism - like a second order motion correlator – to reduce the effect of noise/variability on the signal.

7.2 Arousal

Over the course of these experiments I found that individual CSTMD1 neurons display a significant amount of variability both within and between neurons. I postulate that this variability in the onset time course is an indicator of the dragonfly's neural state, likely a combination of motivation, attention and arousal. This kind of interaction between these higher order neural states and sensory response has been identified in other insects as well as primates (Attention: McAdams and Maunsell, 1999; Treue and Martínez Trujillo, 1999. Arousal: Rind et al., 2008; Aston-Jones et al., 1992. Motivation: Maunsell, 2004.).

I hypothesise that this is likely mediated by octopamine. Rapid rises in octopamine level have already been shown to produce an increase in general arousal, changing the insect from a resting state into an active state, ready to process incoming sensory stimuli and prepared for sustained activity or aggression (Stevenson et al., 2005; Weisel-Eichler and

Liebersat, 1996). Octopamine is released locally within the CNS, to modulate activity of visual neurons (Stern et al., 1995), and in a more widespread way into the blood, increasing the insect's preparedness either to escape a threatening stimulus by flight (Bicker and Menzel, 1989) or to maintain a fight (Stevenson et al., 2005). When locusts or crickets are forced to fly, or the mechano-sensory pathways in their hind legs are stimulated, octopamine levels in the hemolymph and optic lobes increase (Simpson et al., 2001).

7.3 Limitations of the Current Study

There will always be caveats associated with any study and the *in vivo* dragonfly experimental preparation offers some disadvantages that have been overcome in other organisms i.e. recent advances in intracellular recording in behaving dipterans (Maimon et al., 2010; Longden and Krapp, 2010) and the large body of work that exists on the awake, behaving primates (though these recordings are not intracellular). Intracellular recordings require immobilization of the head, which prevents the dragonflies from actively directing their gaze (indicating overt attention) during experiments. By preventing the animals from 'closing' this behavioural loop we, unfortunately, lose some of the natural context for the function of these neurons. Head movements associated with fixation have certainly been observed during free-flight pursuit of prey, using high-speed video techniques (Olberg et al., 2007). Thus far no one has reported a method to train dragonflies, for reward, for covertly attending to specific features. Such controlled, endogenous focus has been used in primates to examine interactions between visual circuits and those responsive to reward, memory, and sensory-motor coupling (Rizzolati, 1983; Maunsell, 2004; Desimone, 1996). Analogues of these primate systems have been identified in insect brains and therefore the potential for such interactions also exists (Maimon et al., 2010; Menzel and Muller, 1996; Wustmann et al., 1996).

The absolute luminance of the experimental display (mean luminance of ~300Cd.m^{-2}) is several orders of magnitude dimmer than the outdoor environment in which the dragonflies behave. Dipteran LPTC's have been shown to encode more information about self-motion when luminance is in the naturalistic range (Lewen et al., 2001).

7.4 Future Directions

The findings of this thesis, as well as the discovery of selective attention, have raised a host of interesting new questions regarding the small target motion detecting pathway and the role that CSTMD1 plays in this pathway. This includes opportunities for further categorisation of the properties of facilitation, particularly interactions with target velocity; modelling the fundamental elements that underlie facilitation; and seeking to advance techniques for recording from larger insects whilst allowing closed loop behaviours.

Two major variables that could potentially affect the scale and extent of facilitation were unaddressed by the experimental paradigms used in this thesis. The velocity of targets was unvaried during experiments and even the spatio-temporal displacements were velocity-matched to give the impression of constant velocity when the target is not on the screen. This was a necessary concession to allow the full stimulus to be of a realistic length. As shown in Chapter 5, facilitation has a more pronounced effect on slower moving targets. However we have no data currently on how this interacts with the variables probed in Chapter 6. Does facilitation linger longer at the last seen location if the target is moving slower? Does the velocity of the target affect the predictive power seen in the spatio-temporal displacements? Further, I did no tests varying the contrast of either the facilitating target or the test target. This would be a direct test of whether facilitation affects the sensitivity of the target detecting system to sub-optimal targets or simply serves to boost the speed at which maximum response is reached.

My thesis has generated information on the time course and spatial scale on which facilitation functions. The next step is to incorporate this new information into a computational model of the target motion pathway that includes the proposed second-order motion detectors. Modelling the basis of this facilitation and associating it with likely neuronal precursors will provide further insights into the physiology and function of target motion detection pathways.

As I mentioned in Section 7.3, a knowledge gap exists regarding the function of the STMD pathway in behaving predatory insects. Recent work has shown the importance of behavioural context on the response of neurons in wide-field motion pathways and extending this to dragonflies and other predatory animals is an important next step. The major issues to overcome are the larger forces exerted by the heavier, stronger insects as compared to the significantly smaller *Drosophila* seen in Maimon et al. (2010). However, this advancement is essential to gain a greater understanding of the type of response generated in these neurons when faced with a naturalistic pursuit, even if the pursuit is of an unnatural target against a plain background.

Biological target motion detection, particularly in insects, is undergoing an exciting expansion and great advances are on the horizon from both electrophysiological and modelling approaches.

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