An Electrophysiological Investigation into Selective Attention in the Dragonfly

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For their name to glorify,

without a doubt they qualify.

Midges and mosquitos try

escaping peril in the sky,

but though they flee and try to hide,

their swarming tactics won't divide

the focus of the steely-eyed

Australian emerald dragonfly.

Ellen Weatherford
Just the Zoo of Us, episode 27

i. Thesis Abstract

All animals, including humans, must contend with distracting sensory input in order to achieve behavioural success. Selective attention, stimulus tracking, and prediction are fundamental computations that drive goal-directed behaviour across species and tasks, but the study of how these computations are implemented in neuronal architecture has been largely focussed on vertebrates. However, there is mounting evidence that despite small brains, insects are capable of complex computations and exhibit behaviour and performance that surpasses even the most advanced modern robotics and artificial intelligence.

Adult dragonflies (*Insecta: Anisoptera*) are predatory pursuit specialists that intercept prey and conspecifics (Territorial rivals, mating partners) mid-air with high success rates, by flying along interception trajectories based on predictive internal models. Additionally, adult dragonflies are immune to the 'confusion effect,' a reduction in predatory capture success experienced by vertebrate predators when hunting targets amidst a swarm.

Previously, we have identified a system of 'Small-Target Motion Detector' neurons in the dragonfly (*Hemicordulia sp.*) optic lobe that are thought to underlie target-pursuit behaviours. In particular, one well-characterised STMD termed 'Centrifugal Small-Target Motion Detector 1' (CSTMD1) readily exhibits both 'selective attention' for a single target within a pair of rival targets and 'predictive gain modulation' that enhances the neuronal response to targets following a predicted trajectory. In comparison to selective attention observed in vertebrate studies, CSTMD1 exhibits absolute encoding of the selected target (rather than weighted, or relative encoding) and responds as if the distractor did not exist. Such robust stimulus representation could be critical for rapid pursuits in dynamic environments and avoid motor-control errors associated with relative stimulus representation.

In order to understand how the dragonfly achieves such behavioural success, we have recorded intracellular electrophysiological spiking activity from CSTMD in vivo during the presentation of small moving targets. We show that target selection in CSTMD1 is able to 'lock-on' to a selected target, even when challenged by an abrupt-onset, highly salient distractor. Intriguingly, CSTMD1 is also able to dynamically switch between targets. In order to achieve such a fine balance between resistance to distraction and flexibility, we show that dragonfly attention system utilises Preattentive enhancement of targets and inhibition of return in combination to 'gatekeep' access to attentional competition mechanisms, providing a filter for transient and inconsistent stimuli but allowing novel, coherent stimuli likely to represent a target of interest (prey, conspecific, or predator) to capture attention.

We further place the neurobiological properties of target selection in the dragonfly STMD system into behavioural and ecological context by investigating the exogenous stimulus properties that drive target selection and switching, and additionally show target selection and tracking in swarm-like conditions that resemble real-life feeding conditions encountered by dragonfly behaving in the wild.

Understanding how dragonflies achieve such remarkable behavioural success with such comparatively limited computational architecture has the potential to inform the development of bioinspired computer vision, artificial intelligence, and Neurobotics platforms, such as self-driving vehicles or search-and-rescue drones, as well as illuminate fundamental mechanisms of neuronal processing and representation for stimulus selection, target-tracking, and prediction.

ii. Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 in my name, 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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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Benjamin Horatio Lancer

Friday, 24 September 2021

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We also thank the manager of the Adelaide Botanic Gardens for allowing Insect Collection.

iv. Dedications and Personal Acknowledgements

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University of Adelaide Visual Physiology & Neurobotics Laboratory, September 1, 2021. From left to right: Steven Wiederman, Edward Luong, Katie Skeen, Joseph Fabian, Benjamin Lancer, Andrew McCauley, Hamish Pratt, Bernard Evans, Matthew Schwarz. Not Pictured: John James, Mahdi Hussaini.

v. List of Included Publications

By date

Lancer, B. H., Evans, B. J. E., Fabian, J. M., O'Carroll, D. C., & Wiederman, S. D. (2019). **A Target-Detecting Visual Neuron in the Dragonfly Locks on to Selectively Attended Targets**. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *39*(43), 8497–8509.

Lancer, B. H., Evans, B. J. E., & Wiederman, S. D. (2020). **The Visual Neuroecology of Anisoptera**. *Current Opinion in Insect Science*, 42, 14-22. *

Lancer, B. H., Evans, B. J. E., Fabian, J. M., O'Carroll, D. C., & Wiederman, S. D. (*in submission*). **Preattentive Facilitation and Inhibition of Return in an Insect Target-Tracking Neuron.** *In submission* at *Nature Communications*.

Lancer, B. H., Evans, B. J. E., O'Carroll, D. C., & Wiederman, S. D. (*in prep*). **Target Properties that Drive Selection in a Dragonfly Target Tracking Neuron.** *Unpublished Manuscript.*

Lancer, B. H., Evans, B. J. E., O'Carroll, D. C., & Wiederman, S. D. (*in prep*). **Performance of a Dragonfly Target-Tracking Neuron in Swarm Conditions.** *Unpublished Manuscript.*

* *The Visual Neuroecology of Anisoptera* is a review article and has been excluded from the main text of the thesis, as heavily edited sections appear integrated in the *introduction*. The full text of this article is reproduced as-published in the appendix.

Chapter 1: Introduction

Attention is the ability to selectively respond to some stimuli while ignoring others. A classic demonstration of attention is known as the *cocktail party effect*, where a partygoer in a busy cocktail bar can direct attention to a single conversation despite a noisy room. The natural world contains many cocktail party situations, for example returning penguins searching for their mate within raucous colony babble (Aubin, 2004) or vampire bats identifying characteristic individual human snoring in polyphonic jungle soundscapes (Gröger and Wiegrebe, 2006). Attention is a fundamental neuronal processing strategy (Lindsay, 2020) that likely emerged early in animal evolution (Sridharan et al., 2014) as it is seen across taxa, including in insects (De Bivort and van Swinderen, 2016; Nityananda, 2016). Robust selective attention is particularly important for visual predators which hunt amidst swarms (Jeschke and Tollrian, 2007), containing potentially hundreds of prey and conspecifics. Many predators hunting in these conditions are susceptible to the 'confusion effect', a reduced capture-success rate due to difficulty tracking a single target amid the swarm (Jeschke and Tollrian, 2007; Schradin, 2019), The confusion effect can be reduced if the predator is able to identify and track individual prey, a process made easier if a particular prey instance is visually distinct (Landeau and Terborgh, 1986).

Dragonflies have recently emerged as a model for visual selective attention (Wiederman and O'Carroll, 2013a). Dragonflies are predatory insects that capture prey in flight with high success rates (Olberg et al., 2000; Combes et al., 2013), even overcoming the confusion effect (Jeschke and Tollrian, 2007; Combes et al., 2012). Possibly underlying this behaviour, the dragonfly visual system contains a class of 'Small Target Motion Detector' neurons which are tuned to small target movement (O'Carroll, 1993). One well-characterised neuron of this class, 'Centrifugal Small Target Motion Detector 1' (CSTMD1, Geurten et al., 2007) exhibits selective responses to single targets when presented with rival target pairs (Wiederman and O'Carroll, 2013a). The focus of this thesis is on the electrophysiological response properties of CSTMD1 in relation to selective responses to paired and swarming targets. Chapter 1: Introduction (Pg. 12) provides an introductory review that covers an overview of the literature on selective attention (pg. 13), a brief natural history of the dragonfly (pg. 29) and dragonfly behaviour (pg. 30), and a detailed overview of the dragonfly visual system (pg. 38). Chapter 2 will focus on the experimental methods employed, serving as a high-level 'methods' section. Chapter's 3-6 present experimental results organised as either published units (Chapter 3), or manuscript-style presentation of unpublished data (Chapters 4,5, and 6). Finally, Chapter 7 will provide an overall discussion and general conclusion to the work.

Attention

"Everyone knows what attention is. It is taking possession of the mind, in clear and vivid form, of one out of what seems several simultaneously possible objects or trains of thought. Focalization, concentration of consciousness are of its essence. It implies a withdrawal from some things in order to deal effectively with others."

- *William James, 'The Principles of Psychology', 1890*

Since foundational psychologist William James first proclaimed 'everyone knows what attention is' in his seminal work *The Principles of Psychology* (James and Drummond, 1890), the body of knowledge on attention has developed into a far less clear, though perhaps far more vivid, state. In contrary to James's proclamation at the turn of the 20th century, recent authors have declared '*no one* knows what attention is´ (Hommel et al., 2019), and questioned whether 'attention' is even a meaningful concept (Anderson, 2011). Yet despite these existential uncertainties, attention has become fundamental in modern Cognitive Science and a key component in the interpretation of both animal and human behaviour and neurophysiology (Lindsay, 2020). Attention is generally considered a limited resource (Alvarez and Franconeri, 2007) that manifests as enhanced processing of 'attended' stimuli and supressed processing of 'ignored' stimuli. Here, we will define attention in general computational terms as any neural process that limits the processing of viable stimuli to a subset (Nityananda, 2016; Lindsay, 2020). This conceptualisation emphasises two key components:

1) Attention is fundamentally about efficient use of limited resources (Alvarez and Franconeri, 2007; Lindsay, 2020) to enhance the processing of some stimuli *over* others; if everything were attended, nothing would be. This component allows attention to be differentiated from concepts of increased arousal, motivation, or vigilance.
2) Attention is flexible, meaning it can be directed towards different stimuli based on behavioural goals and task demands.

Attentional effects have been shown in a variety behavioural tasks and brain regions, and it is likely that 'attention' is a fundamental component of brain function across systems rather than a single event. *Visuospatial attention* is attention given to selected *locations* in the visual field that contain stimuli of interest. One popular metaphor for thinking about visuospatial attention is that it produces a 'spotlight' of enhanced processing which can be directed across the visual field. This metaphor naturally leads to some interesting questions to guide discussion;

1. How is the 'spotlight' directed?
2. What is the effect of 'directing the spotlight' at a particular location?

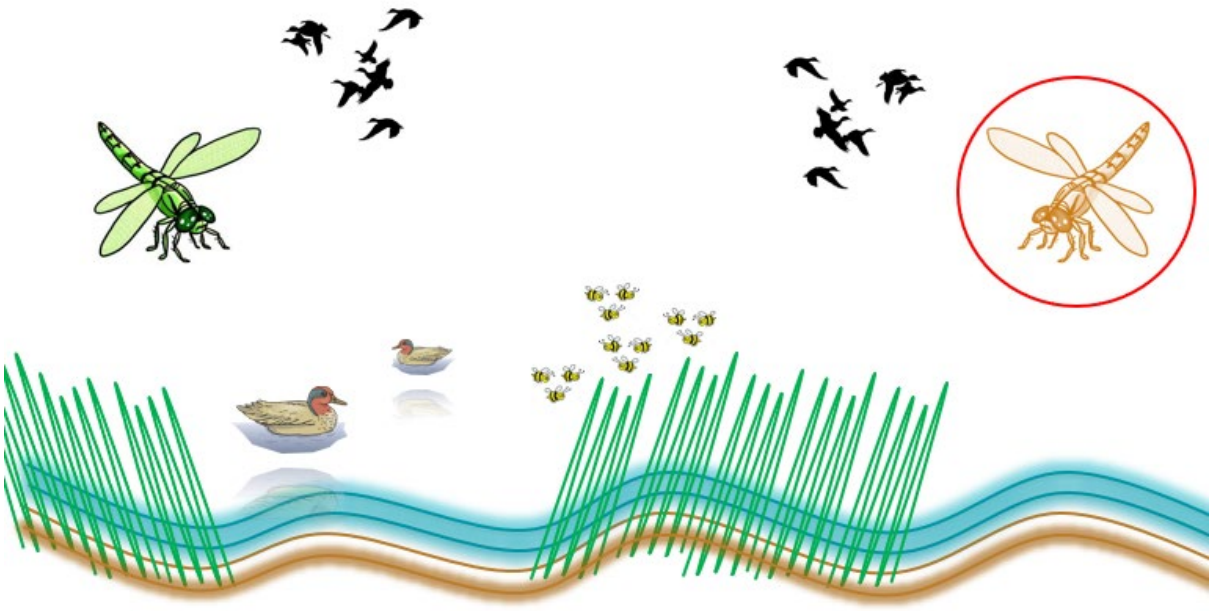

Figure 1: A Naturalistic Selective Attention Task. *A green dragonfly (left) patrolling its territory encounters a competitor (orange). In order to engage the competitor in a territorial defence pursuit, the green dragonfly must track the target amidst other target-like distractors (birds, bees) in a reedy and foliage-cluttered environment. The red circle illustrates the attentional spotlight.*

3. What neural mechanisms contribute to deploying and maintaining the spotlight?

The Spotlight of Attention

How is the Spotlight Directed?

Bottom Up vs. Top Down

Traditional conceptualisations present a dual-process system of attentional control comprised of 'bottom-up' and 'top-down' systems. The cocktail party effect discussed at the beginning of the introduction is an example of top-down attention, where attention is controlled by cognitive, voluntary, and deliberative decision making. In contrast, the involuntary turning of the head when a dropped cocktail glass shatters on the ground would be an example of 'bottom-up' attention that is involuntarily, reflexively directed to a highly salient stimulus (Baluch and Itti, 2011; Pinto et al., 2013). Top-down attentional direction reflects acute behavioural goals and are driven by experience and expectation, while bottom-up attention generally reflects the physical salience of a stimulus (Posner et al., 1980; Itti and Koch, 2001; Theeuwes, 2013).

This classification has recently been challenged (Awh et al., 2012; Gaspelin and Luck, 2018a; Benoni and Ressler, 2020), and different authors emphasise different components of top-down control, such as intention/volition (Theeuwes, 2018), while others argue top-down attention can be involuntary (Gaspelin and Luck, 2018a) or that top-down attention is any direction driven by behavioural relevance (Mysore and Knudsen, 2013).

Attentional Capture

Humans and non-human primates searching for a specific target perform worse if a salient, task irrelevant distractor (even of a different sensory modality) is presented immediately before or during target presentation (Wolfe and Horowitz, 2004). This cost in performance is attributed to the task-irrelevant but highly salient distractor 'capturing' attention, a process known as attentional capture (Hickey et al., 2006; Koelewijn et al., 2009). The interpretation is that the focus of attention is automatically and unavoidably captured by salient stimuli, even when attentional resources are highly engaged in a top-down controlled task (Koelewijn et al., 2009).

Stimuli that readily capture attention include abrupt onset stimuli (Remington et al., 1992), novel objects (Yantis and Hillstrom, 1994), looming objects (Lin et al., 2008), highly salient objects (Horstmann and Ansorge, 2016), and objects previously rewarded (Anderson et al., 2011) via classical conditioning. Attentional capture is thought to be largely bottom-up, stimulus driven, involuntary, and despite task-irrelevance, however the influence of previous reward/punishment history and imaginary cues (Craver-Lemley and Reeves, 1992; Craver-Lemley and Arterberry, 2003) implicates top-down sensitisation to some captured objects.

Attentional capture can also be overcome. In one study, monkeys behaved as if blind to objects presented in an unexpected part of the visual field during an attentional task, while neurons in MT failed to encode such stimuli despite its presence in their receptive field (Harrison et al., 2013). Similar 'inattentional blindness' results have been observed in human behaviour and psychophysics, where under some conditions attention to a task results in subjects completely missing salient events (Simons and Chabris, 1999; Drew et al., 2013; Ruthruff and Gaspelin, 2018).

What are the Effects of Falling Under the Spotlight?

'Weighted' and 'Winner-Takes-All' Attention

The classic demonstration of attentional modulation in an individual vertebrate neuron produces an effect known as 'weighted attention.' These classical experiments were generally conducted in visual stimulus responsive neurons in the primate occipital lobe. Experimenters simultaneously present a pair of stimuli within the cell's receptive field, one of which is drives a strong excitatory response when presented alone (the 'good' stimulus), and one of which drives a weak (but still above noise) excitatory response when presented alone (the 'poor' stimulus). When presented as a neutral pair in the absence of an attentional cue or motivation to select one, a neuron will typically response with the 'average' of its response to either stimulus alone, leading to the somewhat unintuitive result that adding a mildly exciting 'poor' stimulus to a strongly excitatory 'good' stimulus suppresses the neuronal response. However, when one stimulus is cued or rewarded, the neuron responds with a 'weighted' average that draws the response strength closer to that of the attended

stimuli. Such 'weighted attention' has been observed across brain regions and species and with a variety of variations on this classic experiment (Treue and Maunsell, 1996, 1999; Luck et al., 1997; Reynolds and Desimone, 2003)

However, not all cells respond to multiple stimuli in a weighted manner. 'Winner-Takes-All' (WTA) selection refers to a scenario where *only* the winning stimulus is represented in the final neuronal response (Mysore and Kothari, 2020). WTA selection is observed in the inferior temporal cortex of rhesus monkeys (Chelazzi et al., 1998) and Posterior Parietal cortex of Macaques (Oleksiak et al., 2011). WTA and Weighted Attention are not necessarily mutually exclusive, but may represent different strategies used at different levels of hierarchical processing or for different task demands. Recent evidence shows Zebrafish engaging in predator-escape behaviour by fleeing from two predator-like stimuli (looming spots presented on either side of the fish) implement both 'weighted' and 'WTA' strategies within the same individual across repeated trials (Fernandes et al., 2021). In Macaques, Posterior Parietal neurons exhibit approximately WTA responses (Oleksiak et al., 2011) while neurons in the nearby MT exhibit weighted average rules (Snowden et al., 1991; Recanzone et al., 1997). Recently, single neurons in the macaque Lateral Prefrontal cortex were shown to implement either WTA or Weighted rules depending on the location of the stimulus within the cell's receptive field (Duong et al., 2019).

Attention Acts by a Combination of Enhancement and Suppression

In order to direct attention towards a selected location, attentional systems use a combination of enhancement and suppression. At the level of subjective perception and behaviour attention increases contrast sensitivity (Carrasco et al., 2000), spatial resolution (Carrasco et al., 2002), processing speed (Carrasco and McElree, 2001), and response speed (Wilimzig et al., 2008), while unattended locations experience either relative suppression or completely fail to respond to presented stimuli. The effects of attention can be so powerful that in a now-classic humorous demonstration, when human observers were tasked with counting the passes of a ball between players on a basketball court, half of test subjects were completely unaware of a man in a gorilla suit crossing the court during the task, even when the 'gorilla' stopped to beat its chest (Simons and Chabris, 1999). Even expert radiographers examining lung CT-scans for pathological signatures failed to notice a gorilla superimposed over the image (Drew et al., 2013). At the level of neuronal responses, directing attention within a neuron's receptive field increases contrast gain (Spitzer et al., 1988; Treue and Maunsell, 1996, 1999; Luck et al., 1997; Reynolds et al., 1999; Ghose and Maunsell, 2008) and sensitivity (Reynolds et al., 1999, 2000; Carrasco et al., 2000; Pestilli and Carrasco, 2005), sharpens neuronal tuning (Spitzer et al., 1988; Martinez-Trujillo and Treue, 2004), increases response speed (Jancke et al., 2004), as well as reducing neuronal variability (Mitchell et al., 2009) and enhancing

coherence across neuronal populations (Saalmann et al., 2012). Attention can even reshape stimulus tuning properties (McAdams and Maunsell, 1999; Treue and Maunsell, 1999) and receptive field structure (Moran and Desimone, 1985; Fritz et al., 2003; Womelsdorf et al., 2006). Meanwhile, unattended locations and objects experience decreased contrast sensitivity (Pestilli and Carrasco, 2005) and enhanced competitive suppression (Moran and Desimone, 1985; Treue and Maunsell, 1996, 1999; Recanzone et al., 1997; Reynolds et al., 1999; Reynolds and Desimone, 2003; Malek et al., 2017)

In summary, attention towards a stimulus within a neuron's receptive field causes an increase in sensitivity, excitability, sharpened tuning, and reduced variability whilst neurons encoding other spatial locations exhibit general suppression. Taken together, attention enhances the processing of 'attended' stimuli via a pattern of enhancement and suppression of neuronal encoding properties across populations involved in scene representation. Importantly, these attentional effects are usually reported relative to a single stimulus presented alone, suggesting most attentional effects studied in vertebrates triggered by a *distractor* and the need to enhance and supress stimuli in order to bias processing towards the desired target.

What Neural Mechanisms Deploy and Maintain the Spotlight?

Attention is achieved by dynamic interactions between two broad mechanisms, filtering and competitive selection. These mechanisms are not mutually exclusive; an attentional system may use both, and both may be influenced by top-down and bottom-up factors. Filtering involves the suppression of neural channels encoding irrelevant information, such as orientation information in a colour selection task (Itti and Koch, 2001). In contrast, competitive selection involves mutual inhibition by stimuli representations whereby the strongest stimulus 'outcompetes' the rest by supressing all others (Mysore and Kothari, 2020). While salience filtering is useful when attending to a specific feature (e.g., colour), competitive selection is required when salience filtering fails to isolate the target, or when selecting a single stimulus from several similar alternatives such as a predator hunting amidst a swarm.

Biased Competition

The Biased Competition Theory first put forward by Robert Desimone et al. (Desimone and Duncan, 1995; Desimone, 1998) models attention as a bottom-up competitive interaction between ascending neuronal representations that can be biased by top-down feedback which enhance the representation of stimuli under top-down selection and suppress the representation of 'selected-against' stimuli. Primate cortical cells are thought to be 'hard wired' to respond to the highest saliency stimulus (Reynolds and Desimone, 2003), a property that can be exploited by attentional systems which dynamically increase the effective gain on the stimuli to be attended (Schiller and Lee, 1991;

Hillyard et al., 1998; McAdams and Maunsell, 1999; Treue and Martínez Trujillo, 1999; Reynolds et al., 2000; Martínez-Trujillo and Treue, 2002; Reynolds and Desimone, 2003; Reynolds and Heeger, 2009). In this view, a 'poor' or 'weak' but behaviourally relevant stimulus can come to dominate the competitive mechanism when *enhanced* by top-down feedback, allowing it to outcompete exogenously higher-salience stimuli via lateral inhibition (Suzuki and Gottlieb, 2013; Hampshire and Sharp, 2015; Noonan et al., 2017). Such gain enhancement is observed in primate visual cortex when directing attention towards a 'poor' stimulus amidst highly-salient distractors (Treue and Maunsell, 1999; Reynolds et al., 2000; Treue, 2001; Reynolds and Desimone, 2003), but comes at the cost of perceptual accuracy by exaggerating stimulus magnitude (Mehrpour et al., 2020).

However, not all neuronal observations are consistent with enhancement models of attention. Recording from the inferior temporal cortex in macaques, Chelazzi et al. (1998) showed that neuronal responses to paired stimuli settled on a firing pattern identical to that of the selected stimuli alone (Chelazzi et al., 1998). That is, these cells encode an *absolute* representation of the stimulus without enhancement. Similar 'Winner-Take-All' responses are observed in the macaque posterior parietal cortex (Snowden et al., 1991), lateral prefrontal cortex (Duong et al., 2019), and Frontal Eye Fields (Zénon et al., 2009). These results cannot be explained by *enhancement* because the magnitude of the representation of the winning stimulus is preserved. It is interesting to note that all these examples are from 'higher-order' regions, raising the possibility that attention is implemented by different rules depending on the hierarchical level of the circuit. For circuits close to motor output, accurate representation of stimulus magnitude in neuronal responses would seem key in guiding accurate behavioural responses.

Such behavioural demands and neuronal effects may be better served by suppression of distractors. In suppressive models, biased competition can be achieved via direct suppression of distractors without enhancing the target (Chen, 2017; Noonan et al., 2017), or by 'global' pooled inhibition that equally supresses all possible targets coupled with inhibition-of-inhibition that 'releases' the selected target from inhibition, allowing it to propagate 'as if' it were the only stimulus (Mahajan and Mysore, 2019; Mysore and Kothari, 2020). Whatever the mechanism, neuronal suppression is thought to play a key role in distractor avoidance (Mazza et al., 2009; Munneke et al., 2013; Suzuki and Gottlieb, 2013; Gaspelin et al., 2015; Noonan et al., 2017; Gaspelin and Luck, 2018b). Evidence from multiple species and levels of analysis show that attention to an object induces suppression of surrounding areas of space (Moran and Desimone, 1985; Hopf et al., 2006), suggesting spatially localised lateral inhibition plays an important role.

As the dragonfly visual neuron CSTMD1 exhibits winner-takes-all selective attention when presented with paired targets (Wiederman and O'Carroll, 2013a), we will narrow our detailed discussion to mechanisms of Winner-Takes-All selection.

Network Models of Winner-Takes-All Attention

How can a circuit of neurons converge on a winning representation from an array of simultaneous stimuli? The Winner-Take-All (WTA) model is a generalized 'network model' for how a network of neuron-like units may come to a decision between inputs. WTA models have been applied across a range of computational functions, from high-level cognitive phenomena such as action selection (Mink, 1996; Gurney et al., 2001), decision making (Wang, 2002; Furman and Wang, 2008; Schmuker et al., 2014; Barron et al., 2015), and attention (Itti and Koch, 2001; Walther and Koch, 2006), to low-level neuronal computations such as the development of feature selectivity (Chen, 2017). The emerging view is that despite differences in in abstract components of the different tasks, many brain functions rely on *symmetry breaking* between multiple options, which may share common computational principles and neural mechanisms. Mysore and Kothari (2020) have proposed a framework for WTA functions that consists of 6 elemental computations (ECs);

1. *Comparison across options*. In order to be compared, neural representation of selection options must be encoded along a common scale that represents the 'norm' (variously defined as 'value', 'worth', 'saliency', etc. across contexts (Levy and Glimcher, 2012)). In models of visuospatial attention, 'norm' is generally defined as the 'priority' of each stimulus, a combination of innate saliency and top-down motivational modulation (Koch and Ullman, 1987; Fecteau and Munoz, 2006; Awh et al., 2012).
2. *Categorical Selection Boundaries.* Selection boundaries must be sharp and 'step like' – the norm difference between options should not, in principle, influence the final output.
3. *Dynamic Flexibility*: The selection boundary should be flexible, able to shift based on the dynamic range of the input. For example, a simple threshold could select between a high and low saliency option, but a proper selection mechanism must be able to give equally good selection responses to a pair of 'good' stimuli and a pair of 'poor' stimuli.
4. *The ability to select between all competing options*. For example, any two targets within the visual field must be able to compete, regardless of relative location.
5. *The ability to select in the presence of multiple (>2) competing options*, rather than only selecting between pairs.
6. *"The production of a unity choice,"* a competitive selection mechanism should produce exactly one winner.

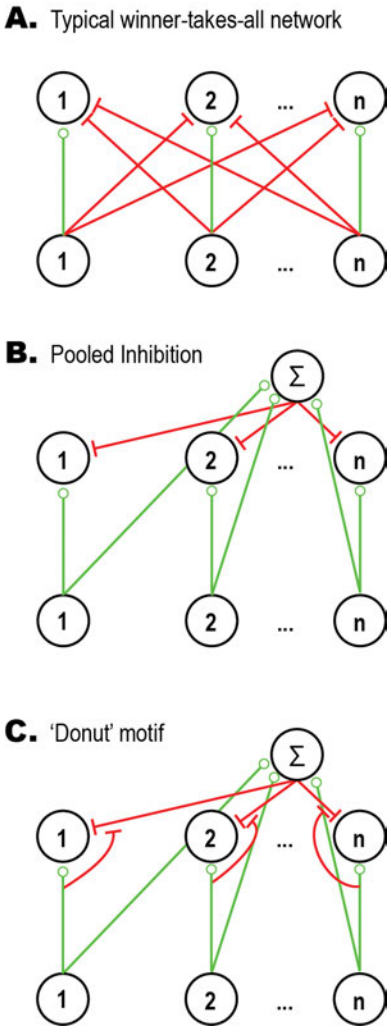

Figure 2: Winner-Take-All Topologies. *Examples are scale-free. Units (circles) representing stimuli (1,2, … n) could be either individual feature-sensitive cells or neuronal populations. Edges (lines) are either inhibitory (red) or excitatory (green), but may represent either an individual axon, the output tract of a larger population, or include a sign-inverting interneuron.* ***A)*** *A typical WTA network utilizing lateral inhibition. Units representing competing stimuli (bottom) project to a second-order representation (top), which also receives inhibition from other stimuli. The strongest stimulus is able to simultaneously suppress alternative options and withstand suppression from competitors.* ***B)*** *Pooled Inhibition. First-order representations project to a pooled inhibitory unit, which projects uniform inhibitory feedback.* ***C)*** *'Donut' motif proposed by Mahajan and Mysore (2019). Inhibitory feedback from the Pooled Inhibition unit is vetoed, allowing the winning stimulus representation to maintain its original strength.*

Typically, a WTA model is conceptualised as a number of computational units representing different options interacting with one another via competitive inhibition, with the strongest option 'outcompeting' all the others and dominating the network response by supressing all competitors (Yuille and Grzywacz, 1989; Lee et al., 1999; Maass, 2000; Mysore and Kothari, 2020). Such responses have been observed in the avian Optic Tectum and a specialised tegmental nucleus, which flexibly signal the strongest of all competing stimuli regardless of absolute strengths (Asadollahi et al., 2011; Mysore and Knudsen, 2011).

In the simplest version of this model, units representing each option compete with each other via lateral inhibition scaled to the norm of each stimulus such that the unit with the highest norm supresses all others (Figure 2A). Such a mechanism satisfies almost all the elemental computations, except EC#4, the ability to select between all competing options. Lateral inhibition mechanisms are difficult to scale up, as every possible option must be directly connected to every other possible option, making them highly wiring inefficient. Alternatively, inhibition can be pooled to a single unit (either a single neuron, or population of neurons) that nonlinearly combines the inhibition from each option and then projects feedback inhibition back to each stimulus representation, a scheme referred to as 'pooled inhibition' (Figure 2B). Under such a scheme, only the strongest original norm will be able to withstand pooled inhibition.

Winner-take-all models typically function as bottom-up selectors by comparing the norm of each input option to come to a decision, but can be influenced by top-down feedback projections to bias the competition between options, as per the biased competition theory described above. Feedback projections artificially enhancing the norm of one stimulus function as a 'finger on the scale' tipping an otherwise equal competition or allowing exogenously weaker stimuli to outcompete stronger stimuli. Such an architecture leads to an archetypical 'centre-surround' topology,

characterised by moderate widespread inhibition and narrow high-gain excitation centred on the selected target (Figure 3A).

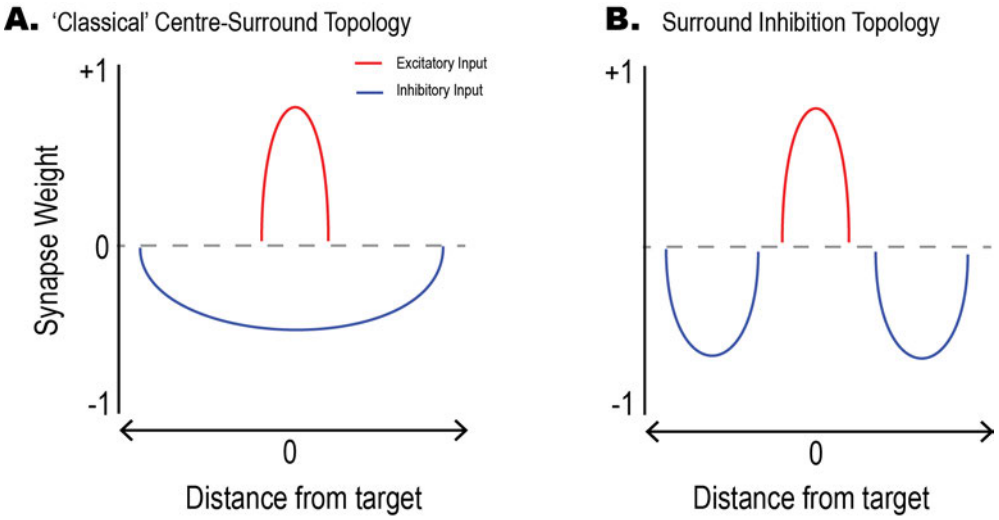

Figure 3: Synaptic Topologies for Competitive Selection. A) *Classical centre-surround topology.* A narrow region around the target is enhanced, combined with suppression of a wide region centred on the target. As a result, the area around the target receives net-enhancement, whilst regions further away are supressed. **B)** Surround Inhibition topology (Chen, 2017). Narrower surround inhibition combined with narrow excitation allows spiking networks to be robust to spike-timing errors.

However, the classical centre-surround topology cannot account for *absolute* encoding of the winning stimulus, as the ultimate activation of the winner is a combination of excitation and inhibition. Furthermore, using computational modelling Chen et al. (2017) found that such centre-surround topology was not guaranteed to converge on a WTA 'winner' when applied to a spiking neural network, due to spike timing differences between different populations of excitatory and inhibitory neurons at different points preventing uniform subtraction (Chen, 2017). Instead, the authors propose a *surround inhibition* topology (Figure 3B), where representation of the winning target is enhanced while spatial locations surrounding the winner are inhibited, allowing the representation of the selected target to be preserved with minimal inhibitory influence.

Recently, Mahajan & Mysore have proposed a model of competitive selection that utilises a self-sparing lateral inhibition motif named 'donut-like' inhibition (Mahajan and Mysore, 2019; Mysore and Kothari, 2020). In donut-like inhibition, each option generates suppression for all other options, sparing itself, resulting in a surround-inhibition like topology. Such topology has been observed in the midbrain selection network of the Barn owl, *Tytus alba* (Mahajan and Mysore, 2018, 2019) as well as *Drosophila* (Jovanic et al., 2016). Mahajan & Mysore's (2020) proposed canonical selection circuit also includes a number of other circuit elements to aid in generating robust winner-takes-all behaviour, including differential amplification via a recurrent excitatory interneuron.

Both surround inhibition and donut inhibition models utilize direct inhibition via inhibitory interneurons, but as we have seen such direct lateral inhibition on large scales is extremely computationally inefficient. Is it possible for a single inhibitory unit (neuron or population) to

implement self-sparing pooled inhibition? One possibility is a donut-like motif where each representation inhibits the pooled inhibition of itself (Figure 2C), allowing the winning option to both contribute to the pooled inhibition and supress competitors, whilst negating inhibition of itself.

Multiple Spotlights?

The discussion so far has been prefaced on the tacit assumption of the selection of one target and suppression of all others. However, there is evidence that humans and other primates are able to track multiple, independent objects simultaneously (Pylyshyn and Storm, 1988), even when there are distractors placed between targets and no 'single spotlight' could select a region large enough to include all targets, without also including distractors. Two broad explanations for this ability have been proposed. First, 'serial switch' theories suggest a single focus of attention rapidly switches between target locations, allowing multiple targets to be 'tracked' via serial updates. Second, parallel process theories suggest that multiple spotlights of attention are directed independently and simultaneously. fMRI research shows spatially independent peaks of activity arise in human V1 when engaged in a multifocal attention task (McMains and Somers, 2004), strongly supporting parallel processing theories. However, multifocal attention comes at a cost. During multifocal attention only task-related target information is tracked (e.g. location), while other feature information (shape, colour) is ignored and can change during tracking without the observer's notice, despite accurate location tracking (Bahrami, 2003; Saiki, 2003). It is thought that since attention is a limited resource, multiple targets must share the same available attentional capacity, so each is reduced to encoding no more than the location required to perform the tracking task (Cavanagh and Alvarez, 2005).

Attention in Behavioural Context

The Competing Demands of Selection and Flexibility

Attention to some stimuli fundamentally implies *inattention* to other stimuli, leading to the phenomenon of *inattentional blindness* whereby observers miss obvious or salient stimuli while attending to another part of a scene (Simons and Chabris, 1999; Drew et al., 2013; Harrison et al., 2013; Horstmann and Ansorge, 2016; Palmer et al., 2018; Ruthruff and Gaspelin, 2018). The most famous example of inattentional blindness is the *invisible gorilla* experiment mentioned above, where observers tasked with tracking a ball thrown around a basketball court miss the appearance of a man in a gorilla suit (Simons and Chabris, 1999). However, inattentional blindness is also seen across species during ethologically relevant tasks. For example, butterflies searching for appropriate oviposition flowers in a mixed-flower field can be primed to a single species of flower (by presenting that flower first), causing them to ignore or respond slower to equally or more viable alternative oviposition sites (Gamberale-Stille et al., 2019). Similar behaviour occurs in birds foraging for seeds or

worms in a cluttered environment, leading birds to miss potential food that does not match the current target and reducing probability of noticing a predator (Plaisted and Mackintosh, 1995; Dukas and Kamil, 2000; Tosh et al., 2007). Thus 'rigid' attention can lead to missed opportunities or increased predation risk. These reductions in ability to respond to predators or alternate targets whilst attending a specific target have been referred to as the 'cost of limited attention' (Dukas and Kamil, 2000).

Meanwhile, too 'fickle' attention results in frequent switches and distraction that reduces overall behavioural efficiency (Milinski, 1990; Krause and Godin, 1996; Dukas and Kamil, 2000; Dukas, 2002). However, it is important to note that 'distraction' does not necessarily imply a failure of attention when placed in behavioural and ecological context, for a system with both evolutionary and developmental history outside the narrow confines of a lab attention task (Benoni and Ressler, 2020). Many animals engaged in attention must remain vigilant for predators, as predator-free environments rarely exist in nature (Lima and Dill, 1990). Behavioural studies in mice reveal they are readily distracted by unexpected sounds (Rogalla et al., 2020), and are unable to suppress responses to sudden distracting visual stimuli even in a straightforward task (Wang and Krauzlis, 2018). 'Readily distractible' attention in the mouse may be an adaptive anti-predator defence. Furthermore, many species show rapid, reflexive behavioural responses to the presentation of multiple stimuli that targets the 'average' stimulus location (Lisberger and Ferrera, 1997; Ioannou et al., 2008; Nummela and Krauzlis, 2011; Fernandes et al., 2021), which has been characterised as a failure of attention. Such a response may be detrimental to a predator attempting to capture one target, but beneficial for prey-species attempting to escape multiple potential predators, where an escape vector opposite the 'average' predator location may be more efficient than an escape vector opposite just one (Fernandes et al., 2021).

The theoretical optimal allocation of attention thus requires a careful balance between two competing drives: the current behavioural goal of the animal, and flexibility to respond to unanticipated opportunities or dangers. Animals must balance the scope of their attention based on environmental challenges (target crypsis, dynamic backgrounds, predation risk) and internal cues (hunger, motivation).

The Confusion Effect

> *"It is easier to hit a single bird than one of a dozen. The number of possibilities in the latter case distracts the attention from any one individual, and in consequence all are likely to escape."*
>
> *- Robert C. Miller, the Significance of the Gregarious Habit (1922)*

The *confusion effect* describes a reduced attack-to-kill ratio experienced by predators hunting in high-density groups of prey (Schradin, 2019), such as a flock of birds, school of fish (Neill and Cullen, 1974), or swarm of insects (Gillett et al., 1979; Milinski, 1984; Ioannou et al., 2008). Intriguingly, the effect does not manifest as behavioural hesitation or reduced reaction time, but as the actuation of an inaccurate attack vector at a 'mean' target location (Lisberger and Ferrera, 1997; Ioannou et al., 2008; Nummela and Krauzlis, 2011), suggesting a failure to select an *individual* target for the direction of response.

The confusion effect is observed in a range of taxa, including in humans (Gillett et al., 1979; Jones et al., 2011), non-human primates (Ottes et al., 1984; Lisberger and Ferrera, 1997; Schradin, 2000; Nummela and Krauzlis, 2011), lizards (Gillett et al., 1979; Schradin, 2000), birds (Brighton et al., 2020), fish (Neill and Cullen, 1974; Milinski, 1984; Ioannou et al., 2008), cephalopods (Neill and Cullen, 1974), and insects (Jeschke and Tollrian, 2007) and has been modelled in artificial neural networks (Tosh and Ruxton, 2006; Tosh et al., 2006; Ioannou et al., 2008), but is most associated with *visual* predation (Jeschke and Tollrian, 2007). The confusion effect even influences non-task-related stimulus processing. For example, hungry three-spined sticklebacks preying on *daphnia* swarms took longer to detect and respond to a predator of themselves with increased prey density (Milinski, 1984). The confusion effect is reduced when the predator is able to 'isolate' individual prey, either due to a unique perceptual feature (Landeau and Terborgh, 1986), spatial exclusion (Milinski, 1977), or a predictable prey trajectory (Jones et al., 2011). Thus, from the predator's perspective, the crucial point in avoiding confusion seems to be the ability to single out individual prey and 'lock on.'

Intriguingly, the confusion effect does not require complex coordination among prey within a group, but is a perceptual consequence of having too many stimuli within the predators' visual field (Ruxton et al., 2007; Jones et al., 2011). Under the standard conceptualization of the confusion effect, the 'confusion' in confusion effect implies that the predator experiences attention or concentration difficulties targeting one of several possible prey items. That is, *confusion* is taken to be cognitive in nature. However, some authors have argued that confusion can be attributed to information degradation of topographic mapping of prey position in highly dense environments (Krakauer, 1995; Tosh et al., 2006; Ioannou et al., 2008) and is thus more of a result of the information capacity of a neural network becoming oversaturated than a breakdown in cognitive attentional mechanisms. Under this conceptualisation, attention can be thought of as an evolutionary response to *overcome* the confusion effect which is inherent in hierarchical sensory processing, rather than as a *cause* of the confusion effect (Tosh et al., 2006). Intriguingly, Artificial Neural Network modelling has found the 'modular' organization of the arthropod visual system is less susceptible to the confusion effect in contrast with the more hierarchical and convergent vertebrate visual system (Tosh and Ruxton, 2006).

Selective Attention in Insects (and other invertebrates)

Although the majority of attention research is conducted in vertebrates, there is increasing evidence that insects and other invertebrates exhibit many attention-related phenomena (van Swinderen, 2011; De Bivort and van Swinderen, 2016; Nityananda, 2016; Winsor et al., 2021).

Selective Orientation Between Rival Stimuli in Drosophila

One important aspect of insect behaviour for studying attention in insects is *orientation* (Winsor et al., 2021). When an insect is placed on an air-supported ball but otherwise tethered in place in the centre of a nearly-blank visual arena with a single high-contrast object, such as a vertical stripe, most insects will turn such that their body is oriented with the stripe along the vertical midline (Taylor et al., 2015). When presented with a pair of such stripes laterally offset from the midline by an equal amount, *Drosophila* choose one to bring into the midline and ignore the other (Sareen et al., 2011; Koenig et al., 2016a), displaying winner-take-all behavioural selection where a single stripe is chosen. The decision to choose one stripe or the other can be directed by horizontally oscillating one of the stripes, resulting in a bias towards selecting that stripe that decays with time (Koenig et al., 2016b), requires dopaminergic signalling (Koenig et al., 2016b) and short-term memory genes (Van Swinderen, 2007). In a similar experiment conducted in honeybees, the contrast of rival stripes were modulated at distinct frequencies, leading to frequency-locked responses reflected in recorded Local Field Potentials from the optic lobes (Paulk et al., 2014). This study revealed that during the presentation of paired stripes, the optic lobe response was dominated by a frequency-locked response consistent with the honeybee's subsequent behavioural choice, predicting which of the two stripes the bee would orient towards (Paulk et al., 2014).

Such selective fixation responses have been observed deeper in the insect brain in the *Drosophila* ellipsoid body and protocerebral bridge, midline neuropil involved in navigation that represent sensory input on an egocentric orientation map (Lin et al., 2013; Seelig and Jayaraman, 2015; Green et al., 2017; Kim et al., 2017; Turner-Evans et al., 2017). When presented with a stimulus stripe, a single peak of activity appears in the ellipsoid body space map consistent with the stripe location relative to the fly (Seelig and Jayaraman, 2015). As the fly turns its orientation relative to the stripe, so the ellipsoid-body 'activity bump' shifts around the space map in order to maintain accurate relative angle. Axons from multiple sensory interneurons converge on the protocerebral bridge, which projects to the ellipsoid body (Lin et al., 2013). In turn, ellipsoid body output projects to premotor circuits involved in locomotion (Guo and Ritzmann, 2013; Martín-Peña et al., 2014; Martin et al., 2015). Computational modelling has demonstrated that the connectivity patterns of neurons in the ellipsoid body network promote Winner-Takes-All competitive selection across a wide dynamic range (Kakaria and De Bivort, 2017; Kim et al., 2017), resulting from lateral inhibition and recurrent projections

(Zalucki et al., 2015; Kakaria and De Bivort, 2017; Kim et al., 2017; Kottler et al., 2017), producing a single coherent activity bump and widespread suppression of alternatives resulting in a cross-sensory 'priority map' (Kottler et al., 2017) potentially homologous to the vertebrate Tectum or Basal Ganglia (Strausfeld and Hirth, 2013; Fiore et al., 2015). Ongoing neural activity represents the chosen destination even when sensory cues are not available, and switch-like changes can shift the destination (Seelig and Jayaraman, 2015; Kakaria and De Bivort, 2017). Intriguingly, recordings from TB and Ring neurons, which form part of an ascending sensory chain conveying visual information from the Anterior Optic Tubercle to the Ellipsoid body, show these cells respond to rival bilateral stripes with *weighted* attentional responses reminiscent of classical primate cortical cell experiments (Sun et al., 2017). However, when either the ipsilateral or contralateral stripe was primed by a prior presentation, response characteristics shifted towards a more characteristic WTA pattern (Sun et al., 2017).

Thus, it appears that insects presented with multiple 'landmarks' for orientation utilize winner-takes-all selective attention to select one stimulus for fixation, but the majority of these studies were conducted when insects were tethered in place, using an air-supported ball to 'move' the stimulus around a central point. How do insects behave during free locomotion? Frighetto et al (2019) presented a distracting visual target to *Drosophila* already engaged in visual goal-directed locomotion. *Drosophila* freely moving in a circular arena move towards and LED stripe by first orienting towards it and then walking forward. However, when a second stripe appeared 60° laterally offset to the target, *Drosophila* exhibited attentional capture by shifting their direction of locomotion to a weighted mid-point between the original and newly appeared target. Although this distractor appeared during original target fixation rather than a two-target choice as in earlier experiments, it is significant that flies exhibited *weighted* rather than *winner-takes-all* attention during this experiment. It is currently unknown whether attentional capture or free behavioural locomotion was the important factor in this (Frighetto et al., 2019).

In vertebrates it is thought that directed attention towards lesser-salience stimuli, and attentional switching between stimuli, involve top-downs suppression signals from higher order brain regions. Evidence suggests that the invertebrate Mushroom Bodies (MB) may serve a similar function for insects. The Mushroom Bodies are a higher order, midline, multimodal sensory integration neuropil primarily involved in experience dependant learning and memory functions (Zars, 2000). MB-deficient mutant *Drosophila* exhibit reduced selection abilities when stimuli are of equal contrast (Xi et al., 2008), in the presence of a cross-modal (olfactory) distractor (Xi et al., 2008), when facing contradictory cues (Tang and Guo, 2001), as well as reduced efficacy of priming (Koenig et al., 2016b), and reduced ability to detect 'pop-out' high-salience stimuli amongst multiple distractors (Xi et al.,

2008). Intriguingly, while flies with in-tact mushroom bodies are able to rapidly 'switch' selection between rival stimuli, MB-deficient flies show more linear, gradual shifts (Van Swinderen, 2007). Together, these effects suggest the Mushroom bodies are critically involved in stimulus selection, and are thought to be the origin of suppressive signals that aid in stimulus selection and background noise filtering (Xi et al., 2008), consistent with other demonstrated roles in object-background segregation (Peng et al., 2007).

Visual Search and Attentional Templates in Honeybees and Butterflies

Other selection behaviours have also been studied in insects, although in less detail. Visual Search tasks involve an observer looking for an object within a cluttered, distractor-rich background, and has been extensively studied in vertebrates (Wolfe, 2020). Detecting camouflaged targets during visual search can be challenging (Wolfe, 1994), presenting time-accuracy trade-offs (Chittka et al., 2003; Chittka and Spaethe, 2007) and increased predation risk (Ings and Chittka, 2008; Wang et al., 2013, 2018). Foraging Visual Search behaviours in butterflies and bees provide an analogue to visual search studies in vertebrates (Spaethe et al., 2006; Nityananda and Pattrick, 2013; Gamberale-Stille et al., 2019), showing evidence for serial search in honeybees (Spaethe et al., 2006) and parallel search in bumblebees (Morawetz and Spaethe, 2012; Nityananda and Pattrick, 2013). While foraging in a mixed-flower field, bees must distinguish between highly and poorly rewarding flowers while avoiding predators, such as crab spiders. To achieve this, bees match the incoming sensory stream to a 'search template' based on colour and shape (Goulson, 2000; Spaethe et al., 2001). When visual cues must be used both to locate rewards and avoid 'robotic' spider predators bees perform poorly at both tasks (Nityananda and Chittka, 2015), especially when spiders are cryptic (Ings and Chittka, 2008; Wang et al., 2013, 2018), but both tasks can be performed successfully when predators can be avoided with visual cues while rewards can found with olfactory cues (Nityananda and Chittka, 2015). Similarly, butterflies performing visual search for a suitable oviposition host plant can be primed to a particular host species with experimental exposure, increasing search efficiency for that plant but reducing ability to find other viable host species (Gamberale-Stille et al., 2019), suggesting priming activates a search template for the primed features, at potential cost for a generalist species.

Attention to Targets in Invertebrate Predators: Mantises, Jumping Spiders, and Dragonflies

Attention and predatory behaviour have been the subject in only a few studies of invertebrate attention. An early study in a praying mantis showed that mantises presented with two moving targets saccade to each in serial, but fixate preferentially on a target that appears closer or prey-like (Rossel, 1996). Although not insects, jumping spiders have been a popular model for studying predatory attention in small brains. Jumping spiders can flexibly 'shift' or 'lock' attention to a stimulus in the face

of peripherally appearing distractions depending on the relative attractiveness of the central and peripheral targets (Bruce et al., 2021). As with honeybees engaged in visual search, jumping spiders must detect targets amidst a cluttered environment. Priming jumping spiders with the scent of blood or colour red enhances the speed with which they can detect obscured targets in cluttered scenes (Cross and Jackson, 2010), and specific olfactory priming for a single type of prey induces a search image that enhances ability to detect that prey at the cost of detecting alternatives (Cross and Jackson, 2009).

Target selection has also been observed in the intracellular spiking response of a higher-order dragonfly visual neuron 'Centrifugal Small Target Motion Detector 1' (CSTMD1,Geurten et al., 2007), which gives a robust winner-take-all response when presented with paired targets (Wiederman and O'Carroll, 2013a) or a single target embedded in clutter (Wiederman and O'Carroll, 2011; Evans et al., 2020). Behaviourally, dragonflies exhibit immunity to the confusion effect (Jeschke and Tollrian, 2007; Combes et al., 2012), and artificial neural network modelling suggests that the highly modular architecture of the insect brain may be less susceptible to confusion than the more convergent vertebrate brain (Tosh and Ruxton, 2006). Having discussed the mechanisms of selective attention in vertebrates and the literature on visual attention in insects, we now turn to specific discussion of the dragonfly visual system, beginning with an account of predation behaviour and then following the path of visual information from the retina and into the brain.

How Dragonflies Pursue and Catch their Prey

Figure 4: *A male dragonfly (Hemicordulia tau) patrols its territory over a lake in the Adelaide Botanic Gardens.*

Dragonflies are incredible aerial pursuit predators, capable of amazing feats of aerial and neuronal performance. The dragonfly lacks a tympanic membrane and has comparatively reduced antennae, implying poor auditory and olfactory senses (Corbet, 1999). As a result, vision is the dragonflies' main source of information about the world, and most dragonfly behaviour is heavily dependent on visual cues (Corbet, 1999). When a dragonfly spots its prey, it may launch itself into the air with an acceleration of 1.52 ± 0.4 g, reaching maximum speed of 2.28 ± 0.46 m/s (Combes et al., 2010) and embarking on an interception path where the future position of the prey is actively predicted (Mischiati et al., 2015) and turns with a radius of curvature as small as 4.1 ± 2.4 cm (Combes et al., 2012), far exceeding the flight performance of typical prey (Ray et al., 2016). Yet during these flights the dragonflies' target rarely spans more than 1 degree of visual space (Lin and Leonardo, 2017), stimulating only two or three ommatidia of the compound eye at a time (Horridge, 1978), against an often cluttered background of variable contrast and in variable luminance conditions, often with swarms of distracters and conspecifics nearby (Edman and Haeger, 1974). Yet despite these sensory and locomotor challenges, dragonflies are able to achieve capture success rates above to 90% (Olberg et al., 2007; Combes et al., 2013).

In order to successfully capture prey, the dragonfly must first visualise the target against variable background clutter and self-generated optic flow, and then track its chosen target in the face of sensory interference derived from background clutter and target-like distractors during swarm conditions. In this section, we review the visual system of the dragonfly with a focus on target detection, beginning with a brief account of dragonfly natural history and behaviour.

Evolution and Development

The earliest known flying animals, *Odonatoptera (*The insect order including modern dragonflies (Anisoptera), damselflies (*Zygoptera*), and their extinct relatives*)* appeared in the carboniferous period approximately 300 Ma (Nel et al., 2009; Petrulevicius and Gutierrez, 2016) in a terrestrial environment dominated by vast swampy forests and moorlands. The very first *Odonatoptera* where more damselfly-like, with slender bodies, narrow wings and separated eyes (Jarzembowski and Nel, 2002). These first proto-damselflies probably evolved flight to practice predatory gleaning as in modern damselflies, where terrestrial prey are plucked from a surface such as the ground, vertical tree trunk, tree branch, or even a spider web. However, by the appearance of

modern dragonflies in the Jurassic, approximately 150 Ma ago (Huang et al., 2017; Nel et al., 2017), dragonflies had already developed a variety of wing, eye, and leg specialisations suggestive of aerial predation (Nel et al., 2018). For example, the paleozoic 'giant dragonflies' (*meganisoptera,* also known as Griffinflies, though not true dragonflies), a now-extinct offshoot of the lineage leading to modern dragonflies, display enlarged holoptic compound eyes that fuse at the dorsal eye, as in extant hawking dragonflies where the compound eye develops a highly specialised zone for tracking targets against the sky (Labhart and Nilsson, 1995; Sauseng et al., 2003). Although early dragonflies probably could not match the aerobatic performance of modern *Odonata* (Nel et al., 2018), the presence of 'smart' mechanisms built into the physical anatomy of the wing to manipulate wing shape in response to air stressors without direct neural control suggest they were already adapting towards high-performance flight and aerial predation (Wootton et al., 1998).

Dragonflies begin life as aquatic larvae, where they emerge as already ferocious predators. Many dragonfly larvae, known as nymphs, are ambush hunters that hide within aquatic foliage, reeds, and even under sand or rock beds (Corbet, 1999) before striking their prey with morphologically specialised lower jaw that acts as a 'spring-loaded catapult' (Büsse et al., 2021). Still others stalk prey on the river bed in order to bring it within range of their jaw (Corbet, 1999). Although dragonfly nymphs, like adults, are primarily visual predators that use specially adapted compound eyes to detect and track prey, the current work is focused on pursuit predation in adult dragonflies and, thus, discussion of the aquatic larval eye and behaviour is beyond our scope (but see Chou et al. (2020) for a review).

Following up to several years of aquatic life, and having undergone several instar transitions, dragonfly nymphs climb out of the water and emerge as an adult dragonfly, where they will live for up to a couple of months (Corbet, 1999). As adults, dragonflies are aerial pursuit specialists that capture prey, guard territory, and mate almost entirely on the wing. It is to these adults that the rest of this work will be devoted.

Dragonfly Behaviour

Dragonflies are extremely agile, capable of high speeds, hovering flight, and acute manoeuvrability due to intricate wing innervation that grants independent control of each of the four wings (Bomphrey et al., 2016), allowing them to overcome the general rule of biology that larger animals are slower than smaller counterparts, and outmanoeuvre their prey (Combes et al., 2012, 2013). During predatory flights, dragonflies approach targets from behind and below (Olberg et al., 2000) in order to take advantage of the target's blind spot and keep their target fixated in a fronto-dorsal position of the eye, at the position of the dorsal acute zone (Olberg et al., 2007), maximising target contrast by holding the target against the bright sky in the area of the high with the highest

spatial resolution (Kirschfeld and Wenk, 1976). Even so, the target rarely spans more than 1 degree of visual space, stimulating only two or three neighbouring ommatidia (Horridge, 1978; Lin and Leonardo, 2017).

Feeding and Territorial Behaviours

Odonates employ a variety of different feeding strategies to feed on a large variety of different prey, all of which are employed by true dragonflies in varying degrees. Dragonflies are broadly generalist predators, but some species may be specialised for particular types of prey (Baird and May, 1997; Combes et al., 2013). For example, different species of dragonflies show different preferences and capture success for prey of different sizes and types (Baird and May, 1997; Olberg et al., 2005; Combes et al., 2013).

Three feeding strategies are found in the Odonata (Corbet, 1999; Wootton, 2020); hawking, typical of *Aeshnidae*, *Corduliidae* and a few *Libellulidae*, where the dragonfly catches prey on the wing, during flight; perch-darting, typical of most *Libellulidae* and Damselflies, where the odonate remains on a perch until prey flies past, and then darts off to catch it before returning its perch to eat, and; Gleaning, typical of *Coenagrionidae* Damselflies, but also observed in some Hawking dragonflies, where the predatory odonate plucks prey from a surface such as the ground, tree trunk or branch, the water's surface, or a spiders web.

It is important to note that, although the behavioural hawking/perching dichotomy among dragonflies is broadly accurate and reflects underlying differences in anatomy and thermoregulation (Corbet and May, 2008), it is based on the *territorial* period of a dragonflies daily activity. As broadly diurnal predators, dragonflies of both types spend the night perched within foliage until first light the next day, where they begin to warm up either by direct action of the sun or by wing-whirring (Corbet, 1999; Corbet and May, 2008). Recently awoken dragonflies engage in 'Swarm Feeding' early in the morning, where large congregations of dragonflies come together to feed on swarming insects also awakening for the day. In these large congregations, territoriality is not enforced and most species do not engage in reproductive chases or behaviours. Notability, most dragonflies act as 'hawkers' during these swarm-feeding congregations. Following this, adult dragonflies disperse to streams and ponds to carve out a territory to defend, while females search for suitable oviposition sites. It is during these periods of activity – usually around mid-morning to mid-afternoon, with a break during the hottest parts of the day, that the hawking vs. perching dichotomy is most prevalent. Here, hawking dragonflies will define a large territory to be patrolled, and will respond vigorously to any male intruders by chasing them off, and to any female intruders with attempted copulation. During these patrols, many

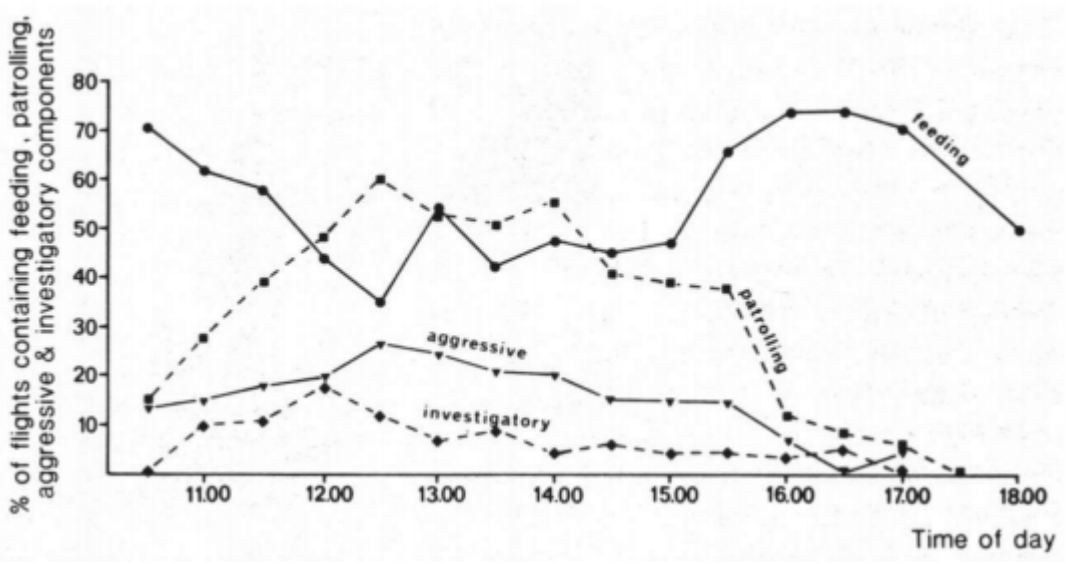

Figure 5: Daily Flight Activity of Orthetrum coerulescens *(Anisoptera: Libellulidae), a perching dragonfly, across the day. (Analysis of 1323 territorial flights from Parr (1983)). Note the bimodality of the feeding behaviour centred on mid-morning (10:00) and mid-afternoon (16:30).*

hawking dragonflies are opportunistic hunters. Perching dragonflies will also take territories, usually defined by the area they can reach within a certain duration of flight-time from their chosen perch, where they will lay in wait for a passing conspecific female. Like hawkers, perchers will vigorously chase away any intruding males before returning to their perch, and will often make sallies to capture passing prey. Following several hours of territorial activity, many dragonflies will return to hawking 'swarm feeding' congregations during the final hours of daylight (Corbet, 1957, 1999; Edman and Haeger, 1974; Parr, 1983; Miller and Miller, 1985; Orr, 2003; Corbet and May, 2008; Kosterin, 2008), including species that are normally perchers, E.g: *Brachythemis lacustris* (Miller, 1982); *Orthetrum coerulescens* (Parr, 1983); *Parazyxomma flavicans* (Dijkstra, 2003); *Stylurus plagiatus* (Corbet and May, 2008); *Sympetrum sanguineum* (Gorb, 1994)*, and Sympetrum striolatum* (Corbet and May, 2008). Thus, most dragonflies, though not averse to opportunistic feeding during territorial behaviours, complete most of their feeding in crepuscular 'swarm-feeding' congregations at the start and end of the day (Figure 5).

High-performance predation is critical for all *odonates*, as the gain in mass associated with predation directly affects reproductive fitness for both females, by increasing fecundity (Anholt, 1991), and males by increasing flight muscle mass, driving the acquisition of territory and mating success (Marden, 1989). However, foraging flight also increases the risk of being predated on by birds or larger odonates (Anholt, 1991), and for perching species a sallying flight introduces the risk of losing their perch to a competitor (May and Baird, 2002). Thus, predatory performance (capture success, pursuit time, distance travelled) has consequences not just in terms of energetics (losses associated with flight Vs. gains associated with feeding), but also increases risk of mortality, territory loss, and missing an arriving female.

Dragonflies are therefore motivated to increase prey capture efficiency as much as possible in order to maximise relative gains and minimise risks. Behavioural studies show that dragonflies achieve relatively high capture success rates, ranging from 90-97% foraging on fruit flies (Olberg et al., 2000; Combes et al., 2013), but success rate drops dramatically with increased prey size and velocity (Combes et al., 2013), down to as low as 20% for *Libellula cyanea* hunting large deerflies (*Chrysops sp.*) (Combes et al., 2013). As dragonflies appear to initiate capture for any target around 1.5° and rarely pursued targets greater than 3° (Combes et al., 2013; Lin and Leonardo, 2017), larger targets were only pursued when further away thus increasing flight time, and decreasing capture efficiency. Moreover, larger prey are more in the habit of active manoeuvring, and are often able to evade dragonflies during their final capture manoeuvre when dragonflies must pitch sharply to bring their legs (on the ventral side of the body) into range of the target, which is held dorsally during pursuit (Olberg et al., 2000; Mischiati et al., 2015) moving the target from their well-adapted dorsal eye into the poorly target-adapted ventral eye, with an erratic evasive turn (Combes et al., 2012, 2013). Despite these difficulties in capturing larger targets when far away, by ignoring any potential target greater than 3° dragonflies may be passing up the most efficient prey – a nutrition-dense large *dipteran* flying within easy reach (Combes et al., 2013).

Target Selection and Pursuit Initiation

Perching dragonflies of North America and Europe have been the most popular models for studying dragonfly behaviour, especially as it concerns predation and target selection. The fact that perching dragonflies continuously return to the same perch after either a successful or unsuccessful sally makes it easier to obtain repeated high-speed recordings required to measure body position and head angle relative to either an experimental fake target or live, introduced prey (Sauseng et al., 2003; Olberg et al., 2007; Combes et al., 2012, 2013). Given the potential costs associated with even a short flight, dragonflies must choose their hunts to carefully balance potential nutritional gain against energetic loss, predation, and territorial loss risks. Although the majority of studies addressing these questions have been undertaken in perching dragonflies, hawking dragonflies likely face similar decisions.

Studies addressing this in perching dragonflies have uncovered that dragonflies only pursue prey within a limited range of distances (Olberg et al., 2005, 2007; Combes et al., 2013) using a series of heuristic rules based on the target's angular velocity (Lin and Leonardo, 2017). Perching dragonflies make rapid head movements ('saccades') immediately before take-off (Miller, 1995; Sauseng et al., 2003), which were previously thought to allow the dragonfly to estimate target distance by computing motion parallax (Olberg et al., 2005). However, a recent analysis discovered that the image speeds during these head movements were too small for accurate parallax computation, and that instead

these head movements serve to orient the dragonfly's direction of gaze (Lin and Leonardo, 2017). The dragonfly eye contains on its dorsal surface a 'dorsal acute zone' specialised for target tracking, within which the image of the prey is fixed during these pre-take-off saccades (Miller, 1995; Sauseng et al., 2003; Lin and Leonardo, 2017) and kept during the interception flight (Olberg et al., 2007; Mischiati et al., 2015). Following this initial fixation saccade, the dragonfly head tracks the prey for ~250 milliseconds while it is estimated the dragonfly makes a decision on whether to engage or not, given not all fixation saccades are followed by a take-off (Lin and Leonardo, 2017). It is currently unknown if hawking dragonflies make this same series of movements while hovering or in flight, before deciding to pursue an observed target.

Work across labs and dragonfly species has found that angular size and speed are the most important factors for a dragonflies' decision to pursue a target (Olberg et al., 2005; Combes et al., 2013; Duong et al., 2017; Lin and Leonardo, 2017). Although different species have different preferences, dragonflies will pursue any target within the correct size and velocity range, suggesting they maintain a broadly turned model of angular target properties matched to the motion statistics of prey within their striking range (Lin and Leonardo, 2017), although there are conflicting reports on whether or not dragonflies area able to determine the absolute distance of prey. In one study, dragonflies appeared to ignore targets larger than their heads even if it subtended the same angular size as previously captured prey (Olberg et al., 2005).

If a pursuit is intended, the dragonfly makes a series of preparatory leg, wing, and body movements before taking off as the prey passes the zenith above the dragonfly. Intriguingly, due to the time required to make such movements and the response latency of visual neurons thought to register the approach of the prey (Gonzalez-Bellido et al., 2013) the dragonfly must predictively engage predatory movements to put the legs, wings, and body in a stereotypical position for take-off timed just as the prey reaches zenith (Lin and Leonardo, 2017).

Interception Strategies and Prey Capture

Once airborne, there are two broad computational strategies a predator can apply to the problem of intercepting a target. The first and simplest is known as classical pursuit (Also known as pure pursuit, direct pursuit, smooth pursuit). This strategy describes a scenario where the pursuer actively follows the target by steering to minimise the angle between its own flight path and that of the target (Collett and Land, 1978). This pursuit strategy yields a simple geometry in which flight is always aimed directly at the target (Figure 6, Classical Pursuit), causing the pursuer to give 'chase' and guaranteeing successful interception if the pursuer is faster than the pursued. Alternatively, proportional navigation (also known as parallel navigation) seeks to maintain a constant acute relative angle to the target (Figure 6, Proportional navigation), allowing the pursuer to ignore many target

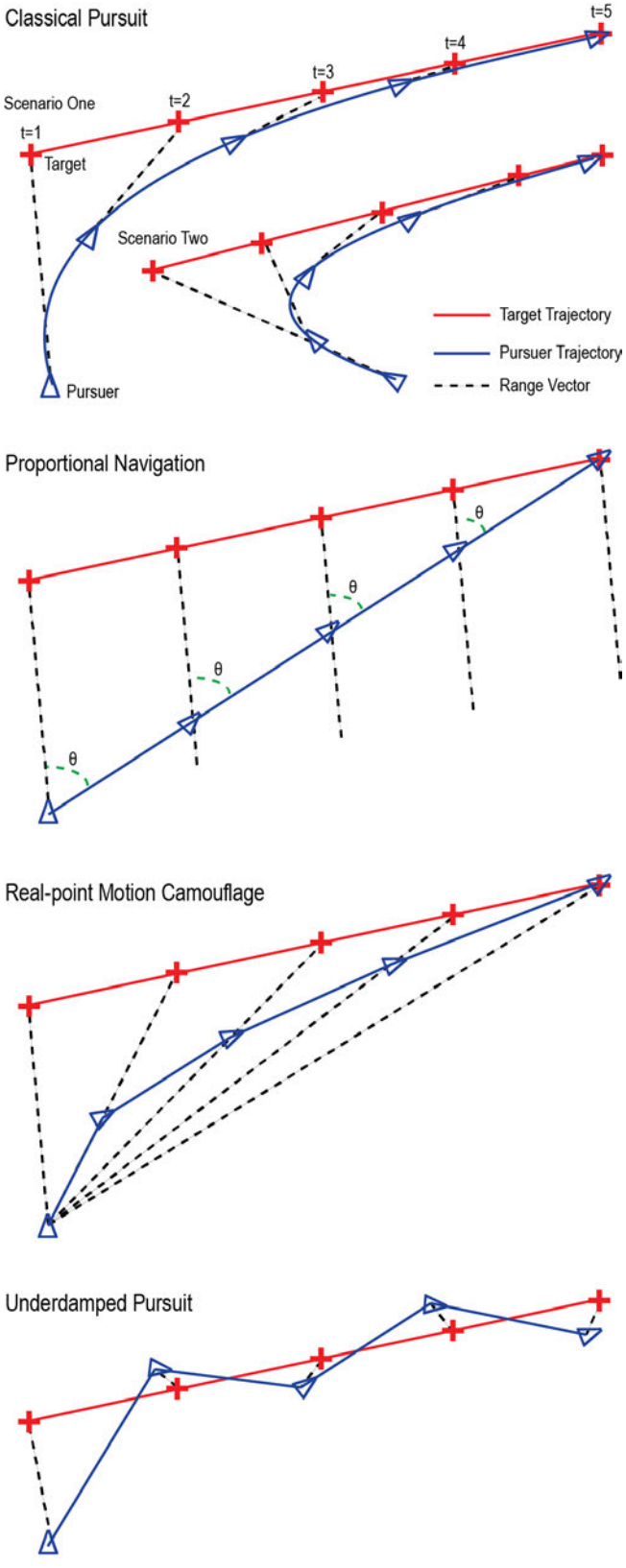

***Figure 6: Pursuit Strategies seen in Dragonflies**, reproduced from Lancer et al. (2020). Classical (also known as tracking, smooth pursuit, pure pursuit, and simple pursuit) and Proportional navigation (also known as Interception or Parallel navigation) are two strategies used to catch a target. In Classical pursuit, the pursuer moves towards the target along the current range vector, resulting in in a 'chase' (Scenario One). If the pursuer notices the target before it has crossed ahead of the pursuer, the pursuer's trajectory will start in one direction and then turn as the target crosses ahead (Scenario Two). In Proportional navigation the target position is held constant relative to the pursuer while the length of the range vector is minimised. There are a number of steering laws that can be used to implement proportional navigation. Here, we have illustrated the Constant Error Model (CEM) where the error between the target and pursuers' trajectory is maintained stable. Real-point Motion Camouflage is a special case of proportional navigation in which a real-point landmark, rather than the target, is held constant relative to the pursuer. Here, the pursuers approach is disguised in background information, by remaining on a path where it is interposed between the target and a constant real point behind the pursuer. Underdamped pursuit is an aggressive strategy involving repetitively overshooting a target that is employed in conspecific engagements where the goal is to evict a territorial intruder, rather than make physical contact.*

uncertainties such as size, speed, etc (Wardill et al., 2017). Changes in the target's trajectory can be countered by steering to minimise the target's relative angular movement (Shneydor, 1998; Mischiati et al., 2015) and keep the target fixed in the dorsal acute zone (Olberg et al., 2007; Mischiati et al., 2015), and as long as the angle is kept acute this strategy is guaranteed to generate an interception point between the pursuer and target flight paths *eventually*, although most dragonflies will give up after an amount of unsuccessful flight time (Lin and Leonardo, 2017). This pursuit strategy can be implemented through a variety of computational mechanisms that maintain constant the angle (Olberg et al., 2000), relative direction (Ghose et al., 2006), or bearing (Olberg et al., 2000; Conchrane, 2005) of the target relative to the pursuer. This guidance law can be epitomised by the 'constant bearing, decreasing range' strategy employed by frigate captains in the Napoleonic wars to chase down a fleeing opponent

(Conchrane, 2005). This strategy has been observed in bats (Ghose et al., 2006), hoverflies (Collett and Land, 1978), Peregrine Falcons (Brighton and Taylor, 2019), robber flies (Wardill et al., 2017), Hawks (Kane et al., 2015), and is implemented in surface-to-air and air-to-air missiles (Shneydor, 1998). Dragonflies often exhibit proportional navigation in both predatory (Olberg et al., 2000, 2007; Mischiati et al., 2015) and territorial (Bomphrey et al., 2016; Lohmann et al., 2019) engagements, but also display classical pursuit in some conspecific engagements (Bomphrey et al., 2016). What factors lead dragonflies to choose one pursuit strategy over another is currently unknown, but classical pursuit may be used primarily by males to chase females.

Until recently it was thought that the proportional navigation employed by dragonflies was driven by a reactive neuronal autopilot (Collett and Land, 1978; Gonzalez-Bellido et al., 2013) where sensory information is passed to motor actuators without significant transformation. However, recent research has shown that many of the body, head, and flight adjustments both before (Lin and Leonardo, 2017) and during (Mischiati et al., 2015) pursuit occur too quickly to be accounted for by a response to live visual input, and thus must be driven by an internal predictive representation of prey movement and body position.

During pursuit dragonflies can achieve speeds of up to 2.28 m S^{-1} and make tight turns with curvature as little as 4.1 cm (Combes et al., 2012). Such flight capabilities far exceed the flight performance of typical prey (Combes et al., 2012; Ray et al., 2016).However, prey are not the only species of target a dragonfly is motivated to track and pursue. Male dragonflies pursue female conspecifics for mating purposes, and will purposefully engage both conspecifics and heterospecifics (Moore, 2000) in territorial defence. Unwarranted male attention can be overcome by female dragonflies by flying faster than or flying loops around males (Rüppell and Hilfert-Rüppell, 2014), or by simply dropping from the sky (Khelifa, 2017). Male-male territorial interactions can be described as an aerial dogfight, in which dragonflies make use of rapid and acute manoeuvres to drive off competitors (Beckemeyer, 2009; Bomphrey et al., 2016; Lohmann et al., 2019). When approaching a conspecific, dragonflies can make use of two distinct behavioural strategies. The first is known as 'real-point motion camouflage,' describing a scenario where the pursuer embarks on a trajectory that keeps its image stationary relative to a fixed point on the target's retina by keeping itself on a line between the target and some fixed point target (Figure 6, Real-Point Motion Camouflage), thus disguising its own motion in optic-flow information and appearing stationary (though looming) to the target (Srinivasan and Davey, 1995; Mizutani et al., 2003). Alternatively, dragonflies can also use an aggressive interception that involves overshooting of the target from alternate directions (Figure 6, Underdamped pursuit) (Lohmann et al., 2019). This strategy could be utilising evoked evasive responses from the target to 'herd' the target towards a territorial boundary (Lohmann et al., 2019).

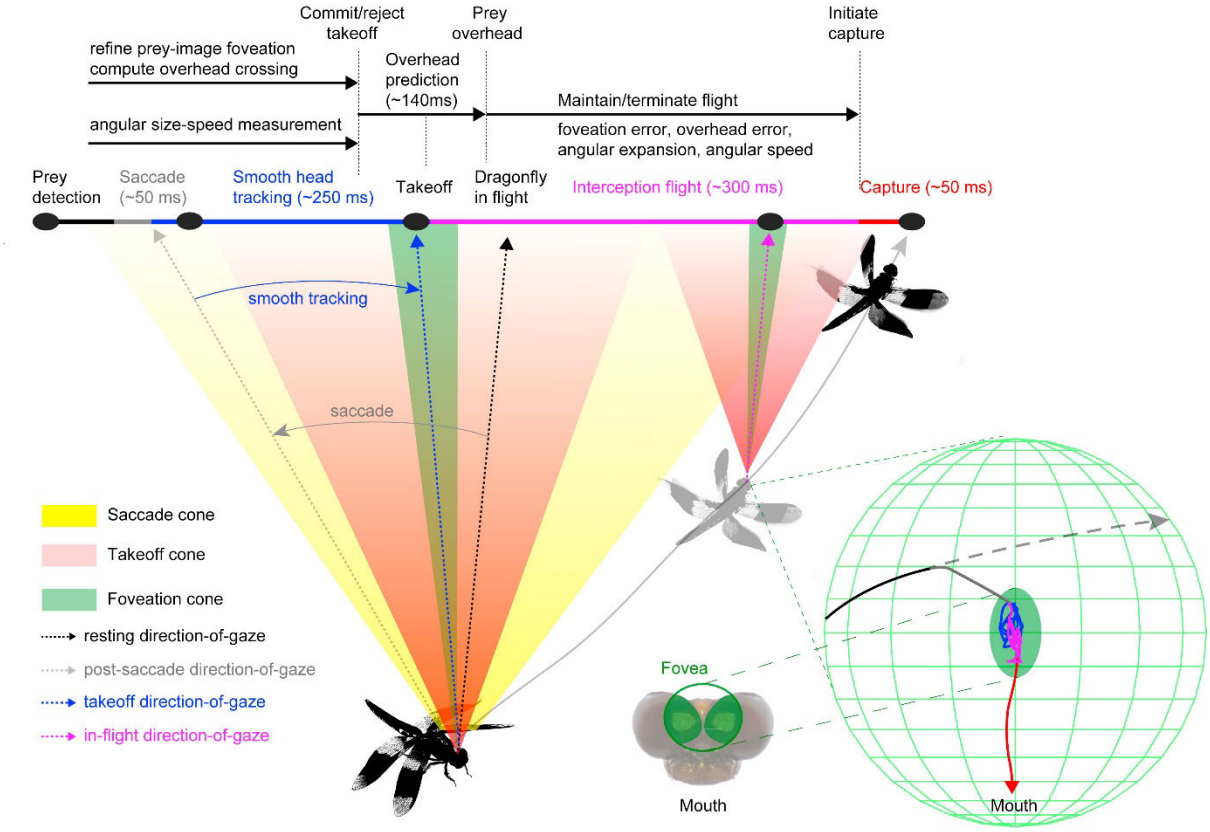

Figure 7: Summary Schematic of Heuristic Rules for Dragonfly Prey Selection and Interception*, reproduced from(Lin and Leonardo, 2017). This illustrates the behavioural movements leading up to the initiation of flight and final capture in a perching dragonfly, although a capture attempt can be terminated at any point. Insert: Target position on the retina is illustrated in the green circle. Following prey detection the dragonfly makes a rapid saccade to fixate the target within its dorsal acute zone. During the following period the dragonfly visually tracks the target with smooth head tracking that decreases the initial foveation error and makes a decision on if it is going to attempt to capture the prey based on angular size and velocity (Lin and Leonardo, 2017). The majority of 'attempts' are aborted at this stage, pre-takeoff. Meanwhile, the dragonfly predictively begins a series of preparatory leg, body, and wing movements timed to initiate take-off as the target reaches the zenith directly above the dragonfly (Lin and Leonardo, 2017). In flight, the dragonfly uses a Proportional Navigation strategy in order to generate an interception path with the target by keeping the target fixated in the Dorsal Acute Zone (the pink line remains within the fovea), by maintaining a fixed relative angle (Olberg et al., 2017) correcting for both predicted and observed deviations (Mischiati et al., 2015). Once the dragonfly nears its target, the body is pitched > 90° in order to facilitate capture with the long, specially adapted forelegs.*

Additionally, as continuous overshooting requires high speeds and repetitive high-magnitude turns, it may function as display of flight performance and an honest signal of fitness. Displays of performance are common in male-male competitive interaction (Enquist, 1985) and in many species play a role in sexual selection (Kuijper et al., 2012). In dragonflies, flight performance is dependent on health (Marden and Cobb, 2004) and wing integrity (Combes et al., 2010), which is in turn dependant on age. Intriguingly, age does not appear to affect mating success in damselflies (Hassall et al., 2015).

In predatory pursuit flights, once the dragonfly comes within approximately 30 mm of its prey, they dragonfly pitches it's body upwards in order to initiate capture with its legs (Lin and Leonardo, 2017), a manoeuvre that brings the prey from its fixation point in the dorsal eye down into the ventral eye with poorer spatial resolution. During this period, some erratically moving insects may be able to

avoid capture by making sharp evasive manoeuvres, although the dragonfly is likely to re-fixate and attempt capture again (Combes et al., 2012, 2013).

The Dragonfly Visual System

"Prey Capture is predominantly a sensory challenge rather than an aerobatic dogfight."

- Richard Bomphrey, Flight of the Dragonflies and Damselflies, 2016

Dragonflies are predominantly reliant on Vision for their everyday behaviour, for everything from flight control (due to a lack of halteres and abridged antennae (Corbet, 1999)), navigation (Bernáth et al., 2002; Eason, Perri K., 2006), habitat selection (Laughlin and McGinness, 1978; Bernáth et al., 2002; Kriska et al., 2009), conspecific recognition (Schultz and Switzer, 2001; Schultz and Fincke, 2009; Brydegaard et al., 2018; Schröder et al., 2018), predatory selection (Olberg et al., 2005; Duong et al., 2017; Lin and Leonardo, 2017) and tracking (Olberg, 2012). In predation the overwhelming aerobatic prowess of the dragonfly far exceeds the capabilities of typical prey (Combes et al., 2013; Ray et al., 2016), and when dragonflies do fail a predatory capture attempt, it is usually because the prey has erratically and unpredictably moved during the final moments of the capture sequence where they prey leaves the dragonflies sight (Combes et al., 2012). To paraphrase Richard Bomphrey; Predatory pursuit is a sensory challenge, not a motor one (Bomphrey et al., 2016). This behavioural reliance on vision is reflected in the complexity of their visual system in comparison to other insects (Evans et al., 2019; Fabian et al., 2020). This section of the introduction will walk through the dragonfly visual system with a focus on adaptations, cells, and pathways that support target tracking and *predatory* behaviour.

Optics, Photoreception, and Retinal Processing

Dragonflies' poses a *Compound Eye* comprised of thousands of individual optical units known as *ommatidia* (or *ommatidium* in singular), or *facets* (Figure 8A). The compound eye architecture consists of a matrix of these ommatidia on a convex surface protruding from the head, each of which presents a crystalline lens structure shaped like an upside-down pyramid to the outside world that focuses incoming light like a funnel onto a single *Rhabdom*, a rod-like structure containing several photoreceptors (Figure 8B). There are several compound eye designs known throughout the arthropod phylum, the most common of which is known as the *apposition eye,* which is seen in dragonflies as well as many other diurnal insects.

In the apposition eye, Individual ommatidia are optically isolated from their neighbours by opaque chitin, which in concert with the focusing effect of the pyramidal lens structure ensures each

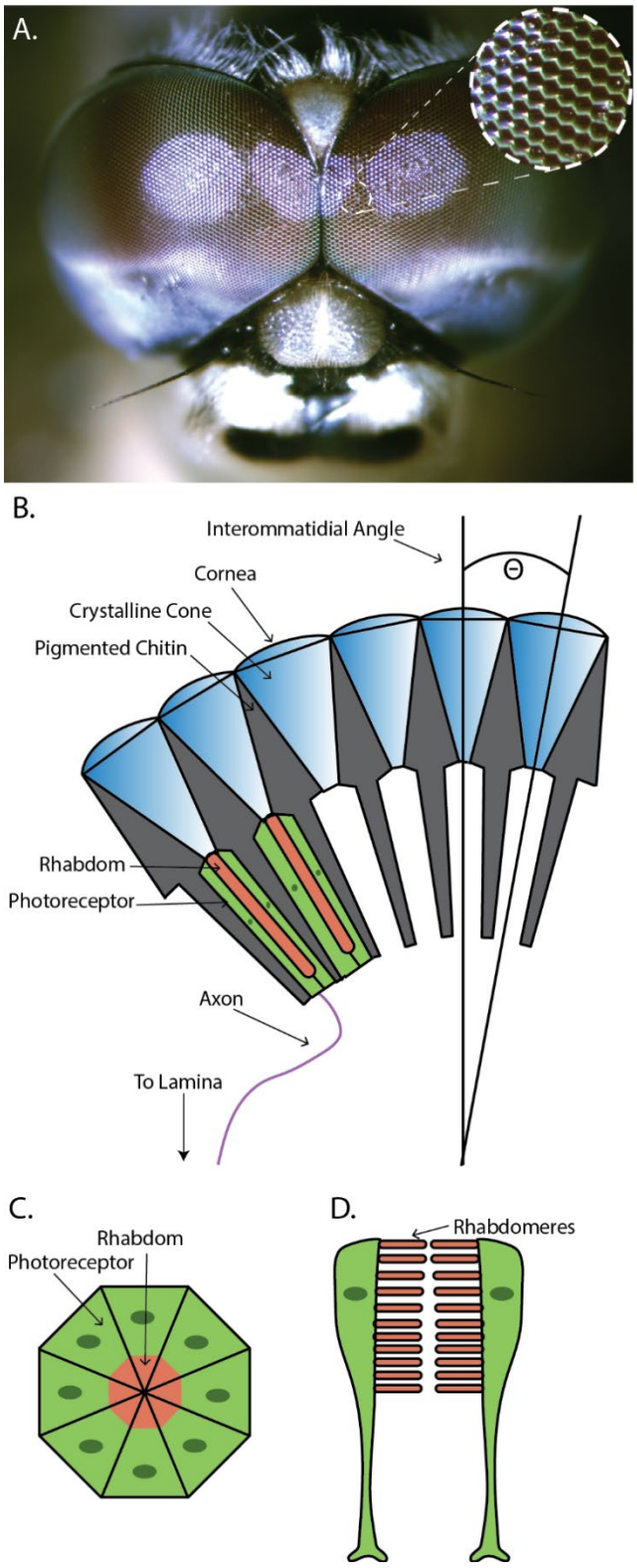

Figure 8: Optics of the Apposition Eye. *Each compound eye is made up of several thousand Ommatidium, the individual compound eye unit containing a Lens, Rhabdom, and several photoreceptors.* ***A)*** *Dorsal view Image of the eye of the Dragonfly Hemicordulia tau. Insert; zoomed-in view showing individual facets.* ***B)*** *Sagittal section of an Opposition-type compound eye. Light is focused through the lens, comprised of the Cornea and Crystalline Cone, onto the Rhabdom below.* ***C)*** *Horizontal section at the level of the Rhabdom. Several photoreceptors form a 'cylinder' packed between the pigmented chitin of the Ommatidium, projecting their pigmented Rhabdomeres into the centre to gather photons. This image shows a Fused Rhabdom as in Dragonflies, but in many insects the Rhabdomeres are unfused.* ***D)*** *A pair of Photoreceptors.*

ommatidia receives photons from a narrow angle, giving rise to a single image point, or 'pixel' of a visual space.

One disadvantage of this ocular architecture in comparison to the vertebrate single-lens camera-eye is decreased *spatial resolution*. On the compound eye, the spatial resolution is defined by the *interommatidial angle*, the angular separation between neighbouring ommatidia. Lower interommatidial angles and increased ommatidia count improve spatial resolution, as each ommatidium is responsible for a smaller region of space. Dragonflies possess the largest compound eyes and smallest interommatidial angles thus-far measured, down to as little as 0.24 degrees in the family of large dragonflies *Aeshnidae* (Land, 1997).

In most insects, the interommatidial angle varies across the surface of the eye (Land, 1989), forming regions specialised for specific visual information and behavioural demands. Many predatory insects, including dragonflies, possess an acute zone analogous to the vertebrate fovea (Horridge, 1978; Land and Eckert, 1985; Wardill et al., 2017), containing decreased interommatidial angles that increase spatial resolution and specialised photoreceptors that improve temporal resolution (Weckström and Laughlin, 1995; Burton and Laughlin, 2003). These optical and physiological specialisations improve the detection of small,

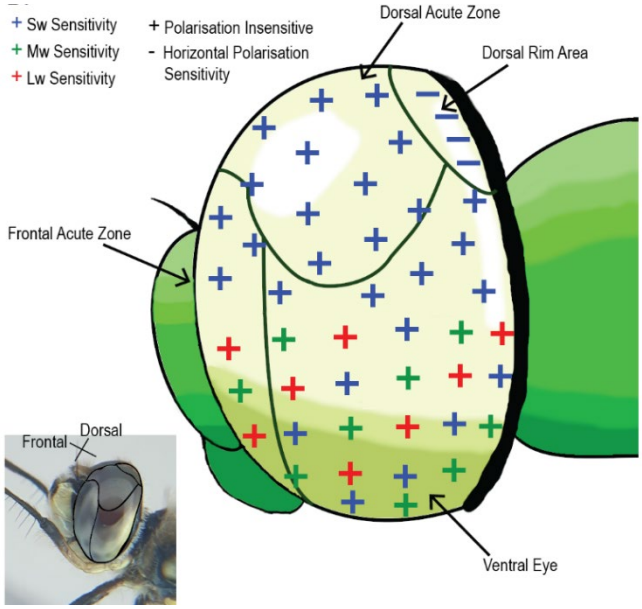

Figure 9. The Compound Eye of the Dragonfly. *Reproduced from Lancer et al (2020). Illustration of the dragonfly eye (Credit: Bernard Evans), illustrating prominent zones, spectral sensitivity (Sw = short wavelength, Mw = Medium wavelength, Lw = Long wavelength), and polarisation sensitivity. Note the ventral eye also contains horizontal polarisation sensitive photoreceptors, but their distribution is currently unknown. Insert: Boundaries of the major zones superimposed on an image of the dragonfly eye. Compass indicates direction. From front in clockwise: the Frontal Acute Zone, the Dorsal Acute Zone, the Dorsal Rim Area and the Ventral Eye.*

fast-moving objects such as prey, conspecific competitors, or potential mating partners. Acute zones are necessary as it would not be feasible to maintain such high levels of visual acuity across the entire eye, as this increased acuity requires more ommatidia, a larger surface aera, and decreased interommatidial angle that implies a 'flattening' of the eye surface from a sphere into an ommatidial sheet. Despite these high costs, many insects – including dragonflies – have evolved eyes that are relatively large compared to the remainder of the head in order to support the acuity required of behavioural demands (Figure 9).

The dragonfly eye contains two contiguous major acute zones, of which the dorsal acute zone is the better. The dorsal acute zone is positioned fronto-dorsally as a laterally-extending 'strip' branching from the midline of the dorsal surface of the head capsule where the eyes are conjoined (Figure 9). Here, it is positioned to capture the image of a potential target at maximum contrast, against the bright sky (Labhart and Nilsson, 1995). During target pursuit and foraging flight, dragonflies fly low and fix the image of the target in the dorsal acute zone (Kirschfeld and Wenk, 1976; Olberg et al., 2007; Mischiati et al., 2015; Lin and Leonardo, 2017).

This pattern of spatial division of the dragonfly compound eye is preserved down to the physiological make-up of photoreceptive cells in the underlying retina, especially in regards to the distribution of colour-sensitive opsins. In both vertebrates and invertebrates, opsins are G-protein coupled transmembrane receptors that, together with chromophores, form a visual pigment that is able to respond to physical interaction with a photon, causing an intracellular signalling cascade that ultimately results in photoreceptor depolarization (or hyperpolarization, in the case of vertebrates.) Opsins thus form the molecular basis of phototransduction and, ultimately, visual sensation.

Different kinds of opsins respond to different wavelengths of light, giving rise to spectral sensitivity. The *Anisopteran* eye contains a wide variety of colour-sensitive opsins that are differentially distributed both spatially across the eye, and temporally across different stages of dragonfly development (Futahashi et al., 2015; Futahashi, 2016). A detailed review of colour-

sensitivity in the dragonfly eye is beyond the scope of this introduction, which focuses on target-tracking behaviour and the neural pathways that support it, but see Appendix 1 for review (Lancer et al., 2020).

The dorsal eye of the dragonfly is dominated by photoreceptors with narrow short-wavelength sensitivity (Figure 9; Labhart and Nilsson, 1995; Futahashi et al., 2015), matching both optical and behavioural specialisations for target tracking and pursuit. During perching, hovering, and target pursuit the dorsal regions of the eye are likely to be pointed towards the sky, where a wide variety of spectral sensitivities is not necessary and where targets appear as a highly contrasting dark silhouette. The dorsal region is used for target pursuit in both conspecific engagement and predatory contexts, suggesting it forms the input to a non-target-specific visual stream. During diurnal territory patrolling flight, male dragonflies are concerned with establishing a territory over a water source, such as a lake edge or stream (using ventrally-directed polarizations sensitive photoreceptors; Laughlin and McGinness, 1978; Kriska et al., 2009), and then setting about patrolling and defending that territory against conspecific males and pursuing and mating with females (Kaiser, 1985; Corbet, 1999). During these hours males are focused on environmental cues about the suitability of their territory that are sensed though the ventral eye, and conspecifics mostly flying around the same vertical plane, sensed through the frontal and lateral eye. When a target is encountered, it is positioned along the midline in the dorsal acute zone, where it appears as a highly contrasting dark silhouette against a bright blue sky (Labhart and Nilsson, 1995; Olberg et al., 2000). However, most dragonflies are not entirely diurnal, but display crepuscular flights shortly after dawn and prior to dusk (Corbet, 1999). During these hours, the sky can be dominated by swarming midges, mosquitoes, and moths emerging for night flight, and it is in these conditions that dragonflies engage in predatory activity (Edman and Haeger, 1974; Corbet, 1999). Light from the sun during these dusk and dawn hours becomes dimmer and short-wavelength skewed (Coemans et al., 1994). Thus, the prevalence of shortwave sensitive photoreceptors in the dorsal eye can help maintain effective contrast for target tracking even in the dimmer dawn and dusk environments.

Rapidly manoeuvrable diurnal flying insects such as dragonflies (but also including a range of other invertebrate species) tend to have fast photoreceptors characterised by high corner frequencies, low gain, low input resistance, low sensitivity, and fast light adaptation (Laughlin and Weckström, 1993; Weckström and Laughlin, 1995), driven by expression of non-inactivating K_v channels on the photoreceptors of animals linked to this ethological niche (Laughlin and Weckström, 1993; Frolov, 2016).

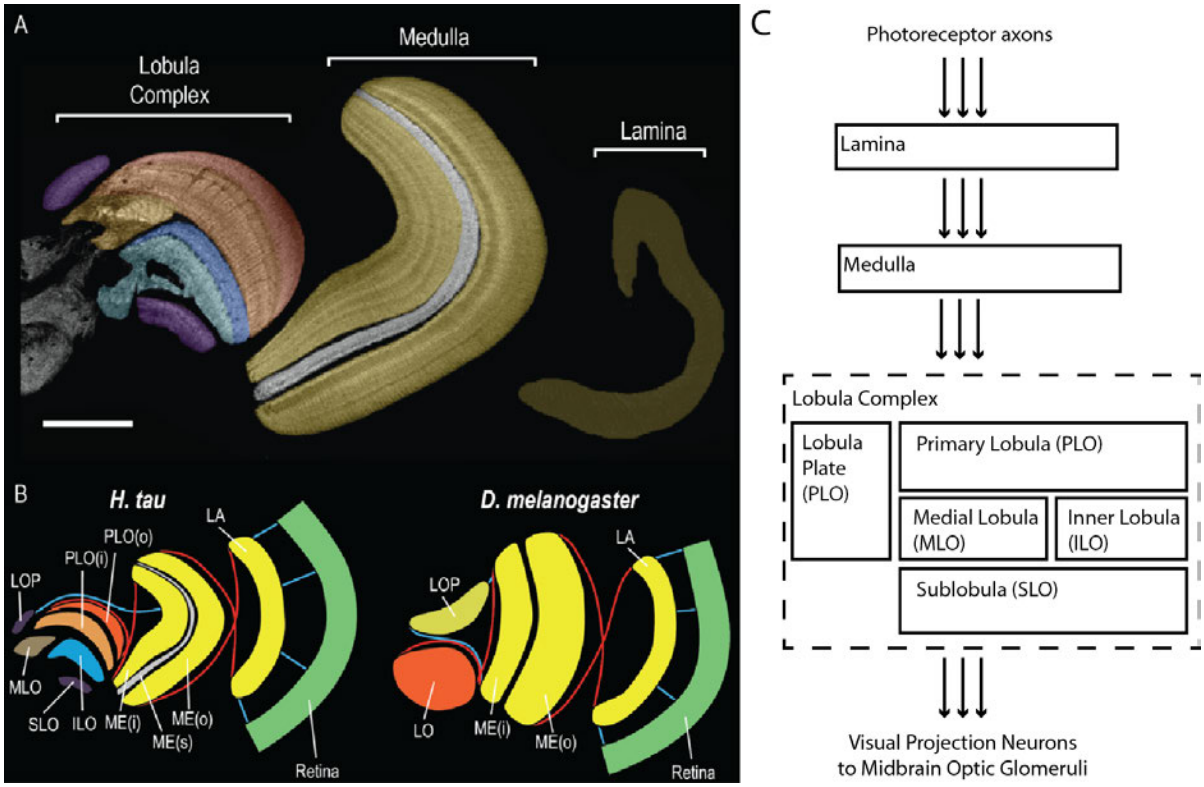

Figure 10. The Dragonfly Brain. A) *Synapsin stained horizontal section through the optic lobe of H. Tau, from Fabian et al. (2017). Scale bar = 200 µm.* ***B)*** *Diagrammatic view of the optic lobe of H. tau (Left) and Drosophila melanogaster (right), also from Fabian et al., 2017. LA = Lamina, ME(o), ME(s) and ME(i) = outer, serpentine, and inner medulla, PLO(o) and PLO(i) = outer and inner subunit of the Primary Lobula, ILO = Inner Lobula, SLO = Sublobula, MLO = Medial Lobula and LOP = Lobula Plate. Blue lines indicate major uncrossed serial connections, red lines indicate crossed connections.* ***C)*** *Block Diagram. The internal circuitry of the Lobula Complex is currently unknown.*

The Dragonfly Optic Lobes

Before we continue our deep drive through the functional physiology of the dragonfly visual system, it will be beneficial to briefly review a general outline of the anatomy of the dragonfly brain, in order to better picture the flow of information. Broadly, the neuropil of the insect brain can be subdivided into several 'supercategories' (Ito et al., 2014), including the optic lobes, mushroom bodies, central complex, and several others unrelated to vision. All of these structures are present in the dragonfly brain (Fabian et al., 2020). Of these, the most pertinent to visual processing are the optic lobes (very roughly analogous to the occipital cortex), a series of peripheral neuropil that receive projections directly from the photoreceptors of the retina and project outputs to midline structures in the central complex and mushroom bodies. In most insects, the optic lobes are comprised of the lamina and medulla, a pair of neuropil in serial, followed by a set of parallel structures together termed the 'lobula complex' variously including the primary lobula, inner lobula, medial lobula, lobula plate, and sub-lobula depending on species (Ito et al., 2014). As we shall see, the dragonfly lobula complex truly lives up to its name, and contains significant complexity in comparison to other insects studied (Ito et al., 2014; Fabian et al., 2020). A general map of the dragonfly brain is presented in Figure 10 (Fabian et al., 2020).

The Optic Lobes are a series of neuropils common across insects, generally split into four discrete neuropils known as the lamina, medulla, lobula, and lobula plate (Ito et al., 2014). However, recent research into the dragonfly optic lobe (including *Hemicordulia tau)* using synapsin staining reveals a more complex picture, with 11 distinct neuropil; the lamina, the outer medulla, serpentine medulla, inner medulla, anterior accessory medulla, and a lobula complex with six subdivisions (Fabian et al., 2020). In the more frequently studied Drosophila, each of these neuropils consist of a repetitive structure of retinotopically-arranged columns analogous to ommatidia that support the parallel processing of visual information from different points in space, and contain hundreds of cell types, many of which may only be present once per column (Strausfeld, 1976).

In general, however, the lamina and medulla are arranged sequentially, while the lobula and lobula plate are downstream from the medulla in parallel, forming the lobula complex. Here, parallel visual pathways split into those focused on wide-field optic flow information in the lobula plate and motion detection in the lobula (O'Carroll, 1993). Cells in the lobula display a diverse range of physiology, with some tuned to moving bars (O'Carroll, 1993), looming stimuli (Rind and Simmons, 1992) and small moving targets (O'Carroll, 1993; Nordström and O'Carroll, 2006; Geurten et al., 2007; Keleş and Frye, 2017). In *Drosophila*, neurons originating the lobula that project to optic glomeruli in the central complex have been shown to link feature detection to distinct, ethologically relevant and context sensitive behavioural responses (Wu et al., 2016).

Early Visual Processing in the Lamina and Medulla

"Keep absolutely still. Its vision is based on movement."

- *Dr. Alan Grant, Jurassic Park* (Spielberg, 1993)

A visual scene is a complex, 2D array of multivariate information about the 3D world, sensed by a 2D plane of optical sensors, described in the dragonfly above. At the earliest levels of the visual system, this complex set of incoming information is decomposed into basic visual features that are then combined at successive processing stages to build a more complex picture of the world from incoming, discrete photoreceptor activity (Gilbert, 2012). Thus, the individual 'visual pixels' (Individual photoreceptors in vertebrates, and whole ommatidia in invertebrates) come together to form line segments of different orientations, line segments of different lengths and orientations come together to form shapes, and shapes come together with other intermediate-level visual features to form objects (Gilbert, 2012).

The first synapse of the Insect visual pathway is the l*amina*, a simple neuropil making the first structure of the optic lobes. Although 'in the brain', the insect lamina receives input directly from the photoreceptors, and can be thought of as roughly analogous to the outer plexiform layer of the vertebrate retina (y Cajal and Sanchez., 1915; Laughlin, 1976). All photoreceptors that sample the

same point in space (I.e., are in the same ommatidia in the *apposition*-type eye) project to the same targets in the lamina. Neighbouring points of space are encoded in neighbouring lamina columns, forming a retinotopic matrix where ommatidia and lamina columns have a 1-1 mapping. In the lamina, the decomposition of visual signals from the photoreceptors into low-level visual primitives begins the division of incoming visual information into a pair of parallel pathways that respond either to luminance increments ('ON') or decrements ('OFF'), the so-called 'ON' and 'OFF' pathways (Joesch et al., 2010), which are also found in vertebrates at the level of the retina (Meister and Tessier-Lavigne, 2012). The ON pathway is subserved by the lamina interneuron L1, while the OFF pathway is subserved by a pair of lamina interneurons, L2 and L3 (Joesch et al., 2010). Intriguingly, the dragonfly lamina is significantly more complex than that of *Drosophila*, with at least two strata (Strausfeld, 1976; Armett Kibel et al., 1977), although it is currently unknown what extra processing may be taking place in the dragonfly lamina.

Lamina output projects into the medulla, a complex neuropil containing both single and multi-columnar cell types (Gilbert, 2013). The parallel columnar retinotopic arrangement with 1-1 ommatidial:neuronal column mapping is maintained in the medulla. Recent evidence using colabelled anti-synapsin and anti-serotonin immunohistochemistry distinguishes 21 layers in the dragonfly medulla (Fabian et al., 2020), in comparison to the 8-11 layers observed in other insects (See Fabian et al., 2020 for comparisons). The medullary columnar units contain a number of distinct cell types that respond to different elementary features of motion, as well as multi-columnar cells that facilitate spatial pooling across ommatidia outputs (Gilbert, 2013). Neither the function of the majority of the ~100 cells in each medullary column is known, nor is exactly why the dragonfly medulla should contain around double the synaptic layers as that of other insects.

One of the most important elementary features for the dragonfly is *motion*. The fictional palaeontologist Dr. Alan Grant's famous line in *Jurassic park* (Spielberg, 1993) 'Keep absolutely still, It's vision is based on movement' may have been incorrect about the *Tyrannosaurus rex* (Stevens, 2006), but Dr. Grant could well have been talking about a dragonfly (Perhaps a prehistoric, giant, mega-anisopteran; Beattie and Nel, 2012; Huang et al., 2017; Nel et al., 2017). *Motion* stimulation is generally split into two broad categories – *global motion*, when the whole visual space is moving synchronously, is generally associated with ego-movement of the animal; and *Local* motion, the movement of stimuli between two discrete points on the retina, generally associated with the independent movement of an object in the world. These motion signals are related in that global motion is constructed from the combination of lower-level local motion signals; If there is lots of 'local motion' across the visual field in coherent directions and velocities, it is assumed to be global motion. The smallest perceivable motion would be local motion between just two points – say, neighbouring

ommatidia. Computationally, this kind of motion is detected by the 'Elementary Motion Detector,' (EMD) a theoretical model of local motion computation by a neural system. At the scale of neighbouring ommatidia, an insect's visual system must extract luminance changes across both space (neighbouring ommatidia) and time (the time it takes for the signal to cross from one to the other) in order to produce a signal that responds to the appearance of a stimulus in one ommatidia and *then* the other. The key here is that motion must have a *velocity,* both a direction and a speed, in contrast to a flicker stimulus. The medulla is thought to be the location of the neuronal realization of the Elementary Motion Detector, (Gilbert, 2013; Takemura et al., 2013). Before we discuss the insect implementation of the EMD with reference to specific medullary neurons, let us first describe the idealised Elementary Motion Detector.

The Idealised Elementary Motion Detector

The Elementary Motion Detector (EMD) was first proposed on the basis of studies in *Chloropharus* beetles (Hassenstein et al., 1956), it has since been validated in a range of species (Borst and Helmstaedter, 2015), including in human psychophysics (Clifford and Langley, 1996). The simplest EMD, known today as a 'Richardt Correlator' (Or sometimes 'Hassenstein-Reichardt Detector', or 'HR Detector') is a simple microcircuit motif containing only three cells (Figure 11c). First, two subunits receive input from nearest neighbour (or further) ommatidia, each detecting luminance changes (ON/OFF). The two subunits then project to a third 'comparator' unit which acts as a 'coincidence detector,' comparing the outputs of each subunit. In order to distinguish a stimulus in motion, the input of one of the subunits is *delayed*. Thus, if both the delayed and the 'live' signal reach the coincidence detector at once it responds, signalling movement from one of the inputs towards the other at velocity determined by the delay (Borst and Helmstaedter, 2015). Motion in the other direction would result in the 'live' signal reaching the coincidence detector well before the delayed signal and there would be no signal integration. Thus, the simple Richardt Correlator is said to be *direction selective*, with a *preferred direction* to which it responds and a *null direction* to which it does not.

The complete Hassenstein-Reichardt Detector contains two of the 'Half-detectors' described above with temporal delays on opposing input channels (leading to opposite directional selectivity) and their outputs pooled via a fifth unit which subtracts the output signals of each half-detector, resulting in a fully opponent signal. This motif is known as the 'Fully-opponent Richardt Correlator' (Figure 11I). This complete HR detector signals motion in opposite directions with output signals of equal time course and amplitude, but opposing sign (excitatory, inhibitory). In the case of a flicker stimulus with temporally correlated ON/OFF signals across space, the two opponent signals will cancel out, and no net signal will be produced. Thus, the EMD detects true motion. In this model, the

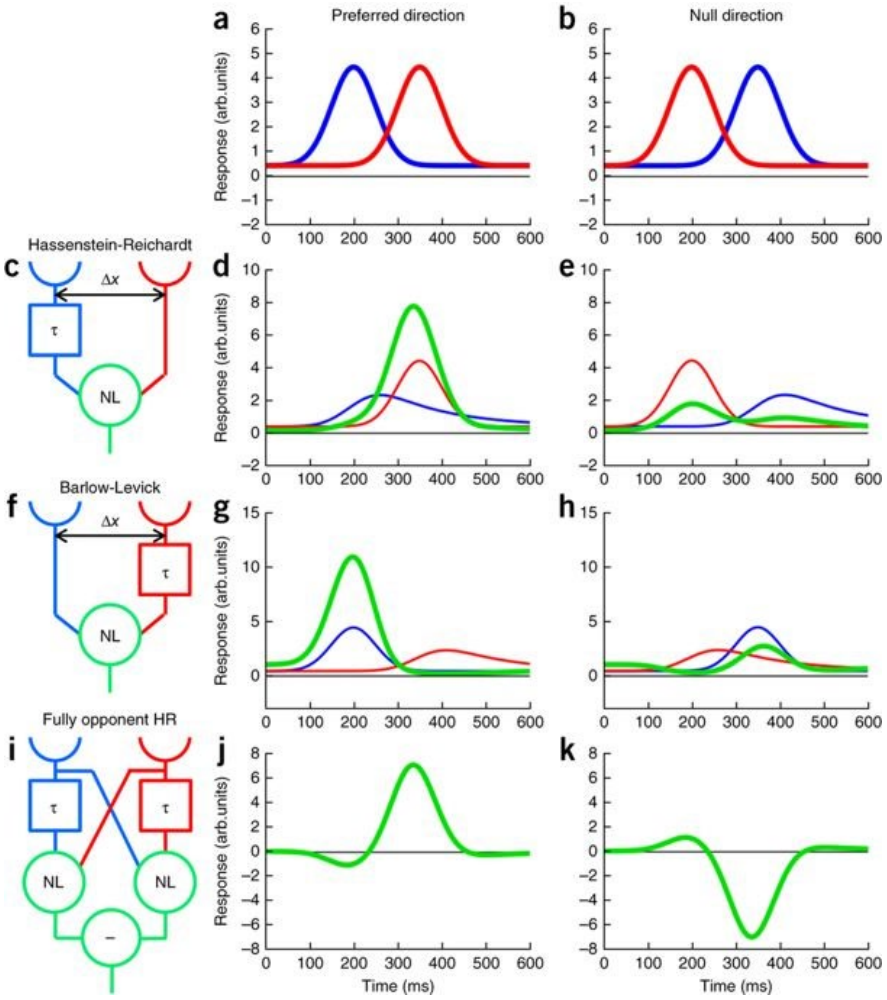

Figure 11: Models of the Elementary Motion Detector. *Image from Borst & Helmstaedter (2015).* ***a-b)*** *Responses of two photoreceptors, colour-coded to the EMD diagrams.* ***c)*** *The basic Reichardt Correlator, showing three subcomponents; two photoreceptors (red, blue), and one coincidence detector (green) implementing a nonlinear correlation (NL), in the case of the Richardt Correlator, multiplication. This example places a time delay (t) on the blue channel.* ***d-e)*** *Responses of the output unit of a Reichardt correlator (green unit in c) to the input specified in a/b. Red trace = red input alone, blue trace = blue input alone, green trace = paired input.* ***f)*** *The Barlow-Levick model. The essential nonlinearity at the confidence detector in the Barlow-Levick model is divisive.* ***g-h)*** *Responses of the Barlow-Levik model to the input in a/b.* ***i)*** *A fully Opponent Reichardt Correlator.* ***j-k)*** *Responses to the output unit of the fully opponent Reinhardt correlator (Subtractor unit in i).*

temporal frequency tuning of the system is dependent on the time delay constant, and the spatial frequency tuning is dependent on the distance between the compared subunits – it is thought that for a wide range of motion tuning, fully-opponent Richardt Correlator with different time delay constants that compare ommatidia of different distances would need to be developed in parallel.

The classic HR detector relies on nonlinear multiplicative amplification of incoming signals using feedforward excitation at the 'coincidence detector'. However, this is not the only possibility. In the *Barlow-Levick* model (Figure 11f), the delayed signal acts to veto or supress the 'live' signal, resulting in suppression of motion in the null direction (Barlow and Levick, 1965).

Dragonflies (Olberg, 1986) as well as other insects (Buchner, 1976; Borst and Egelhaaf, 1989; Borst et al., 2010) were thought to use multiplication-like operations consistent with the fully opponent HR model, whereas vertebrates implement a Barlow-Levick reminiscent subtraction operation (Barlow and Levick, 1965; Adelson and Bergen, 1985; Livingstone, 1998; Clark and Demb, 2016) via GABAergic signalling from starburst amacrine cells (Wyatt and Daw, 1976; Yoshida et al., 2001). However more recent evidence suggest both vertebrates and invertebrates use a combination

to varying degrees (Borst and Helmstaedter, 2015; Fisher et al., 2015b; Haag et al., 2016; Leong et al., 2016; Gruntman et al., 2018). Despite the differences in fundamental implementation of EMDs – and some 700 million years of evolutionary separation (Parfrey et al., 2011) – dragonfly and macaque neurons show remarkably similar response properties (Nitzany et al., 2017) suggesting convergence on an optimised motion computation.

Implementation of the EMD in *Drosophila*

The idealised EMD proposed by Hassenstein & Reichardt in 1956 is a theoretical algorithmic model, and although its predictions have received much empirical support (Borst and Egelhaaf, 1989; Yang and Clandinin, 2018), a definite circuit implementation remains to be found in any species. Research taking advantage of the genetic toolkit available in the fruit fly *Drosophila melanogaster* have revealed possible neuronal substrates of both the HR- and BL-type EMDs situated in medulla and lobula complex interactions.

The first neurons in the insect visual system to exhibit directionally selective true-motion only (flicker insensitive) responses are the T4 and T5 cells, which respond to ON and OFF motion, respectively (Maisak et al., 2013; Takemura et al., 2017). Although the cell body lies near the Lobula Plate, Direction Selective motion responses are exhibited as early as the cell dendrites within the medulla, suggesting this is where the core computations take place (Fisher et al., 2015b). These cells exhibit many of the functional properties that would be expected of the HRC, including direction opponency (Badwan et al., 2019) and temporal frequency tuning (Creamer et al., 2018). When these cells are blocked via mutation, down-stream wide field motion sensitive cells lose all responses to visual motion (Schnell et al., 2012) and flies become motion blind (Bahl et al., 2013).

Within both the T4 and T5 cell class, there are four subtypes labelled T#a-d, (T4a, T4b … T5c, T5d) which respond robustly to motion in one of the four cardinal directions relative to the insect, upwards, downwards, front-to-back (as in receding), and back-to-front (as in proceeding) (Maisak et al., 2013). T4 and T5 are thought to represent a potential output cell for EMDs on both the ON and OFF channels (Maisak et al., 2013; Strother et al., 2017). In principle, if T4 & T5 received input from lamina Interneurons L1 & L2/L3 from spatially offset ommatidia, all that would be required for an EMD implementation is a temporal delay on the signal from one side.

Recent advances in electron microscopy and genetic silences, particularly in the last five years, have uncovered the synaptic inputs into T4 and T5 cells in great detail (Serbe et al., 2016; Strother et al., 2017; Takemura et al., 2017; Shinomiya et al., 2019), building a somewhat more complex picture than the 'in principle' idealised HRC would predict (Figure 12). T4 cells, representing the ON pathway, receive inputs from three linearly arranged spatial locations, with medullary interneurons Mi1 and Tm3 inputting excitatory input from the central point, and the neurons Mi9 and Mi4 projecting input

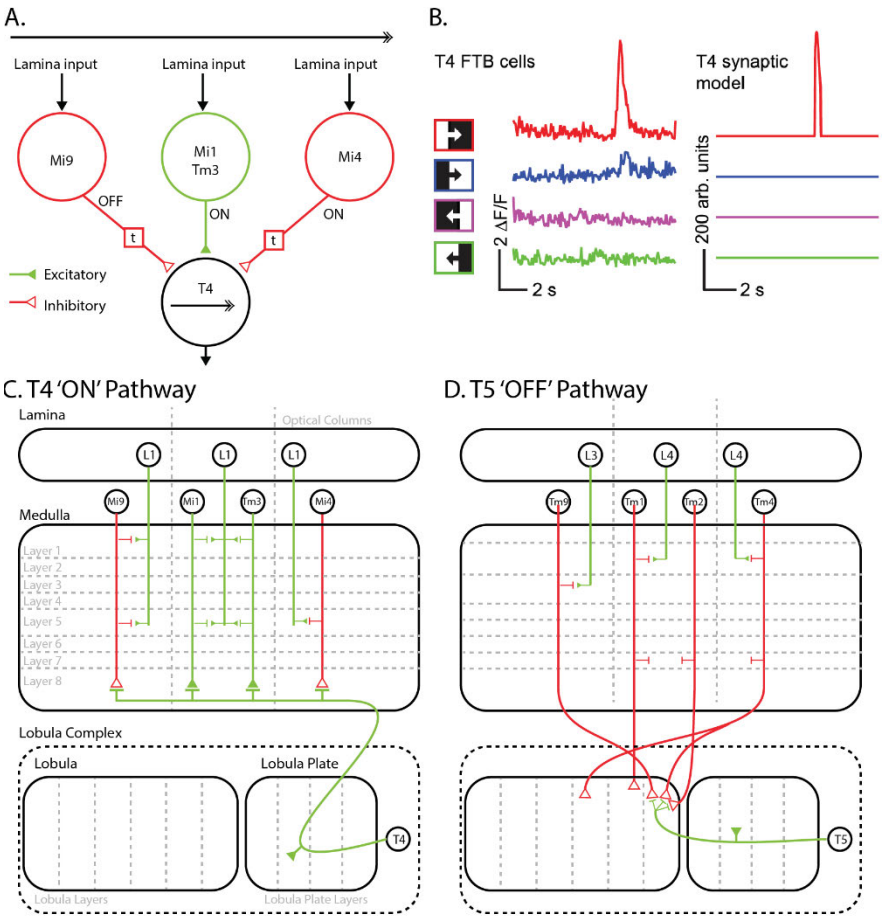

Figure 12: Purported EMD Instantiation in the Drosophila Medulla. A) *Illustration of relationships between T4 and its main presynaptic inputs, based on the synaptic model in Zavatone-Veth et al. (2020). This could be considered roughly analogous to a fully opponent HRC (Figure 11I).* ***B)*** *cell responses (left) and synaptic model output (right) from Zavatone-Veth et al. (2020). Both the electrophysiological T4 response and the minimal synaptic model (A) are responsive to leading-edge ON movement in a single direction, indicating that both the real T4 cell and this minimal synaptic model capture essential functions of the HRC.* ***C)*** *The same circuit as A. in anatomical context. Medullary interneurons Mi1, Mi4, and Mi9 receive excitatory input within the medulla from the ON pathway Lamina output cell L1, and project to T4 dendrites in the deepest layer of the Medulla. T4 integrates dendritic information at the level of the medulla and projects to direction-selective cells in the Lobula Plate.* ***D)*** *Analogous circuit for the purported 'OFF' pathway EMD T5. This circuitry is less well characterised and understood, and in this case dendritic collocation of T5 and medullary inputs occurs in the Lobula.*

from the two peripheral points, respectively (Takemura et al., 2017). T5, representing the OFF channel receives input from different set of medullary intrinsic neurons but with a similar spatial structure (Shinomiya et al., 2019). In addition to these major inputs, both T4 and T5 receive spatially-localised input from other neurons, whose functions are not yet understood (Takemura et al., 2017; Shinomiya et al., 2019). Of the T4 and T5 circuits, T4 is the better understood and thus will provide for a more comprehensive example of Elementary Motion Detection in *drosophila* (Behnia et al., 2014; Arenz et al., 2017; Shinomiya et al., 2019). Central spatial information is relayed to T4 via fast excitatory ON input from Mi1 and TM3 (Behnia et al., 2014; Arenz et al., 2017; Strother et al., 2017; Takemura et al., 2017; Gruntman et al., 2018). On the side of T4's preferred direction, delayed inhibitory ON input is relayed by Mi4 (Arenz et al., 2017; Takemura et al., 2017; Meier and Borst, 2019; Shinomiya et al., 2019). Meanwhile, on the anti-preferred side, delayed inhibitory OFF input is relayed by Mi9 (Arenz et al., 2017). Currently, the patterns and origins of the time delays on these three input channels to T4, which would be required for motion computation are poorly understood. However, the primary central input neurons Mi1 and Tm3 show slightly different low-pass characteristics with different time constants (Behnia et al., 2014). There is also some

evidence that the inhibitory inputs to T4 arrive through di- or tri-synaptic pathways, potentially introducing a time delay (Takemura et al., 2017). The recent expansion of connectomic insight into the T4/T5 EMD circuit has led to an explosion of modelling efforts in the last five years; e.g., see Clark et al., 2011; Behnia et al., 2014; Haag et al., 2016; Leong et al., 2016; Serbe et al., 2016; Arenz et al., 2017; Strother et al., 2017; Creamer et al., 2018; Gruntman et al., 2018; Badwan et al., 2019; Zavatone-Veth et al., 2020 and many more not otherwise cited here. The majority of this work is beyond the scope of this thesis, however a recently published minimal synaptic model of T4 connectivity shows only these basic circuit level connectivity patterns (without implementation of complex subcellar components, ionic currents, other synaptic inputs, dendritic computations, etc.) is required to reproduce HRC-like output and many electrophysiological properties of T4 cells (Zavatone-Veth et al., 2020), including direction-opponency and temporal frequency tuning as predicted by the HRC. Thus, although it is clear that the complete T4 circuit and subcellular components of T4 itself are significantly more complex than the idealised HRC model in both function and anatomy, these more complex properties are likely implemented via additional circuit elements on top of a HRC base.

T5 likely represents an analogue to T4 for the OFF pathway, as it receives inputs from a similar set of medullary interneurons with a similar spatial structure (Shinomiya et al., 2019). However, the T5 microcircuit appears to be substantially more complex, as thus far only inhibitory inputs have been identified and there are many currently unresolved circuit elements.

Despite the neuroanatomical and physiological evidence pointing towards this core neuronal circuit, the behavioural consequences of interrupted interneuron function are surprisingly subtle (Ammer et al., 2015; Fisher et al., 2015a; Serbe et al., 2016; Strother et al., 2017), suggesting even at this early level the motion detection system uses a form of distributed coding and high redundancy.

Why might the dragonfly medulla be so much bigger? In order to detect motion at a wide array of spatial and temporal scales, a large array of HR-detectors with different time and space constants would need to be developed in parallel. Most insects limit the regions of spatio-temporal feature space their EMDs are tuned for by species-specific visual ecology (O'Carroll et al., 1996). However, the dragonfly engages in a broad range of behaviours that would require both slow (hovering, drifting) and fast (pursuit, aerobatics) tuning. Thus the dragonfly visual system may have developed a larger neuropil with more diverse tuning properties in order to support a larger range of behaviours, as it has downstream with more complex visual neurons (Evans et al., 2019).

Intermediate Visual Processing in the Lobula Complex

Following the computation of a directional motion signal by an EMD, motion information is divided into two parallel pathways based on spatial scale for continued processing; Optic Flow information, and feature motion information. Broadly, Optic Flow information is synonymous with

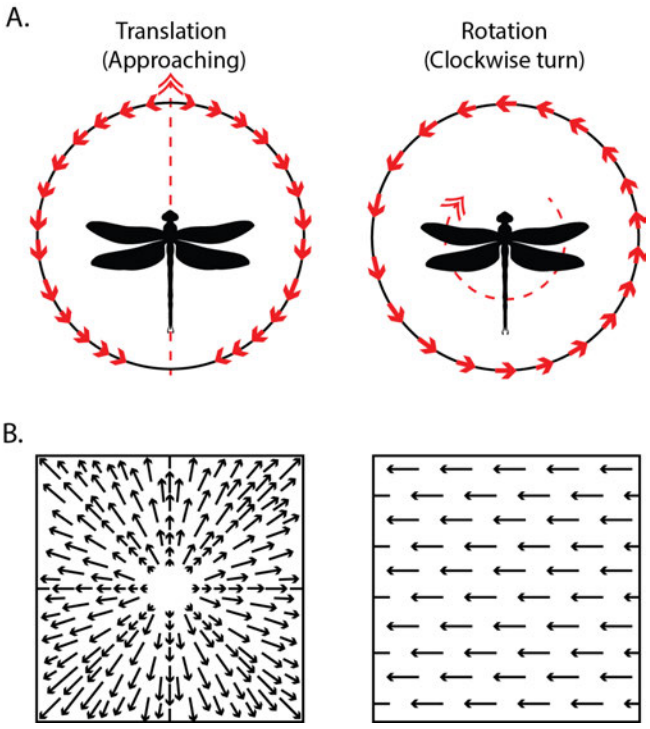

Figure 13: Patterns of Optic Flow during Dragonfly Movement. A) *Perceived Optic Flow Patterns (Red Arrows) during dragonfly movement (Red Dotted line). Left: Translational optic flow for a dragonfly moving forward. Right: Rotational optic flow for a clockwise turn.* ***B)*** *Optic flow patterns perceived on the Dragonfly's Retina for the movements in A.*

Global motion, the synchronous movement of many parts of the visual field at a matched speed and direction, associated with ego-movement. Although Global Motion is built out of the summation of many individual Local motion signals, Local Motion in one part of the visual field that differs substantially from surrounding areas is likely to represent a *feature*, such as a potential prey item, conspecific, or predator, rather than movement of a whole scene. Thus, the most pertinent pathways for target tracking is the detection of highly salient local motion that differs from the surround, a computation achieved in dragonflies by the Small Target Motion Detector system.

Optic Flow Information

Optic Flow refers to the pattern of apparent motion of an entire visual scene caused by relative motion between the observer and the outside world. Many animals, including both insects and humans, use Optic Flow information to guide motor navigation and other behaviours (Srinivasan et al., 1998; Srinivasan and Zhang, 2004; Straw et al., 2010; Schwegmann et al., 2014; Creamer et al., 2018; Stöckl et al., 2019). Optic flow information is particularly important for navigation in odonata, including both damselflies and dragonflies, which lack the Halteres and complex antennae other species use to determine motion (Fraenkel and Pringle, 1938; Pix et al., 1993; Sane et al., 2007; Dahake et al., 2018). Broadly, Optic Flow can be divided into two components, translational and rotational. Translational optic-flow results from linear motion, for example where the world appears to move 'towards' an observer as an observer moves towards it – as the observer moves forwards (Figure 13, left). Rotational optic flow is derived from the rotational motion of the viewer relative to the world (Figure 13, right). If T4 can be thought of as the output of an insect EMD representing local motion, than Global Motion/Optic Flow could be represented by the summed activity of the all T4 cells with a similar preferred direction across different points in space. Such a summation is exhibited in the insect lobula plate, a neuropil within in the lobula complex containing four layers. Each of these four layers receives input from all T4/T5 cells tuned to each direction, resulting in the pooling of all similarly

direction selective cells across space. Here, the T4/T5 cells synapse onto so-called Lobula-Plate Tangential Cells (LPTCs) which integrate local motion cues across large regions of space (Borst et al., 2010). In most insects, LPTC's are split up into the 'Horizontal System' (HS) and 'Vertical System' (VS) cells and as a population exhibit only a narrow range of spatiotemporal tuning characteristics (Hausen, 1982; Hausen and Egelhaaf, 1989; Ibbotson, 1991; O'Carroll et al., 1997; Theobald et al., 2010) matched to their species specific visual ecology (O'Carroll et al., 1996). However, analogous neurons in dragonflies (Termed 'Lobula Tangential Cells', LTCs) are significantly more complex with wide ranging variation in velocity tuning, spatial tuning, and motion adaptation over time that may be matched to different behavioural demands (Evans et al., 2019).

The STMD system in Dragonflies and Other Insects

Predatory species such as dragonflies must be able to track the motion of prey against a complex, dynamic, visually cluttered background. Small-Target Motion Detectors (STMDs) are a class of neuron resident across the insect lobula complex and midbrain that are specialised for this task. The STMD system contains a diverse array of neurons with different receptive field sizes, shapes, locations, directionality, and tuning properties (O'Carroll, 1993; Frye and Olberg, 1995; Geurten et al., 2007; Dunbier et al., 2012), some of which exhibit higher order properties such as selective attention (Wiederman and O'Carroll, 2013a), neuronal facilitation (Nordström et al., 2011; Dunbier et al., 2012), and prediction (Wiederman et al., 2017). However, the major component all STMD system neurons have in common is their robust response to small, independently-moving objects thought to match retinal signatures for prey and conspecifics. Such STMD and STMD-like neurons have been observed in a number of taxa, including moths (Collett, 1971, 1972), hoverflies (Nordström and O'Carroll, 2006), dragonflies (O'Carroll, 1993; Geurten et al., 2007), *Drosophila* (Keleş and Frye, 2017) and blowflies (Gilbert and Strausfeld, 1991; Strausfeld, 1991)**.** Specific tuning properties of STMDs are likely to vary considerably, both between cells within the same animal, and across animals based on the species' size and behavioural demands. Most identified STMDs in dragonflies (predominantly *Hemicordulia sp.*, a medium-sized genus) are tuned to the movement of small (1°-3°) dark targets against a bright background (O'Carroll, 1993; Geurten et al., 2007; Wiederman et al., 2013), matching visual stimuli that trigger a behavioural pursuit (Labhart and Nilsson, 1995; Olberg et al., 2005, 2007; Duong et al., 2017; Lin and Leonardo, 2017).

List of Identified STMDs and STMD-like cells

Although the majority of this thesis is focused on one particular characterised STMD in the dragonfly (CSTMD1, see below), below follows a list and brief description of identified STMD and STMD-like cells in dragonflies & other insects:

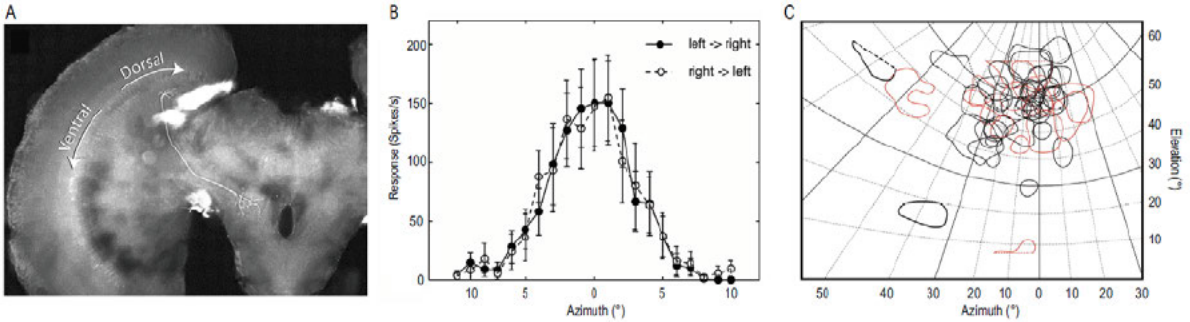

Figure 14: SF-STMD Neurons in the Hoverfly. *Figures reproduced from Barnet et al., 2007.* ***A)*** *Lucifer yellow staining of a single SF-STMD in the hoverfly. Inputs are visible in the dorsal optic lobe and outputs are visible in the midbrain.* ***B)*** *Mean neuronal response to drifting targets (Black, rightward movement; White, leftward movement) centred around the receptive field middle (Azimuth = 0). The response is broadly gaussian with a width of ~10 degrees.* ***C)*** *Receptive field locations in retinotopic space.*

SF-STMDs: 'Small-field' STMDs (SF-STMDs) are found early in the optic lobe in both dragonflies and hoverflies (Figure 14) and exhibit a small, approximately Gaussian receptive field spanning only a few degrees of visual space (generally 5-20°) and varying degrees of direction selectivity (O'Carroll, 1993; Barnett et al., 2007). SF-STMDs exhibit low spontaneous firing rates and robust responses to small targets.

LF-STMDs: 'Large-field' STMDs (LF-STMDs), found in dragonflies and hoverflies (Nordström and O'Carroll, 2006, 2009) exhibit much larger and less homogenous receptive field structures and position invariance, often (but not always) eschewing direction selectivity (Geurten et al., 2007; Bolzon et al., 2009; Dunbier et al., 2012). LF-STMDs are presumed to sum input from multiple SF-STMDs in order to combine the population SF-STMDs response to a larger area of space.

CSTMD1: An identified LF-STMD in dragonflies (*Hemicordulia sp.*; Geurten et al., 2007). Centrifugal Small-Target Motion Detector 1 (CSTMD1) is the most well characterised dragonfly STMD, and is the major cell of focus for this thesis. Physiological and anatomical properties are summarised in Figure 15. CSTMD1 exhibits a relatively high spontaneous spike rate of ~12-25 spikes/s when not stimulated, and a robust response reaching up to 300 spikes/s when presented will an optimally-tuned small moving target in the excitatory hemisphere (Figure 15A). This spiking response makes use of burst encoding, where a rapid burst of spikes is followed by a period of inactivity (Fabian and Wiederman, 2021). When presented with small target motion in the inhibitory portion of its receptive field, CSTMD1's response is driven down to 0 spikes/s. CSTMD1 exhibits a whole-field receptive field sharply divided at the visual midline into excitatory (ipsilateral to the cell body) and inhibitory (contralateral to the cell body) components (Figure 15B). CSTMD1's receptive field covers the dorsal portion of the

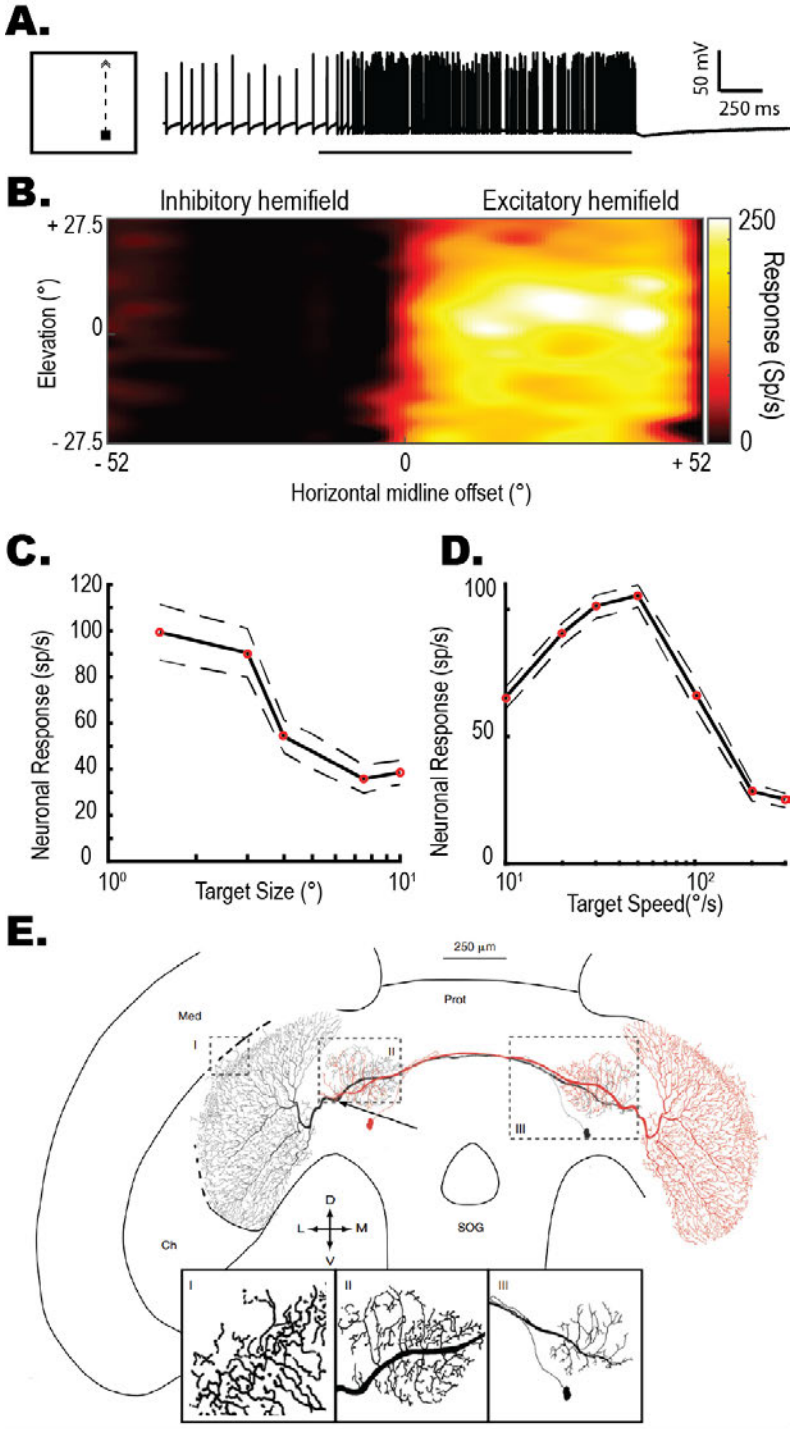

Figure 15: Physiology and Morphology of Centrifugal Small Target Motion Detector 1 (CSTMD1). ***A)*** *CSTMD1 exhibits a robust spiking response to small target motion. Left: Stimulus pictogram illustrating a small target ascending the right side of a presentation monitor (Black Square). Right: an example CSTMD1 spike train in response to this stimulus. Reproduced from Lancer (2019).* ***B)*** *CSTMD1's Receptive Field shows a sharp divide between Inhibitory and Excitatory hemifields. This receptive field was mapped with a Small (2° x 2°) target moving left-to-right at 80°/s.* ***C)*** *Target Height Tuning, reproduced from Chapter 5.****D)*** *Target Velocity Tuning, reproduced from Chapter 5.* ***E)*** *Morphology of CSTMD1 reproduced from Geurten et al. 2007. CSTMD1 was dye-filled with Lucifer Yellow (Black cell), against a mirror-image projection (red) to emphasise collocations. Arborization field I and II represent output arborazations while field II represents input arborizations (for the black CSTMD1). Output arbozations from one CSTMD1 coloccalise with input dendrites from the other hemisphere. Arrow indicates the site at which we are able to regularly pierce CSTMD1 for intracellullar recording. Med = medulla; Ch = inner optic chiasm; Prot = protocereberum; SOG = sub-oesophageal ganglion. Compass labels clockwise from the left = Lateral, Dorsal, Medal, ventral.*

dragonfly eye, matching the location at which dragonflies fixate prey during pursuit (Olberg et al., 2000; Olberg, 2012; Mischiati et al., 2015). Although CSTMD1 responds robustly to targets moving in any direction, targets moving up and to the right (from the dragonfly's perspective) elicit slightly stronger responses. Furthermore, CSTMD1 exhibits higher-order response properties such as spatial facilitation, Predictive Gain Modulation, and selective attention, which will be elaborated on below (pg.59). Anatomically (Figure 15E) CSTMD1 is a large neuron whose input dendrites lie in the ipsilateral protocerebrum, with two distinct output arborisations, one small arborisation in the contralateral protocerebrum that overlaps with the presumed input arborisation for the contralateral CSTMD1, and one large output arborisation throughout the contralateral optic lobe (Geurten et al., 2007). CSTMD1's anatomy is thus consistent with a potential role as an output integrator for the upstream ipsilateral STMD system that is involved in modulation or processing in the contralateral optic lobe and midbrain. A CSTMD1-like cell has also been observed in the female hoverfly (Nordström and O'Carroll, 2006).

BSTMD1: An identified LF-STMD in dragonflies (Dunbier et al., 2012). Bilateral Small Target Motion Detector 1 (BSTMD1) exhibits a binocular excitatory receptive field and is not direction selective. Unlike CSTMD1, BSTMD1 does not make use of burst encoding (Fabian and Wiederman, 2021). The midbrain dendrites of BSTMD1 collocate with the midbrain arborisations of CSTMD1, suggesting BSTMD1 could form synaptic contact with either ipsilateral or contralateral CSTMD1. BSTMD1 also shows Spatial Facilitation.

LC11: Lobular Columnar neuron 11 (LC11) is a homologue SF-STMDs found in the drosophila optic lobe (Keleş and Frye, 2017). LC11 is turned for small targets approximately 2.2° in size, with receptive fields approximately 20° wide. In contrast to identified dragonfly STMDs, LC11 is supressed by motion and flicker in the surround (Keleş et al., 2020). The Lobular Columnar neurons (LCs) comprise a group of at least twenty distinct functional classes (Defined by response properties to different stimuli) of object-selective neuron that project from Lobular Columns to the Optic Glomeruli in *Drosophila* (Wu et al., 2016). Optogenetic activation of LCNs is often sufficient to cause a variety of visually-mediated behaviours such as grasping, turning, and escape take-off (Card and Dickinson, 2008; Wu et al., 2016).

MLG1/2: the Male Lobula Giants (MLGs) are a class of 12 heterogeneous motion sensitive cells found in the blowfly (*Calliphora sp. & Sarcophaga sp.)* Lobula (Gilbert and Strausfeld, 1991; Strausfeld, 1991). As a group MLGs respond to a variety of visual stimuli, but MLG1 and MLG2 in particular are tuned to directional small target motion within the male fly's acute zone. MLG1 exhibits a robust spiking response to movement in the preferred direction and no

response to motion in the null direction, while MLG2 exhibits a fully opponent response to small target motion within its receptive field. MLG1/2 axons terminate broadly terminate on descending neurons involved in motor circuitry in the contralateral protocerebrum (Gronenberg and Strausfeld, 1991).

FDs: 'Figure Detection' cells are the female blowfly equivalent of MLGs (Egelhaaf, 1985a), but show strong responses to bar and textured stimuli, including wide-field motion, suggesting they are less tuned for small target motion (Egelhaaf, 1985b; Warzecha et al., 1993; Kimmerle and Egelhaaf, 2000).

TSDNs: Although not traditionally part of the STMD system due to their location in the Ventral Nerve Cord (Rather than Lobula), Target Selective Descending Neurons are similarly tuned and here considered an 'honorary' member for the purpose of a comprehensive list. TSNDs are a group of 16 efferent neurons (8-pairs representing opposite visual hemifields) that project through the ventral nerve cord of odonates (Olberg, 1986; Supple et al., 2020) and hoverflies (Nicholas et al., 2018). TSDNs are thought to encode a population vector representing target position and direction and convey this information to thoracic motor nuclei in order to drive wing steering (Olberg, 1986; Frye and Olberg, 1995; Adelman et al., 2003; Gonzalez-Bellido et al., 2013; Nicholas et al., 2018), consistent with a proposed role for trajectory steering during pursuit behaviours. Dragonfly TSDNs receptive fields largely cover the dorsal acute zone around the midline (Gonzalez-Bellido et al., 2013), and are generally large and strongly direction selective (Olberg, 1986; Frye and Olberg, 1995). TSDNs show slightly broader size-tuning (1-4°) then lobula STMDs (Nicholas et al., 2018). Anatomically, TSDNs show input arborisations in the lateral midbrain/optic protocerebrum (Olberg, 1986) that collocate with many STMD output arbitrations, leading to speculation that TSDNS may receive input from Lobula STMDs (Barnett et al., 2007; Nordström and O'Carroll, 2009).

Interaction with the Wide field System

How do STMD system neurons interact with the wide field system? One important distinction between the Lobula STMD system neurons found in dragonflies and similar neurons in other species or systems (Such as TSDNs) is that STMDs are even able to respond to a small-target stimulus embedded in cluttered background motion of the same direction and velocity (Nordström and O'Carroll, 2006; Wiederman and O'Carroll, 2011; Evans et al., 2020), effectively ruling out relative motion cues as a driver of STMD activity in dragonflies. In contrast, previous descriptions of STMD-like neurons in other species are generally inhibited by wide-field background motion (Collett, 1971; Reichardt et al., 1983; Gilbert and Strausfeld, 1991; Kimmerle and Egelhaaf, 2000; Trischler et al., 2007), and relative motion cues take a prominent role in the models of target detection a prominent

component in many models (Egelhaaf, 1985a; Higgins and Pant, 2004). Hoverfly TSDNs show suppression in response to background motion in the same direction as a presented target (Nicholas et al., 2018), but enhancement when background motion is presented counter-directional to target motion (Nicholas and Nordström, 2020). A pattern of counter directional background and target motion would be expected in a pursuit where the target is faster than the pursuer, but where the pursuer is faster than the target both the background and target should appear to be moving towards the pursuer in the same direction. However, recent work in drosophila object-detecting LCs has found that a target-response during background-motion was rescued with the application of octopamine (Staedele et al., 2020), a neurohormone related to norepinephrine that serves a functionally equivalent role in invertebrates (Orchard et al., 1993; Roeder, 1999).

Neuronal Mechanisms of Size Selectivity and Direction Invariance

End-Stopped Inhibition

The classical mechanism for size tuning, 'end-stopped inhibition', was first proposed in the mid 1960's on the basis of now-classic experiments in the anesthetised cat (Hubel and Wiesel, 1962, 1965), and is now thought to underlie the responses of so-called 'hyper-complex' cells throughout the mammalian visual cortex. These hyper-complex cells classically have a multi-part receptive field consisting of a pair of inhibitory end zones on either side of an excitatory centre, such that an elongated line traversing the receptive field will elicit both excitatory and inhibitory stimulation, resulting in a reduced response to large stimuli as a function of the ratio between excitatory and inhibitory stimulation they provoke. Physiologically, such a receptive field mapping results in a tuning curve were larger stimuli evoke progressively more inhibition, until the inhibitory signal equals the excitatory signal and they cancel each other out, resulting in a loss of response. In such a regime, only a target small enough to traverse the excitatory receptive field without stimulating the inhibitory end-zones will produce a maximum response. However, STMD neurons typically respond to a translating stimulus anywhere within their receptive field with position invariant size tuning, and even the smallest SF-STMD receptive fields ~4 times larger than optimum target size (O'Carroll, 1993; Barnett et al., 2007). Together these suggest that if end-stopped inhibition does play a role in shaping STMD responses it must take place upstream of the STMDs themselves.

The Object Motion Sensitive Model

The Object Motion Sensitive (OMS) model is another class of model that relies on differentiation of relative motion cues between targets and distractors. This model was first proposed to account for the response of target motion sensitive retinal ganglion cells in vertebrates (Ölveczky et al., 2003; Baccus et al., 2008). Object Motion Sensitive cells identified in vertebrates include the rabbit ON Brisk Transient cell and Salamander Fast OFF cell, which respond to local grating motion

(independently of direction, phase and scale) with a quickly initiated and fast adapting burst of spikes (Ölveczky et al., 2003). Local specificity is achieved via lateral inhibition from a population of inhibitory horizontal cells which signal in similar brief bursts precisely timed to stimulus motion, such that if both the 'centre' and 'surround' of a given OMS cell are stimulated at once, the resulting excitatory and inhibitory bursts will cancel each other out (Ölveczky et al., 2003; Baccus et al., 2008), but the short timescale of bursts and precise timing ensure small differences in the motion statistics of centre-surround regions will be able to pass. Thus, the OMS ganglion cell is able to respond to local motion independently of the actual trajectory, as long as it is decoupled with global motion in the surround (Ölveczky et al., 2003; Baccus et al., 2008).

The Elementary STMD (ESTMD) Model

Both the End-stopped inhibition and Object Motion Sensitive model were proposed on the basis of studies in vertebrates, but have inspired related models grounded in insect visual anatomy and physiology which none-the-less operate with similar conceptual mechanisms (Egelhaaf, 1985a; Higgins and Pant, 2004). However, evidence that dragonfly STMD neurons can discriminate a target and respond robustly when the target is embedded within a highly textured, cluttered background and without relative velocity cues background (Nordström and O'Carroll, 2006; Wiederman and O'Carroll, 2011) suggests that rather than relying on relative velocity, the mechanism for STMD target detection must use some other information. How can dragonfly STMDs respond without reference to relative motion cues?

This property is captured by the Elementary Small-Target Motion Detector (ESTMD) model (Wiederman et al., 2008). It is instructive to think of the ESTMD model as a conceptual advance on the EMD model described above (*The Idealised Elementary Motion Detector*, pg. 45), except instead of comparing signals across a distance the ESTMD model compares signals at the same spatial location with a delay between signal onset and offset. A dark target drifting across the surface of the eye will produce an OFF signal (i.e., a contrast decrement) at the target's leading edge followed by a corresponding ON signal (i.e., a contrast increment) at the target's trailing edge, and this pattern will be repeated for every location along the target's trajectory. This sequence of OFF-delay-ON signals at a single spatial location produces a temporal signature for a target moving across a single spatial location, independent of wide-field optic flow information.

This computation can be achieved by delaying the OFF signal in a manner similar to the EMD model, such that when the delayed OFF signal and the undelayed ON signal arrive simultaneously at a comparator cell, the signals can be multiplied to produce a response. A separate but parallel channel with the delay on the ON signal would result in equal responses to bright targets on dark backgrounds, but as physiological STMDs do not respond this way (Nordström et al., 2006; Wiederman et al., 2013),

this mirroring is not biologically required. As with the EMD model, the time constant of the signal delay plays a key role in determining the tuning properties of the system. Longer delay constants would allow for a longer period between the intersection of the sample point and the leading/trailing edge, thus tuning for larger or slower target. Conversely, shorter delays would shape tuning for smaller or faster targets. An important corollary of this computation is that size and velocity are fundamentally ambiguous: A target 5° wide but moving at 100°/s will have a 50 ms delay between the initial OFF signal and the following ON signal, but so will a target 1° wide moving at 20°/s. The physiological characteristics of CSTMD1 are consistent with this prediction, with large-target responses increasing with target velocity (Geurten et al., 2007). However unlike the EMD model, the ESTMD model is inherently direction insensitive, as comparisons are made at the same point in space. Directional selectivity can be incorporated into the system by assessing the sequence of ESTMD activation across the eye surface (Wiederman and O'Carroll, 2013b).

One important caveat is that target 'width' and 'height' are handled separately. For example, a 'high but narrow' stimulus (e.g., the trunk of a tree) may still evoke a rapid OFF-pause-ON signal at an individual ESTMD, but dragonfly STMDs do not respond to these kinds of bar stimuli which are tall orthogonal to the direction of motion. How might such signals be filtered out? Lateral inhibition between neighbouring ESTMDs ensure that when a sequence of OFF (or ON) channels are simultaneously triggered (I.e., by a dark tree trunk) they are mutually inhibited, preventing a response. However, when a sequence is triggered serially (as in, a target moving from one to the next over time), this spatial antagonism arrives too late to affect the previous ESTMD unit.

The final prominent feature of the ESTMD model is rapid adaptation of both the ON and OFF channels following their division, inspired by physiological responses of 'Rectifying Transient Cells' in the blowfly (Jansonius and van Hateren, 1991) and cricket (Osorio, 1987). This adaptation serves as a filter preventing response to low-contrast textural changes and flicker, whilst preserving response to high-contrast 'breakthrough' luminance changes characteristic of object motion. By filtering out much of the low-contrast, low-frequency information within a scene at the level of the input into ESTMD units, the ESTMDs themselves are able to respond robustly to a target's own spatiotemporal signature (the OFF-delay-ON sequence) without need for relative motion cues or interference by clutter (Wiederman et al., 2008).

An algorithmic implementation of the ESTMD model (combined with components mimicking the early visual system before the model, such as optical blur etc.) has shown good performance in natural environments in both virtual reality simulations (Bagheri et al., 2017b), and on-board a robot (Bagheri et al., 2017a).

Hyperacuity

Insect target-detection pathways are able to respond to targets considerably smaller than the sampling resolution of the eye (O'Carroll, 1993; Vallet and Coles, 1993; Nordström and O'Carroll, 2006; O'Carroll and Wiederman, 2014; Somanathan et al., 2017; Wardill et al., 2017), and smaller than an individual photoreceptor's receptive field (Rigosi et al., 2017). How is it possible for the brain to detect and localise a target smaller than the receptive field of an individual photoreceptor?

Even a small target will result in decreased photon catch as its shadow passes over the receptive field of a photoreceptor, resulting in a reduced photoreceptor response and dimmer image. However, as target size decreases this response approaches the same magnitude as noise induced by the stochastic nature of light and transduction variability (Lillywhite, 1977; Laughlin and Lillywhite, 1982). This floor threshold in photoreceptor signalling imposes an absolute restrained in both neuronal and behavioural tests of target detection (Rigosi et al., 2017). Of the four insect species studied, the dragonfly *Hemicordulia tau* showed the highest signal sensitivity and lowest photoreceptor noise, and capable of detecting a target sized 0.0256 deg^2 and a 1.4% change in contrast sensitivity (Rigosi et al., 2017). The position of such a moving target could then be, theoretically at least, estimated from the responses of multiple, overlapping STMD receptive fields integrating across multiple photoreceptors by divisive normalization (Evans et al., 2016).

Higher-Order Response Modulation in CSTMD1

Beyond the basic tuning characteristics that build up STMDs as a 'matched filter' for small target motion detection, many STMDs display higher-order properties that shape the cell's response to small targets presented in different scenarios. These modulatory properties may aid the dragonfly in particular tasks or amidst challenging sensory environments. As CSTMD1 is one of the most regularly-encountered and well-characterised dragonfly STMD – as well as the focus of the empirical work presented in later chapters – the remainder of this discussion will focus on CSTMD1, with only passing reference to other STMDs where relevant.

Predictive Gain Modulation

Following an initial response to the presentation of a small target in motion, CSTMD1 exhibits a prolonged and gradual enhancement of response magnitude to targets moving on a continuous, linear trajectory that builds up over approximately 100 milliseconds (Nordström et al., 2011; Dunbier et al., 2012; Fabian et al., 2019), but exhibits reduced response to a target presented for the same total time on a discontinuous trajectory that laterally 'jumps' horizontally around in the receptive field as it ascends (Dunbier et al., 2012). This spatial facilitation can be easily observed in the relative response of a small target ('Facilitation probe', or 'probe') presented with and without a preceding

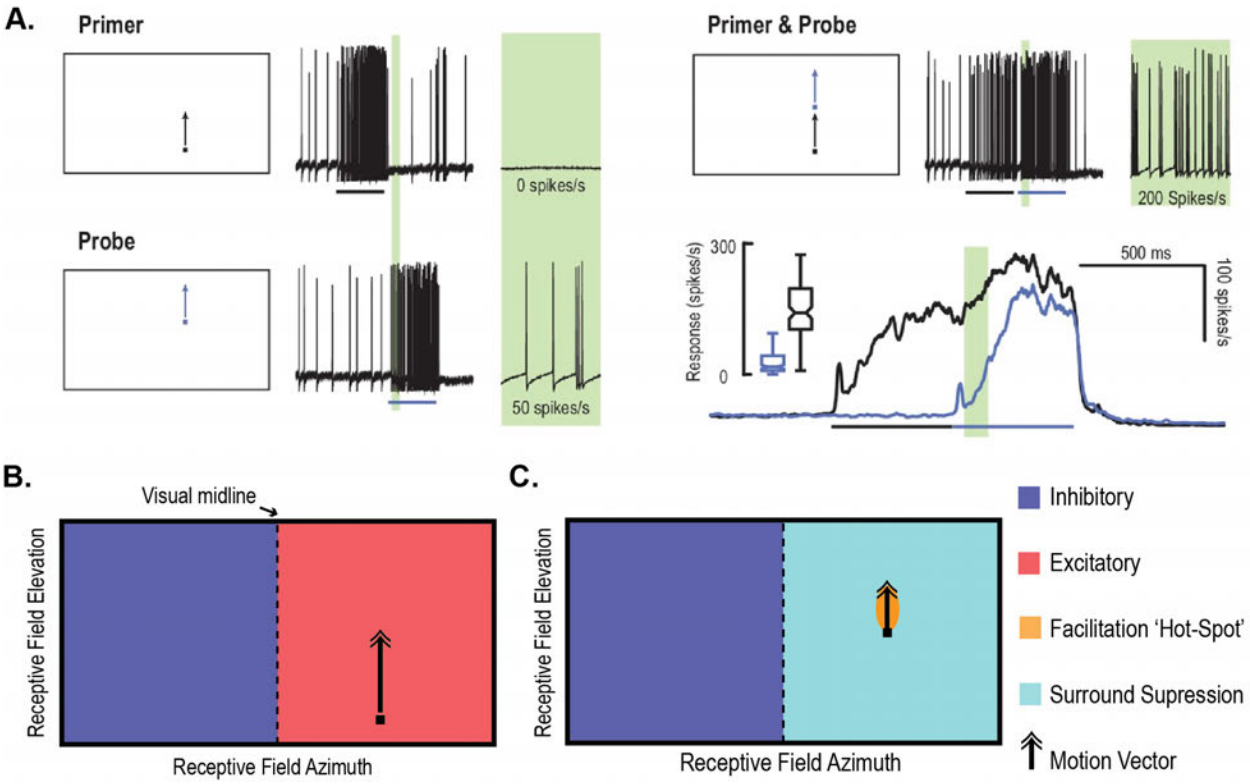

Figure 16: Predictive Gain Modulation in CSTMD1*. A. adapted from Wiederman & Fabian (2017), B. & C. adapted from Lancer et al (2020).* ***A)*** *The effects of a primer stimulus on CSTMD1's response to a probe stimulus at the same spatial location within a short window (before the probe has a chance to build its own facilitation, green window). Peristimulus time histogram reflects 140 trials in a single CSTMD1 for probe alone (blue) and probe + primer (black line) conditions.* ***B)*** *CSTMD1's receptive field is divided into two parts, an inhibitory hemifield (dark blue) and excitatory hemifield (red) divided along the midline of the animal.* ***C)*** *Following the appearance of a target in the excitatory receptive field, the area ahead of the target undergoes gain enhancement that facilitates the cell's response to a target entering the enhanced zone (orange), while the remainder of the excitatory receptive field is supressed (light blue).*

primer on its trajectory (Wiederman et al., 2017; Fabian et al., 2019). In such a case, comparing the first 100 ms of the probe target allows comparison between the cells 'un-facilitated' (Probe Alone) and 'facilitated' (Probe matched with a preceding primer) state for a the same target at the same receptive field location, revealing the strength of facilitation (Figure16A). This spatial facilitation further manifests as a 'spotlight' of gain enhancement directly ahead of the target's current trajectory that enhances responses to the target as it continues into the spotlight (Wiederman et al., 2017). This spotlight of gain enhancement persists for several hundred milliseconds following the disappearance of a target and continues to move on the targets predicted trajectory, allowing a target that temporarily disappears (for example, a prey item flying behind a tree-trunk and becoming temporarily occluded) to elicit a fully facilitated response upon its reappearance (Wiederman et al., 2017). Spatial facilitation also alters the underlying tuning properties of CSTMD1. In its un-facilitated state, CSTMD1 is weakly directionally selective – exhibiting a slightly higher neuronal response to targets moving up and to the right, but nevertheless responding robustly to targets moving in any direction. However, as the facilitation hotspot enhances the area directly ahead of the target's current trajectory, targets changing direction elicit a comparatively reduced response (Wiederman et al., 2017). Thus spatial

facilitation in CSTMD1 predictively encodes a target's trajectory, and is thought to drive the neuronal response to saturation in order to render it insensitive to the transient changes in saliency that might be expected when pursuing a target through a cluttered scene (Fabian et al., 2019), minimising neuronal variability and maximising target detectability despite noise visual input (Fabian et al., 2019). This is a critical computational function for target tracking in highly cluttered environments, where the angular size, velocity, and contrast of a moving target may change drastically throughout a pursuit, or a pursued target may be temporarily occluded (e.g. by an intervening tree branch). For effective target tracking under such conditions, neuronal representation of the target should be robust to a wide range of dynamic stimulus properties. Some earlier STMDs also exhibit facilitation (Wiederman et al., 2017).

Concordant with the spotlight of spatial facilitation generated ahead of a moving target, responses to targets presented in distant areas of the receptive field are actively supressed (Wiederman et al., 2017). This 'surround suppression' easily accounts for an earlier finding where a single target ascending the receptive field but laterally 'jumping' around to different horizontal locations generated a supressed response compared to a single target of the same size and velocity on a continuous path (Dunbier et al., 2012). Together, this spatially distinct pattern of surround suppression and predictive spotlight enhancement is termed 'Predictive gain Modulation.'

Selective Attention

Objects rarely exist alone in the world, and any target detection task must be undertaken against the backdrop of visual clutter, including amidst swarms and against foliage. Many insects attempt to minimise the impact of potential distraction by detecting targets from a perch or by stationary hovering, thereby reducing the background optic flow and enabling target motion to 'pop out.' However, such a strategy is only effective for target acquisition, as optic flow is generated during pursuit movement. Dragonflies may also attempt to reduce the effect of background noise by positioning themselves such that their target is between them and the clear sky, in the dorsal acute zone of the eye (Olberg et al., 2007). However, these strategies rely on the ability to select and respond to only a single target in order to bring it into the preferred position for a pursuit. Such a selective computation is critical of visually-guided behaviour; where there are multiple potential 'targets' within the visual field of an animal, a pursuit trajectory must none-the-less be directed to an *individual* target rather than the 'average' of all targets (Figure 17A).

The visual neuron CSTMD1 has been shown to exhibit *winner-takes-all* selective attention (Wiederman and O'Carroll, 2013a). When presented with a target pair of near-equal salience

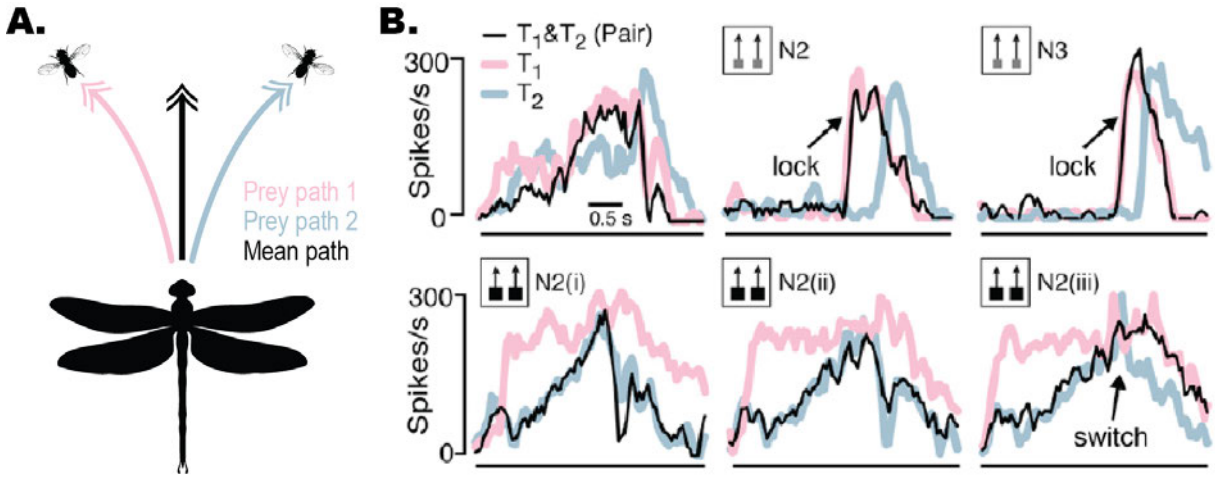

Figure 17: Selective Attention in CSTMD1. A) *In order to capture a tasty fruit fly, this Dragonfly must commit to Prey path 1 (pink) or Prey path 2 (blue), as pursuing a mean path (black) would result in failure.* ***B)*** *Neuronal response to single and competing targets, reproduced from Figure 2 in Wiederman & O'Carroll 2013. In this experiment, small targets were presented on two possible paths through the cells excitatory receptive field, labelled Trajectory-1 (T_1) and Trajectory-2 (T_2). Pink lines illustrate the neuronal response to T_1 targets alone, blue lines illustrate the neuronal response to T_2 targets alone. Differences in spike rate reflect inhomogeneity of the underlying receptive field that the target's traverse. In paired-target trials (black lines), the neuronal response closely tracks the response of one target alone, rather than an average, indicating selection of that target. In some individual trials (I.e., bottom-right subplot), the selection is able to 'switch' partway through the trial, indicating a switch of selection from one target to the other.*

(matched size, contrast, and velocity – but at different locations in the excitatory receptive field), CSTMD1 'selects' and responds to one of these targets by encoding the *absolute strength* of the selected target, without interference from distractors as is characteristic of vertebrate attentional systems (Recanzone et al., 1997; Treue and Maunsell, 1999; Ghose and Maunsell, 2008; Asadollahi et al., 2010). In order to see this, Wiederman & O'Carroll (2013) first presented single-targets alone at two different trajectories within CSTMD1's excitatory receptive field, 'Trajectory 1' (T_1) and 'Trajectory 2' (T_2). Despite analogous target properties (size/speed/contrast), these stimuli evoked different responses based on their position in the receptive field due to the inhomogeneous nature of CSTMD1's response across space. These single-target presentations lead to the characterisation of response 'fingerprints' for each target that were stable over time (Figure 17B, pink and blue traces), where the location of a presented target could be determined by this temporal response 'fingerprint'. Wiederman & O'Carroll (2013) found that when paired targets were simultaneously presented, the neuronal response closely matched the 'fingerprint' for *one* of the presented targets, rather than either an average or summed response (Figure 17B, black traces), indicating selection of and response to just one of the targets in the presented pair, without apparent influence by the distractor. Furthermore, there was inter-trial variability of which target was selected within the same neuron, and on some trials the neuronal response 'switched' from matching one target to the other without a period of average or summed response in between. Intriguingly, the response appears to 'lock' on to a single target, even after that target falls outside of the receptive field hot-spot, as the response then falls rapidly in accordance with the locked trajectory, despite the continued presence of the ignored

target in strong parts of the receptive field. CSTMD1 can therefore select a target independently of its relative salience at any given moment.

The ability of CSTMD1 to respond with the same strength to target presented alone, or when selected in a swarm, combined with the facilitation of selected targets to reduce noise (Fabian et al., 2019) may have evolved to allow some families of dragonflies to so successfully hunt amidst swarms of prey (Combes et al., 2012) and in highly cluttered conditions, helping to overcome the perceptual bottleneck of the confusion effect by selectively locking on to a target.

Beyond the Optic Lobes

Beyond the optic lobe lies a large series of modular neuropil that lie across the midline and are collectively known as the 'midbrain' or 'central brain.' The midbrain neuropil functionally comprise 'the rest' of the insect brain, with a variety of sensory, behavioural, and homeostatic functions, including higher order functions such as learning and memory. The midbrain contains three neuropil of particular relevance to the current work, the Optic Glomeruli, Central Complex, and Mushroom Bodies. Of these, the Central Complex and Mushroom bodies are associated with neuronal processing of attention, and were discussed above. The remaining major components of the insect visual system are known as the Optic Glomeruli.

The optic glomeruli are series of discrete synapse-rich neuropil situated in the ventrolateral protocerebrum, posterior lateral protocerebrum, and anterior optic tubercle, which are broadly adjacent to the optic lobes, that are the major projection site for Visual Projection Neurons (VPNs) leaving the optic lobe, including Lobular Columnar neurons such as LC11 (Strausfeld et al., 2007; Wu et al., 2016), MLGs, CSTMD1 and BSTMD1, as well as the input region for TSDNs. The majority of work on the optic glomeruli has been undertaken in *Drosophila*, and it is currently unknown how this part of the visual system may present different in in dragonflies. In *Drosophila*, each optic glomerulus pools input from across the retinotopic space presented in the lobula, usually receiving input from only single functionally specialised LC type responsible for representing a specific kind of visual stimulus (Strausfeld et al., 2007; Wu et al., 2016). The retinotopic representation in the lobula suggests spatial information is important for computations at the local level (e.g., retinotopic lateral inhibition) and for the extraction of visual features of spatiotemporal patterns that ultimately reflect photoreceptor activity, but the convergence of LC subtypes from across the lobula into single glomeruli without spatially consistent representation (with some exceptions) suggests such spatial information is no longer required (Wu et al., 2016). The optic glomeruli share structural and functional similarities with two sets of glomeruli also in the protocerebrum (olfactory and antennal glomeruli), which serve other sensory modalities (Strausfeld et al., 2007; Mu et al., 2012).

The Current Body of Work

The preceding introductory review covered a broad range of topics, but each descended from a central question – what are the neural mechanisms that underlie the dragonflies' success hunting amidst swarms and cluttered environments? How does the dragonfly visual system avoid the confusion effect? The current set of work aims to elucidate answers to this question by focusing on electrophysiological recordings of CSTMD1 during visual stimulation, drawing inspiration from the literature on selective attention in vertebrates and other insects.

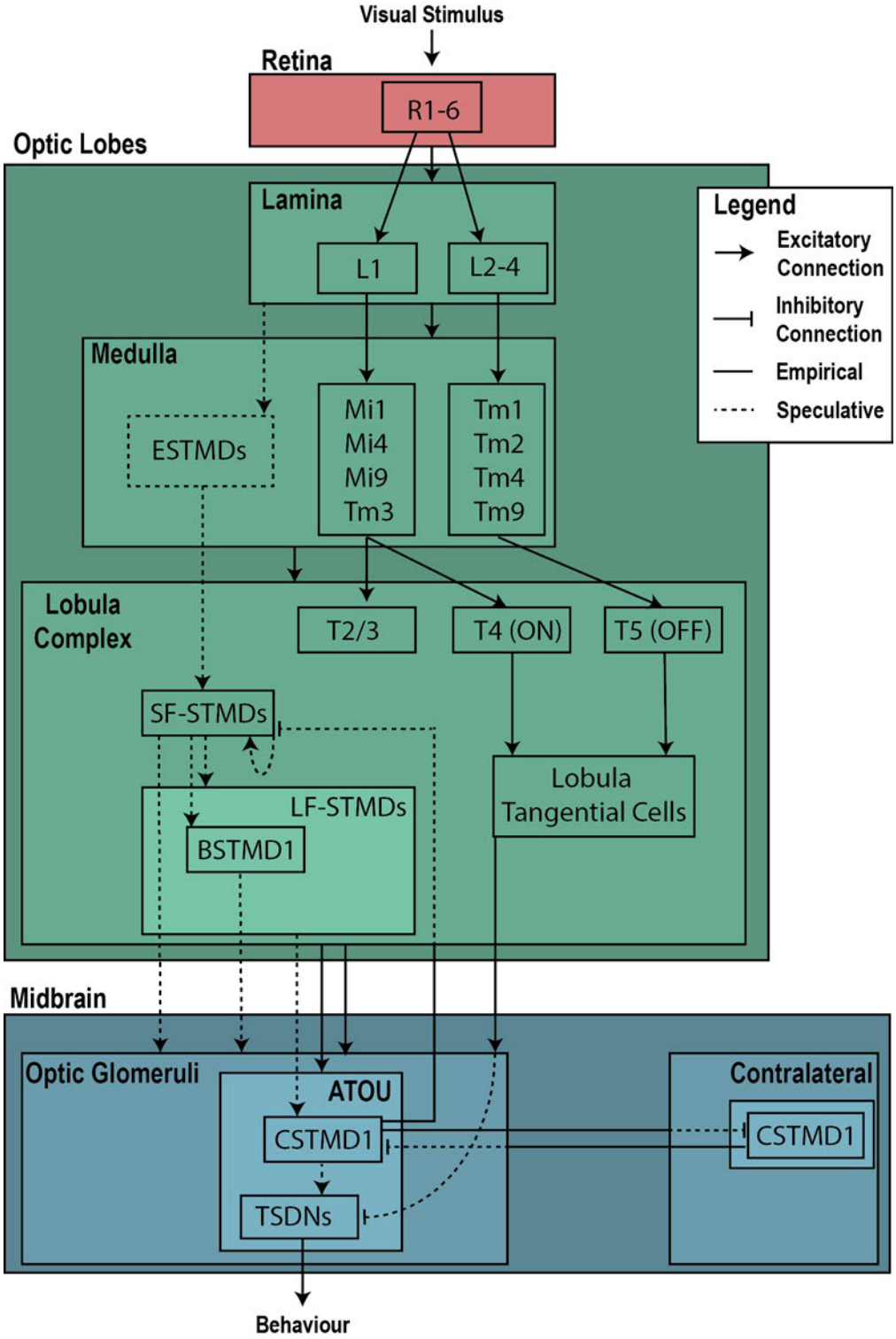

Figure 18: Summary Schematic of the Dragonfly Visual System. *Based on evidence from Dragonflies where possible, and Drosophila research where there is no evidence from Dragonflies. Early optic lobe circuitry (Lamina, Medullla, and T# cells) based largely on findings in Drosophila. STMD connections are largely speculative based on functional physiology in the absence of anatomical evidence. Visual stimuli transduced in the retina and split into ON and OFF pathways before projection through the Medulla and early Lobula Complex where Elementary Motion Detection is thought to occur. Lobula Tangential cells extant in Dragonflies (residing in the Lobula Plate in flies) respond to wide-field optic flow information. STMDs are thought to receive input from Medullary Interneurons instantiating an ESTMD computation, and form a hierarchy of STMDs of increasing receptive field size. LF-STMDs as well as other Visual Projection Neurons (not shown) project to the optic glomeruli in the lateral midbrain, including the Anterior Optic Tubercle (ATOU), where CSTMD1 arborizes a patch of input dendrites. Target Selective Descending Neurons (TSDNs) which convey target positioning information to wing motor centres in the thoracic ganglia also project input dendrites to this zone. Speculative elements of this proposed circuitry are highlighted with dashed lines.*

Chapter 2: Methods

Detailed descriptions of experimental methods will be presented in each results chapter alongside the specific results. Here, I will focus on general principles.

Experimental Animals

The major focus of this thesis was on two species of dragonfly, *Hemicordulia tau* and *Hemicordulia australiae*. *H. tau* and *H. australiae* are both commonly found in South Australia and can be readily collected wild at the Adelaide Botanic gardens, a 5-minute walk from Adelaide Medical School where the laboratory is located. *H. tau* and *H. australiae* are both highly amenable to electrophysiological recording and share behavioural and physiological properties.

Dragonflies are typically available between September and May (in the southern hemisphere), with a bimodal distribution that peaks during October/November in the beginning of the season and around April near the end, representing successive generations over each summer season. Behavioural research in other species has shown no difference between Male and Female performance on capture efficiency (Combes et al., 2013) as both male and female dragonflies feed via pursuit predation. Despite this, I used exclusively male dragonflies in order to limit the effect of capture on the local population. All dragonflies were wild caught as adults, and teneral adults (Identified by the relative brightness of their colouration and softness of the chitin) were released.

In addition to these two focus species, I attempted recordings from a variety of other locally captured species, including: the Australian Emperor (*Anax papuensis*), Blue Skimmer (*Orthetrum caledonicum)*, Blue-spotted Hawker (*Adversaeschna brevistyla),* and Wandering Percher (*Diplacodes bipunctata).* Many of these recordings were unsuccessful, especially for *A. papuensis* and *A. brevistyla*, which are large dragonflies from the *Aeshnidae* family and much harder to restrain for electrophysiological recording. These recordings were attempted in preparation for a comparative study across multiple Australian and European species scheduled to be undertaken in Sweden during the Swedish summer in 2020, but this project was cancelled due to international travel restrictions.

Intracellular Electrophysiology

The major experimental focus of this thesis comprised intracellular recordings from a specific, well-characterised dragonfly visual neuron (CSTMD1) *in vivo* during visual stimulus presentation.

Dragonflies were first immobilised to an articulating magnetic stand using a 1:1 wax:rosin mixture applied to the chitin, wings, and mouth using a heated dental probe (~85°C), in order to prevent movement as much as possible to ensure a stable electrophysiological recording. Once

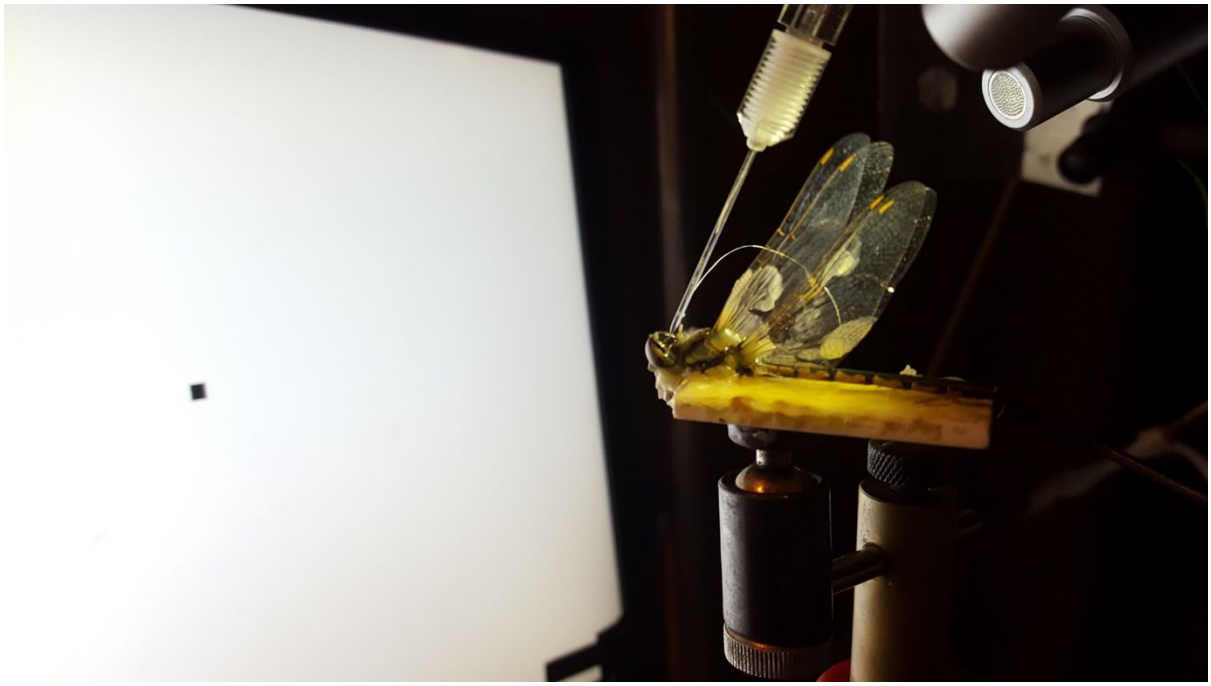

Figure 19: Intracellular Electrophysiology. *A* dragonfly *(Hemicordulia tau*) waxed in position on an articulating magnetic stand. An aluminosilicate glass capillary attached to the electrode holder is carefully placed within a hole dissected on the posterior surface of the head capsule. The reference wire is inserted into the contralateral optic lobe. A small target is present on the stimulus monitor.

immobilised, the head was tilted forward (~45° to the body) and a small (1 by 1 mm) patch of chitin was dissected from the medial posterior surface of the head, adjacent to the oesophagus.

Aluminosilicate electrodes (typical resistance between 40-140 KΩ) filled with 2M KCL solution were placed adjacent to the medial Lobula with the perineuronal sheath intact, where a tract of axons running between the Lobula and Central brain is known to reside. The electrode was manoeuvred above the brain manually, and then finely manipulated with a piezo-electric actuator (PM 10, SDR Scientific) set for 5 µm increments.

Once a neuron was encountered it was characterised via a battery of stimuli designed to test responsiveness to different visual signals, including whole-screen flicker (white-to-black-to-white), sinusoidal gratings (four cardinal directions), a gyrating texel pattern (clockwise, anticlockwise), loom (expanding black square), moving bars (4 cardinal directions), and testing with manual control of a small (1.5° by 1.5°) dark target. CSTMD1 was recognised by stereotypical stimulus response characteristics; insensitivity to gratings, texels, and bars; robust response to small targets in a characteristic inhibitory/excitatory receptive field pattern (Figure 15B) with inhibition in the ipsilateral (to recording site; left optic lobe) and excitation on the contralateral side, as well as spike characteristics (size, waveform, and resting spike rate).

Once CSTMD1 was identified, experiments were run on a custom-made software suite based on MATLAB and PsychToolbox (www.psychtoolbox.org). Each experiment consisted of a ‘sequence’ of trials of different conditions (control trials and test trials) generally lasting 1-2 seconds each (with an

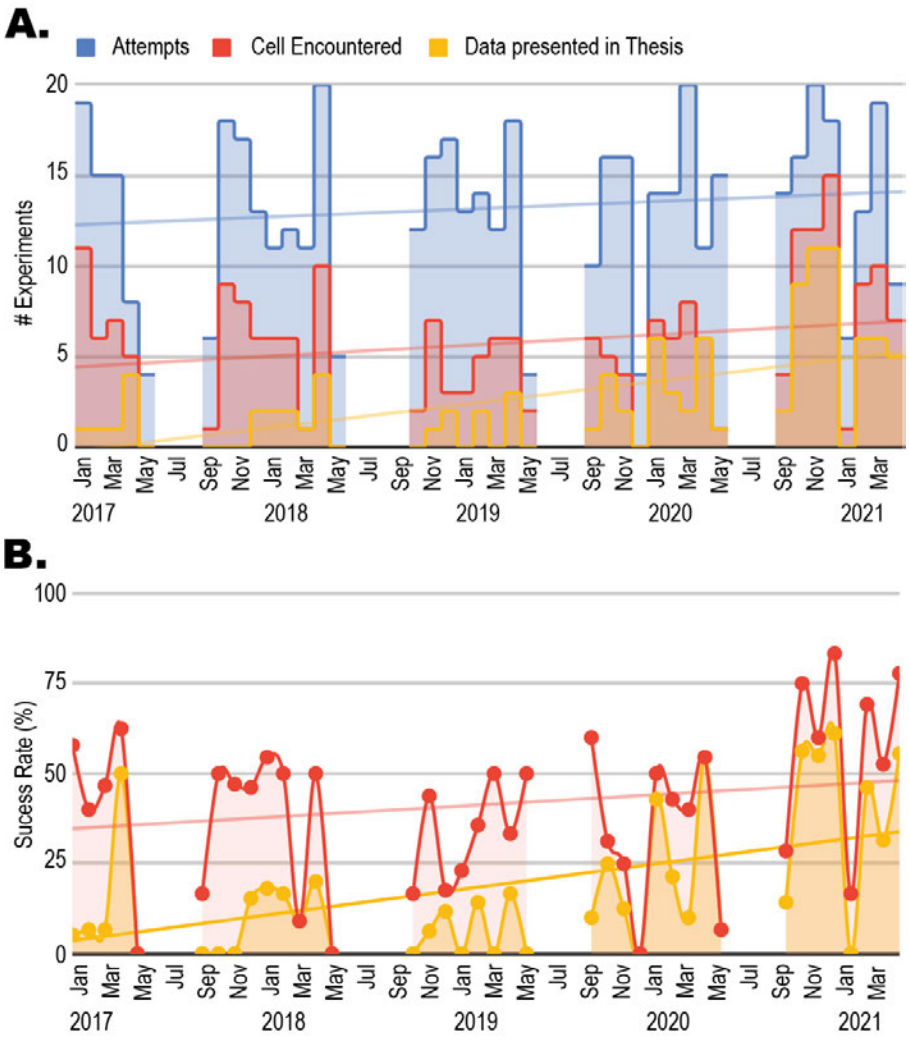

Figure 20: *Experimental performance over time.* ***A)*** *Histograms illustrating count of experiments per month from January 2017 to April 2021, # attempted (blue), # in which a spiking neuron was encountered (Red), and # in which CSTMD1 was encountered with enough quality to include data (Yellow). ~July dips reflect lack of dragonflies during the winter season. Although the overall number of experiments attempted varied widely by month, overall # per season remained stable.* ***B)*** *Success rate (% of experiments attempted) for encountering spiking neurons (red) and collecting quality data (yellow). Data collection success rate increased with time.*

additional second of pre- and post-stimulus recording). Trials were randomly interleaved, and each sequence generally contained 2-4 repeats of each trial. Sequences were preferentially run at least twice in each cell (unless the cell was lost prematurely) at slightly different receptive field locations to avoid habituation. As an additional measure to avoid habituation, a 12-30 second rest period of no stimulation was included between each trial. Most experiments required between 5 and 20 minutes of uninterrupted recording (with some requiring up to 90m), so that on most CSTMD1 encounters I was able to run multiple experiments, including repeats.

Data was sampled at either 5 kHz (< winter 2019) or 10 kHz (> winter 2019) and digitally saved via MATLAB for offline analysis.

Electrophysiological Recording Success Over Time

I attempted a total of 515 intracellular recordings from dragonflies. In total, I encountered neurons in 223 experiments (43.3% of attempts), with multiple cells encountered in most experiments where at least 1 cell was encountered. However, as my electrode was inserted into a large tract of axons communicating between the left Optic Lobe and Midbrain, I encountered a wide variety of other kinds of neuron, including Lobula Tangential cells (encoding wide-field optic-flow), other STMDs, flicker-responsive neurons, as well as unidentifiable neurons with no clear visually evoked response.

These cells were often 'gifted' to other students or lab members who had experiments for cells of that type, or otherwise stepped past in attempt to find CSTMD1. In addition, many encounters with CSTMD1 failed to result in reliable data if the neuron did not fit strict recording quality standards for publication, including minimum signal-to-noise ratio and the absence of pathological physiology (abnormally high firing rates, abnormal bursting, and unstable resting membrane potential) or habituation. Such recordings were excluded from analysis.

I successfully collected publishable-quality data from CSTMD1 from a total of 101 individual dragonflies, for a total PhD-long success rate of 19.61%. Overall performance is shown in Figure 20. However, successful recordings were not evenly distributed. In my first recording season (Jan 2017 – May 2017) I attempted a total of 61 experiments and successfully recorded data from 7 CSTMD1 neurons, a success rate of just 11.5%. In my last recording season (September 2020 – April 2021) I attempted a total of 115 experiments and successfully recorded from 50 CSTMD1 neurons, a success rate of 43.5%.

Visual Stimuli

Visual stimuli were presented on an LCD (Liquid-Crystal Display) computer monitor (either an Eizo Foris FG2421 LCD at 120 Hz framerate or Asus ROG Swift PG279Q IPS at 165 Hz) placed 20 cm away from the insect, centred on the vertical midline. Data from the first recording season (Jan 2017 – May 2017) used a 120 Hz monitor (400 cd/m2), but all subsequent experiments used a 165 Hz monitor (350 cd/m2). Visual stimuli generally consisted of small (1.5° squared) dark targets presented against a bright white background, moving at 50°/s. However, these stimulus parameters were varied depending on the specific question each experiment was designed to approach, and specifics will be detailed in individual results chapters.

Experimental Design

Selective attention is a moment-to-moment, trial by trial process where the neuronal system's ability to change its response to the same stimulus is the phenomena under study and could be masked by averaging trials based on stimulus condition. As such, any individual trial must be treated as an independent observation. In order to ensure statistical robustness, we repeat all trials multiple times in each of several dragonflies. We use 'n' to denote the number of trials and additionally report the number of dragonflies, however, due to the stochastic nature of electrophysiology it is not always possible to record the exact same number of trials in each dragonfly (due to prematurely losing the cell or random non-responsive trials). When presenting data, we visualise all trial data points (rather than averaging across condition or dragonfly). Specific details relative to each experiment are included in the *methods* section of each results chapter.

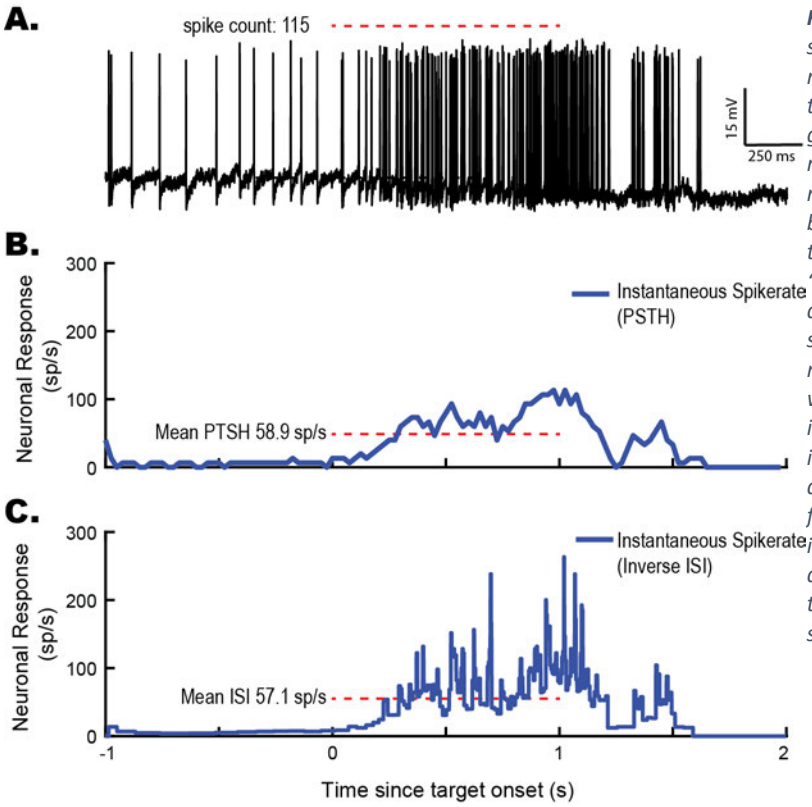

Figure 21: Methods of quantifying neuronal response. A) *A spike-train illustrating an example neuronal response (CSTMD1 responding to a target from t = 0 – 1.5). I counted 115 spikes in the 1 second period following target onset (dashed red line), giving an overall spike rate of 115 sp/s for the first second of response.* ***B)*** *Peristimulus time histogram (PSTH) of the neuronal response presented in A. For a PSTH, the neuronal response is binned into smaller periods (this example uses 20 ms bins, and the response in sp/s is calculated for each bin, resulting in an 'instantaneous spike rate' at the resolution of the bin size. The average PSTH for the first second of stimulus presentation is 58.9 sp/s, but this includes an early period of non-response due to neuronal delays, highlighting the importance of window choices when analysing neuronal responses.* ***C)*** *Inverse Interspike interval (Inverse ISI) of the neuronal response presented in A. ISI is calculated by measuring the time between each pair of adjacent spikes, resulting in a 'high' ISI (in time) when spikes are far apart (not shown). The raw ISI is then inverted and brought into units of sp/s. ISI measures appear 'spikier' as information about short bursts of spikes are preserved, represented as transiently high inverse ISI. The average inverse ISI for the first second of stimulus presentation is 57.1 sp/s.*

Data Analysis

Understanding how neurons represent and encode information is a fundamental concern of Neuroscience. Neurons are thought to encode information via action potentials – transient changes in electrical polarity mediated by ion channels in the cell membrane – which are commonly referred to as 'spikes'. CSTMD1 has a spontaneous spike rate of approximately 12-25 spikes/second (sp/s), which is increased when the cell is presented with an excitatory stimulus (I.e., a target in the excitatory receptive field) and decreased when the cell is presented with an inhibitory stimulus (i.e., a target in the inhibitory receptive field.) CSTMD1 – like other STMDs, and many visual neurons across taxa – respond to increasing stimulus contrast with an increased spike rate (O'Carroll, 1993; Geurten et al., 2007; O'Carroll and Wiederman, 2014), suggesting that these neurons use a 'rate code' to represent stimulus information. Despite apparent simplicity, there are still several ways to measure a neuronal 'rate code.' The simplest method is to count the number of spikes within a window – for example, during stimulus presentation – resulting in a raw spike count (Figure 21A). A finer resolution 'instantaneous spike rate' can be derived by counting the spikes in smaller windowed 'bins' across the period of interest, resulting in a Peristimulus Time Histogram (PSTH; Figure 21B). E.g., If 20 spikes were counted in a 100 ms window the spike rate would be 200 spike/s over that window. Spike counting methods are widely used throughout neuroscience, but ignore potentially important

information about spike timing, such as the relative time between individual spikes and absolute timing of spikes relative to a stimulus (Gollisch and Meister, 2008).

Precise spike-timing and high frequency fluctuations in spiking activity may also carry information content but would be difficult to distinguish with spike counting methods. CSTMD1 has recently been shown to exhibit a burst code where several spikes are fired in quick succession, followed by quiescence (Fabian and Wiederman, 2021), potentially allowing more rapid signalling than a pure rate code integrating over a longer time period would allow. An alternative method to quantify neuronal activity that preserves some timing information is the *interspike interval (ISI)*, the length of time between two adjacent spikes in a spike train. For a pure rate encoding strategy the raw ISI would be expected to steadily decrease as the firing rate goes up, but biological ISIs vary considerably and it is not fully understood if these variations are part of a signal, or biological noise (Stein et al., 2005). In such a scheme, short bursts such as those observed in CSTMD1 would appear as a transient period of low ISI, with a quieter period of high ISI on either side (the time between bursts). For clarity and ease of comparison, the ISI is often plotted as an *inverse* with neuronal response in sp/s on the *y* axis rather than raw ISI (Figure 21C). Similarly, to the spike count methods described above, the neuronal response in a window can be calculated by taking the average ISI over a window of interest. The work in this thesis has made use of Spike Count, PSTH, and ISI methods to quantify neuronal response where appropriate.

Unless otherwise specified, all statistical hypothesis testing was undertaken in MATLAB using a two-tailed, nonparametric test (Generally the Mann-Whitney two-sample test) and corrected for multiple comparisons using the Bonferroni-Holm correction.

Chapter 3: A Target-Detecting Visual Neuron in the Dragonfly Locks on to Selectively Attended Targets

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Abstract: The visual world projects a complex and rapidly changing image onto the retina of many animal species. This presents computational challenges for those animals reliant on visual processing to provide an accurate representation of the world. One such challenge is parsing a visual scene for the most salient targets, such as the selection of prey amidst a swarm. The ability to selectively prioritize processing of some stimuli over others is known as 'selective attention'. We recently identified a dragonfly visual neuron called 'Centrifugal Small Target Motion Detector 1' (CSTMD1) that exhibits selective attention when presented with multiple, equally salient targets. Here we conducted in vivo, electrophysiological recordings from CSTMD1 in wild-caught male dragonflies (*Hemicordulia tau*), whilst presenting visual stimuli on an LCD monitor. To identify the target selected in any given trial, we uniquely modulated the intensity of the moving targets (frequency-tagging). We found that the frequency information of the selected target is preserved in the neuronal response, whilst the distracter is completely ignored. We also show that the competitive system that underlies selection in this neuron can be biased by the presentation of a preceding target on the same trajectory, even when it is of lower contrast than an abrupt, novel distracter. With this improved method for identifying and biasing target selection in CSTMD1, the dragonfly provides an ideal animal model system to probe the neuronal mechanisms underlying selective attention.

Significance Statement: We present the first application of frequency-tagging to intracellular neuronal recordings, demonstrating that the frequency component of a stimulus is encoded in the spiking response of an individual neuron. Using this technique as an identifier, we demonstrate that CSTMD1 'locks on' to a selected target and encodes the absolute strength of this target, even in the presence of abruptly-appearing, high-contrast distracters. The underlying mechanism also permits selection to switch between targets mid-trail, even among equivalent targets. Taken together, these results demonstrate greater complexity in this selective attention system than would be expected in a winner-takes-all network. These results are in contrast to typical findings in the primate and avian brain, but display intriguing resemblance to observations in human psychophysics.

Statement of Authorship

Title of Paper	A Target-Detecting Visual Neuron in the Dragonfly Locks on to Selectively Attended Targets
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Principal Author

Name of Principal Author (Candidate)	Benjamin H. Lancer
Contribution to the Paper	Experiment & methodology conceptualisation and design; animal collection; electrophysiology; data curation, analysis & interpretation; figure generation; manuscript original draft, review, and editing.
Overall percentage (%)	60%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 4/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Bernard Evans		
Contribution to the Paper	Experiment & methodology conceptualisation and design; software development; animal collection; electrophysiology; data curation, analysis, validation, and interpretation; computational modelling; figure generation; manuscript review & editing.		
Signature		Date	11/10/21

Name of Co-Author	Joseph Fabian		
Contribution to the Paper	Experiment conceptualisation; electrophysiology; data analysis & interpretation; manuscript review & editing.		
Signature		Date	6/10/21

Name of Co-Author	David O'Carroll		
Contribution to the Paper	Experiment & methodology conceptualisation; data interpretation; manuscript review & editing; supervision; funding acquisition.		
Signature		Date	29 Sept 2021

Name of Co-Author	Steven Wiederman		
Contribution to the Paper	Experiment & methodology conceptualisation; software development; data interpretation; manuscript review & editing; supervision; provision of resources; funding acquisition.		
Signature		Date	7/10/21

Thesis Contextual Statement

Selective attention was first shown in the dragonfly visual neuron CSTMD1 by my supervisors Steven Wiederman & David O'Carroll in 2013 (Wiederman and O'Carroll, 2013a), but remained an interesting offshoot of dragonfly Target Tracking research until It became my primary focus.

The problem was, then, that the initial method used to establish selection relied heavily on different response 'fingerprints' to targets moving on different trajectories within CSTMD1's inhomogeneous receptive field. This method was good enough to establish a target was being selected in the confines of a narrow stimulus space but precluded any 'interesting' experiments which would be likely to influence the spike rate and thus alter the 'fingerprint.'

The goals of this paper were thus twofold. First, to establish a method for identifying the target of selection, and second, to begin to utilize that method to answer some of our biggest questions regarding selection. Taking inspiration from human electroencephalogram (EEG) and insect Local Field Potential (LFP) research, we applied the 'Steady-State Visually Evoked Potential' (SSVEP) method, which we re-named 'frequency tagging' as SSVEP refers specifically to aggregate potential recordings of neuronal populations, whereas we were looking at the intracellularly recorded spiking response of a *single* neuron. Once frequency tagging was validated as a reliable identifier (though, not without its own limitations), we moved on to more interesting *scientific* questions, rather than just methodological ones.

The first aim was to identify if CSTMD1 was amenable to attentional priming/cueing. The ability to experimentally direct (or at least bias) attention is an important part of experimental design throughout the vertebrate attentional literature and broadens the possible experiments that can be attended. The second aim was to utilize priming and cuing as an experimental tool to understand the process of selective attention in this system.

With priming firmly in place, we moved on to what seemed like the biggest question – 'locking on.' Part of what makes attention an interesting phenomenon to study is that it is *context sensitive*. In most systems, attention is not simply a 'max operator' that chooses the highest raw saliency stimulus at any one time. Instead (and depending on the system in question), attention is influenced by behavioural goals, internal states (hunger, motivation, fear) and stimulus history. To begin testing this in CSTMD1, we assessed the selection mechanism's resistance to a) an abruptly appearing, novel distractor and a distractor of higher contrast. We found in both cases CSTMD1 was able to flexibly 'lock-on' to the initially attended stimulus in some trials, and switch in others. This was an important first step in defining the scope of the selective attention in our model.

While this paper is in the majority my own work and all co-authors assisted in experimental conceptualisation, design, and interpretation, I would like to thank Bernard Evans who undertook the modelling component that provides the capstone to my electrophysiological data.

Introduction

The visual world contains a wealth of information about the environment and surroundings, yet even the most sophisticated visual systems lack the capacity to encode all the information in a scene over time. Instead, animals must parse a scene for behaviourally relevant information and discard the remaining clutter. One solution to this problem is selective attention, the ability to selectively respond to one stimulus amongst multiple alternatives. Selective attention is observed across taxa, from humans and other primates (Treue, 2001) to insects (De Bivort and van Swinderen, 2016; Nityananda, 2016). Selective attention is particularly important in visual predatory animals, such as the dragonfly, which hunt among swarms containing potentially hundreds of prey and conspecifics (Edman and Haeger, 1974; Baird and May, 1997). Many predators hunting in these conditions are susceptible to the 'confusion effect', a reduced success rate due to difficulty tracking a single target amidst the swarm (Landeau and Terborgh, 1986; Jeschke and Tollrian, 2007). Some dragonfly species, however, show particularly good performance hunting among swarms (Jeschke and Tollrian, 2007; Combes et al., 2012).

Successful prey capture relies on the ability to filter irrelevant information, such as background clutter and conspecifics, whilst selecting and tracking prey amongst equally valuable alternatives. The confusion effect is diminished where predators are able to identify individual prey (Landeau and Terborgh, 1986). In order to achieve this, the underlying neuronal system should be able to 'lock-on' to an individual target, whilst also being capable of switching targets when this would increase the chance of success.

We have previously identified an individual visual neuron in the dragonfly optic lobe that exhibits a 'winner-takes-all' selective attention(Wiederman and O'Carroll, 2013a). Named 'Centrifugal Small-Target Motion Detector 1' (CSTMD1), this binocular, efferent neuron resides in the optic lobes and midbrain (Geurten et al., 2007) and is thought to represent the output integrator of a network comprised of many lower-order, small-target motion detector neurons (STMDs). CSTMD1 is tuned for the movement of small (1°-3°) dark targets against a bright background (O'Carroll, 1993; Geurten et al., 2007), matching the demands of an ethologically relevant target-detection system (Labhart and Nilsson, 1995; Olberg et al., 2005, 2007). CSTMD1's receptive field spans the whole visual field, but exhibits a sharp distinction between excitatory (contralateral relative to recording site) and inhibitory (ipsilateral) visual hemispheres (Geurten et al., 2007). When presented with two targets in the excitatory receptive field, CSTMD1 encodes the absolute strength of the selected target without

interference from distracters(Wiederman and O'Carroll, 2013a). In contrast, typical findings in primates (e.g. Recanzone et al., 1997; Treue and Maunsell, 1999), owls (Asadollahi et al., 2010) and other insects (Tang and Juusola, 2010; van Swinderen, 2012) show a response that is modulated by the presence of distracters. Encoding an absolute representation of a selected target (i.e. ignoring the distracter) has been observed in the auditory system of crickets (Pollack, 1988) and in primate MT neurons (Harrison et al., 2013). An analogue exists in humans termed 'inattentional blindness', whereby an object in the visual field is ignored when attention is focused elsewhere (Simons and Chabris, 1999).

Previously, we have shown that CSTMD1 exhibits properties important for a prey-tracking system. Firstly, the observation that selection could sometimes switch between targets mid-way through a trial (Wiederman and O'Carroll, 2013a) raised the possibility that an ongoing competitive mechanism drives selection, even after an initial target has been selected, and that this mechanism can direct switches at opportune moments. Secondly, CSTMD1 exhibits 'predictive gain modulation' whereby a local facilitatory 'spotlight' of increased gain spreads forward along the predicted trajectory of a target (even accounting for occlusions), with inhibition elsewhere in the receptive field (Dunbier et al., 2012; Wiederman et al., 2017). This facilitation may represent a mechanism for 'locking-on' to a selected target, for example, a chosen fruit fly in a swarm.

Here, we have developed a technique to frequency-tag targets by exploiting the contrast dependant neuronal response (O'Carroll and Wiederman, 2014), permitting us to determine which target has been selected at any moment. Frequency-tagging has previously been used during higher-order brain measurements (e.g. EEG) and in extracellular recordings measuring local field potentials (LFP) in insects (van Swinderen, 2012; Paulk et al, 2014). However, it is not yet known whether frequency components within these frequency-tagged signals originate at the level of single neurons, or are an emergent property of a neuronal population code. To our knowledge, here we present the first application of this identification technique at the intracellular level. We thus demonstrate that, due to the contrast-sensitivity of CSTMD1, the frequency component of the stimulus is preserved in the individual neuron's response.

Applying this technique to intracellular spike trains, we show that CSTMD1 is both able to switch selected targets mid-trial and lock-on to selected targets, even in the presence of a higher contrast distracter. We therefore describe a neuronal system more complex than the traditionally modelled winner-takes-all framework. This provides important insight into how selective behaviours are implemented by underlying neuronal processing.

Materials & Methods

Experimental Preparation

We recorded from a total of 26 male, wild-caught dragonflies (*Hemicordulia tau*). Dragonflies were stored at 7°C for up to 7 days before experimentation. Dragonflies were warmed and then immobilized to an articulating magnetic stand with a 50/50 wax-rosin mixture. The head was tilted forwards to allow access to the back of the head, and a small hole was dissected in the rear of the head capsule adjacent to the oesophagus to allow visual and physical access to the lobula complex and lateral midbrain.

We pulled aluminosilicate electrodes (Harvard Apparatus) using a Sutter Instruments P-97 electrode puller, which were filled with a 2M KCl solution. Electrodes were then inserted into the lobula complex using a piezo-electric stepper with a typical resistance of 40-140 MΩ. Intracellular responses were digitised at 5 kHz for offline analysis with MATLAB.

There are two mirror-symmetric CSTMD1 neurons in each dragonfly brain, with one cell body residing in each hemisphere. We record from the left optic lobe where a tract containing a large-diameter section of the contralateral CSTMD1's axon is known to reside. We can therefore record from a maximum of one CSTMD1 per dragonfly.

Visual Stimuli

We presented stimuli on high-definition LCD computer monitors (120 – 165 Hz) using a custom-built presentation and data acquisition suite based on MATLAB (RRID: SCR_001622) and Psychtoolbox (RRID: SCR_002881. Available: http://psychtoolbox.org/). The animal was placed 20 cm away from the monitor and centred on the visual midline, thus minimizing off-axis artefacts. Stimuli consisted of a single or pair (~20° separation) of 1.5° by 1.5° squares of modulated contrast ascending the receptive field at a speed of 40°/s.

We applied to our intracellular recordings a frequency-tagging paradigm inspired by human electroencephalography research (Norcia et al., 2015) and local field potential research in insects (van Swinderen, 2012). We presented two competing, flickering targets each with varying contrast at two different frequencies. As neuronal responses are themselves modulated by the contrast, spikes become entrained to the high contrast phase of the flicker so that modulation of the observed response permits identification of the selected target. To test that the technique was not dependent on the choice of the tagging frequency (i.e. used only for identification and not saliency), we presented non-harmonic frequency-pairs of either 8 Hz (F1) and 12 (F2) Hz, or 11 Hz (F1) & 15(F2) Hz. These frequencies were not multiples of one other but were divisible by the monitor refresh rate, thus ensuring the full range of intensities were presented within each period. We tested with both

sinusoidal and square wave flicker. These results were subsequently pooled because there was no discernible difference in their power to identify selection.

Frequency tagged targets flickered between a minimum Weber contrast of 0.06 and maximum of 1 (mean contrast of 0.51 and a white background of 337 Cd/m2). In single target trials, one target contrast varied at either F1, F2, or 0 Hz (i.e. a non-flickering control at maximum contrast) and was presented moving vertically up the display at one of two spatial locations, T1 or T2 (locations separated 20° horizontally within CSTMD1's excitatory, receptive field). In paired-target trials, targets of different flicker frequencies were simultaneously presented at both T1 and T2 locations. The choice whether the spatial location T1 or T2 was either F1 or F2 (e.g. 8 Hz or 12 Hz), was pseudo-randomized to control for any preferred frequency response.

Experimental Design and Statistical Analysis

For the trial by trial selection processes, any given trial must be considered an independent event as averaging (as in technical replicates) would mask the observation. However, to ensure statistical robustness of the result we repeated experiments across several dragonflies. Here we use 'n' to denote the number of trials and additionally report the number of dragonflies. We visualise all trial data points and describe similarities or differences across animals.

We report exact P except when less than 0.001. All tests are nonparametric, two-tailed and corrected for multiple comparisons (Bonferroni-Holm correction). Box & Whisker plots indicate median, interquartile and minimum/maximum range. Unless otherwise stated outliers are indicated with Δ.

All data analysis was conducted in MATLAB 2017a (RRID: SCR_001622), including the Wavelet Toolbox. Continuous Wavelet Transforms (CWT's) used an analytic Morlet wavelet with gamma = 3.

Results

Neuronal responses can be frequency-tagged.

To test the validity of the frequency-tagging technique, we presented a single flickering target moving vertically up the display within the dragonfly's field of view (Figure 22A). The target ascended at 40°/s within the excitatory region of CSTMD1's receptive field (Wiederman and O'Carroll, 2013a; Wiederman et al., 2017). We use the term 'frequency-tagging' to refer to the modulation of Weber contrast: $(Intensity_{target} - Intensity_{background}) / Intensity_{background}$, over time at a set frequency (in Hertz). Since CSTMD1 is selective for dark targets(Wiederman and O'Carroll, 2013a), we flickered a black-to-grey target against a white background (Figure 22B, upper). An example of an individual data trace in response to a 15 Hz target shows the spike activity during the stimulus presentation (Figure 22B, lower). To extract any frequency-tagged response modulation, we first determine spike locations and

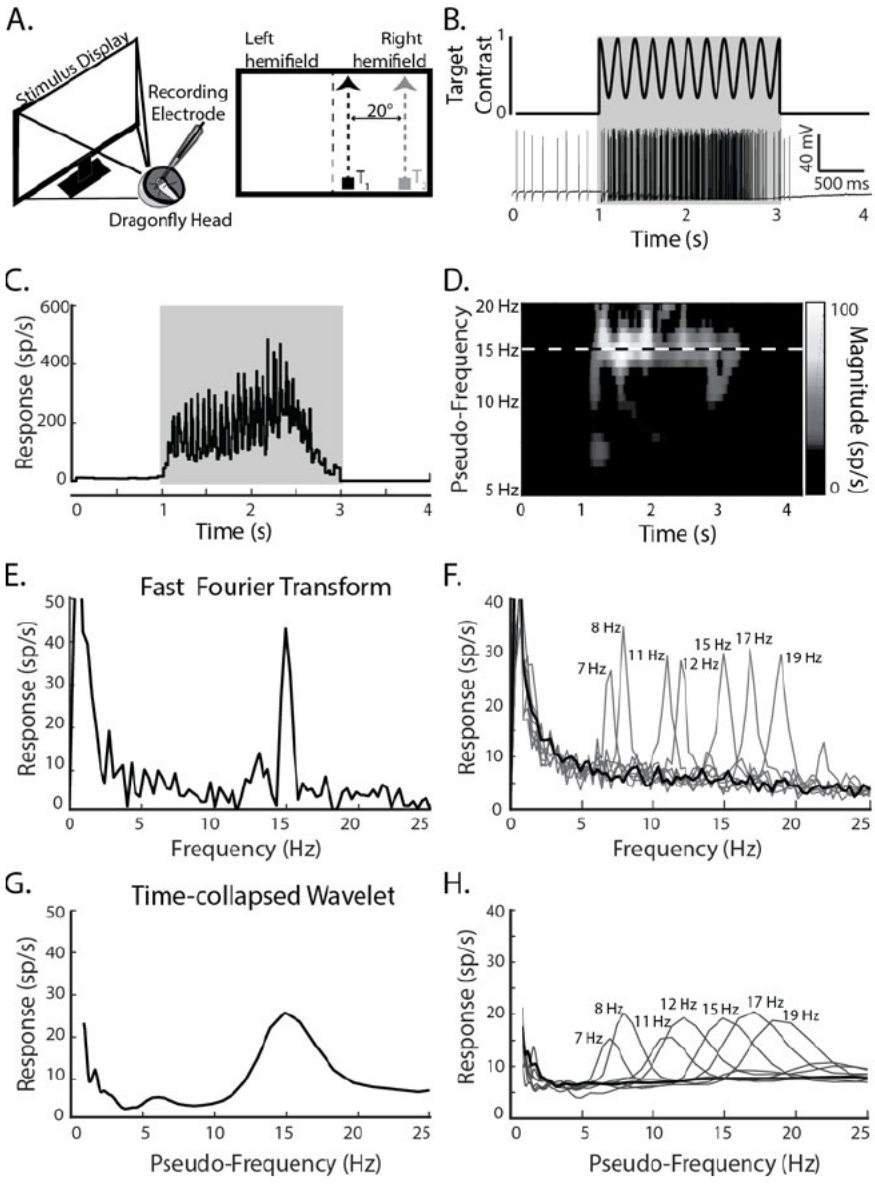

Figure 22: The frequency of the tagged target is preserved in the intracellular responses of CSTMD1. *A) Left: intracellular in vivo electrophysiology involves inserting an electrode into the intact brain to record single-neuron responses to stimuli presented on a computer screen. Right: stimulus pictogram, a single small target (black) ascends CSTMD1's excitatory receptive field. Relative positions of T_1 (black) and T_2 (grey) indicated. B) Upper: Frequency-tagging involves modulating the contrast of the stimulus over time at a specific frequency (5 Hz in this illustrative pictogram). Lower: An example spike train in response to a stimulus modulated at 15 Hz, presented at 1 s for a duration of 2 s. C) The inverse inter-spike interval (ISI) is calculated to determine the spike rate over time. This calculation provides a continuous signal that is amenable to frequency-domain analysis. D) A continuous wavelet transform of the signal in C showing magnitude across time and pseudo-frequency (Logarithmic) reveals response magnitude at a range of pseudo-frequencies over time. In this example, power is centred around 15 Hz for the duration of the trial. E) A Fast Fourier Transform of the signal in C reveals a distinctive peak at 15 Hz, corresponding to the frequency-tagged stimulus. F) Averaged FFT of responses to trials of varying frequency (n = 119 trials across 4 dragonflies) G) The output of the wavelet analysis in D, collapsed across time to be visually comparable to the FFT in E. H) Averaged time-collapsed continuous wavelet transform for the same data presented in F, which although less peaked, still reveals statistically distinctive responses at the relevant frequencies.*

calculate the instantaneous spike rate (Inverse Inter-Spike Interval) over time (Figure 22C). We applied two mathematical transforms to this data; a Continuous Wavelet Transform (Figure 22D), and a Fast Fourier Transform (Figure 22E). The application of a Fast Fourier Transform (square root to provide amplitude) reveals a peak modulation of the neuronal rate code in the frequency domain at 15 Hz, equivalent to the target contrast modulation (a response at 0 Hz is due to the non-zero mean over time). We repeated this process for a series of different frequencies (averaged across neurons) to determine appropriate frequencies for the experiments (Figure 22F). This data shows that from 7 to 19 Hz the frequency content of the stimulus is well preserved in the intracellular response of single neurons. However, we have previously shown that CSTMD1 can 'switch' selection mid-trial (Wiederman and O'Carroll, 2013a). In this circumstance, power from the FFT would be distributed between the two target frequencies, corresponding to the total time each target was selected. Therefore, Fourier analysis of the entire spike train cannot distinguish when: (1) trials where modulation was genuinely shared between T1 and T2 (indicative of a lack of competitive selection, such as neuronal summation) or (2)

those where selection switched from T1 to T2 or T2 to T1 part-way through the trial. To account for possible switches, we instead applied Continuous Wavelet Transforms (CWTs) which provides power across pseudo-frequencies over time (Figure 22D). Averaging the wavelet analysis across time is comparable to a FFT, though reveals a broader peak in the frequency domain centred at 15 Hz (Figure 22G). The broader shape observed in the CWT is inherent to the wavelet analysis and is the cost of providing information of how frequency components vary over time. Although in the frequency domain CWT responses are blurred in comparison to their FFT counterparts, there are statistically significant differences for any two frequencies separated by at least 2 Hz ($P < .001$). Thus, we were able to analyse all further data using CWTs to derive the benefit of examining the frequency response evolution over time of the individual trials.

Frequency-tagging reveals which target is selectively attended.

To test the ability of the frequency-tagging technique to discriminate selected and unselected targets, we first attempted to reproduce our earlier demonstration of selective attention in CSTMD1 (Wiederman and O'Carroll, 2013a), where the response to two competing targets presented simultaneously closely resembles the unique response for the individual targets presented alone. To this end we presented either single targets (pseudo-randomly at either f1 or f2) at either spatial location T1 or T2 (both within CSTMD1's excitatory receptive field). Randomly interleaved with the single target trials (Figure 23A), we also presented paired-targets (i.e. simultaneously at both target locations T1 and T2) which were frequency-modulated at the two different frequencies (pseudo-randomly between T1=f1, T2=f2 and T1=f2 and T2=f1). As our interest is in the chosen target (T1 or T2), rather than the frequency of the 'identifier', we pooled data across the frequency-pairs.

In single target trials (Figure 23B, location T1 orange dots; location T2 blue dots), we usually observed strong modulation at the frequency of the presented target and weak modulation at the alternative frequency (i.e. a frequency that does not exist in the stimulus, therefore representing a form of experimental and analysis noise). However, some individual trials had insufficient modulation in the transform to enable accurate identification of the selected targets. This likely results from two factors: (1) neuronal habituation in the receptive field diminishing the strength of the modulation, or; (2) neuronal saturation from a highly responsive cell limiting the possible strength of the modulation. To analyse trials free of these effects, we used single-target responses to determine a threshold for data inclusion. For each location, T1 and T2, we calculated the average magnitude at the frequency not presented, which provides an estimate of the noise inherent in the frequency domain. This floor was defined as the mean power at the non-presented frequency plus twice the standard deviation. This provided an objective level of the modulation noise at the other frequency. That is, the expected, non-zero modulation at f2 when the neuron has selected a target modulated at f1, and vice-versa

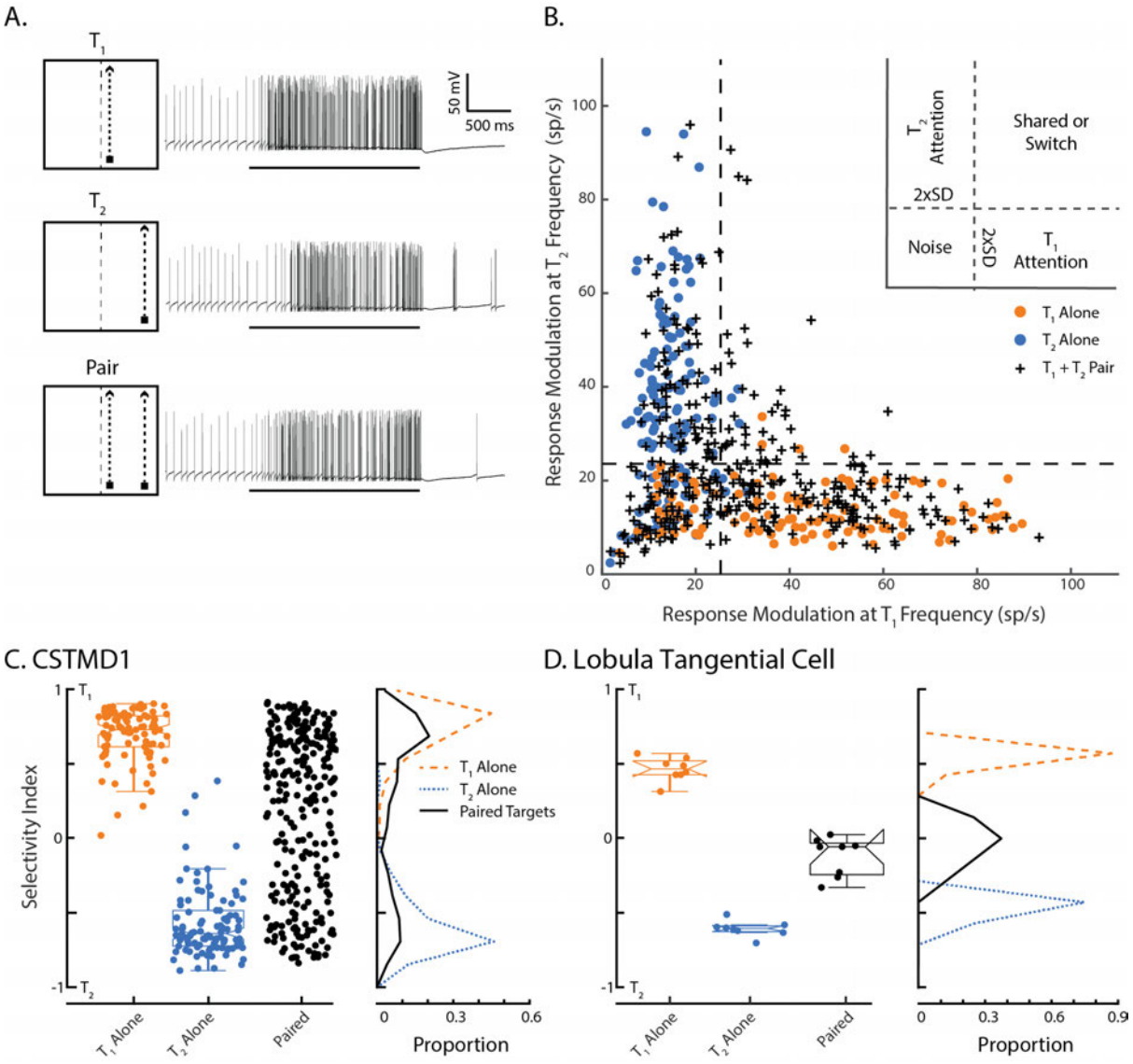

Figure 23: Frequency-tagging identifies the selected target in a paired-target trial. A) *Illustrative pictograms and corresponding single-trial electrophysiological responses for the 3 stimulus conditions. From top-to-bottom: T1 Alone; T2 Alone; Paired-Targets.* ***B)*** *The response modulation at the T2 frequency plotted against response modulation at the T1 frequency. Data is plotted in response to either a single target at the T1 location (orange dots) or at the T2 location (blue dots) when presented alone. Crosses represent CSTMD1 responses to the paired stimulus (total n = 447 trials across 13 dragonflies). Dashed lines indicate the derived noise threshold. Responses to the paired-targets mostly elicit modulation at either one or other of the target flicker frequencies (not both together), indicative of selective attention.* ***C)*** *The Selectivity Index represents the degree to which the response is locked to one of the frequency-tagged stimuli over the other. Values around zero indicate that both frequencies are equal components of the overall modulation. Frequency polygons illustrate the relative proportion of these points, with the bimodal distribution to the paired stimulus clearly revealing the selection of one target or the other.* ***D)*** *In comparison, results from a Lobula Tangential Cell (an optic flow sensitive neuron) in the dragonfly show no selective attention (n = 8 trials in 1 dragonfly), with a unimodal distribution around zero to the paired-targets, indicative of the expected shared modulation to both target frequencies (neuronal summation rather than selection).*

(Figure 23B– dashed lines). Trials in the lower-left corner of Figure 23B thus fail the acceptable signal-to-noise threshold for both frequencies. Using this measure, we rejected 172 trials (27.6% of the total) from any further analysis, revealing that our frequency-tagging technique worked for 71.4% of the total trials presented. There was no significant difference in the number of identification failures between any of the three conditions (X2-test, P > 1, Bonferroni-holm correction), therefore there was

no effect of this data exclusion on the further testing of hypotheses with respect to the presence of selective attention. We applied these exclusion criteria to all further data analysis.

Responses above threshold, at either f1 or f2, indicated significant identification of either one, or both, of the targets. Qualitatively, we observe that the responses to paired-targets (Figure 23B, crosses) were mostly either modulated at the frequency of the target at location T1 or T2 (but not both, i.e. only a few crosses within the 'Shared or Switch' region).

The absolute modulation above this noise threshold (i.e. the distance of the data points along the abscissa or ordinate in Figure 23B) is related to the trial-by-trial sensitivity, rather than to the degree of the selective attention to one or either of the targets. To quantify our data, we therefore defined a Selectivity Index (Figure 23C), which measured the degree of target selection, independent of the strength of response modulation (providing it is above the noise exclusion threshold as previously described). For each data point, we calculated the following:

$$Selectivity\ Index = T_1 - T_2 / \sqrt{T_1^2 + T_2^2}$$

T1 and T2 values are averages of the pseudo-frequency amplitude (known as 'scale') over the trial duration (i.e. collapsed across time from the CWTs), for each of the corresponding target frequency-tagging modulations. The selectivity index ranges between +1 and -1 and represents the selection of T1 (+1) and T2 (-1), respectively. Here 'selectivity' is referred to in the original definition of 'selective attention' as selection of one from multiple competing stimuli, as would be expected in a winner-takes-all network. A value of 0 would occur if the response magnitude at f1 and f2 were equal (irrespective of the absolute distance from the origin), indicating either shared (co-varying) selection across the trial, or a switch in selection during the trial.

In Figure 23 C, we observe significant differences in the Selectivity Index distribution between paired and both T1-alone and T2-alone conditions ($P < 0.001$, Bonferroni-Holm correction). In single-target conditions, the Selectivity Index is narrowly distributed (T1 $\mu = 0.68$, $\sigma = 0.17$; T2 $\mu = -0.58$, $\sigma = 0.23$), whereas in paired-target trials the Selectivity Index is non-normally distributed ($p > 0.001$, one-tailed Kolmogrov-Smirnov test) with peaks at approximately 0.65 and -0.55. The bimodal distribution of responses to paired-targets reveals the selection of either T1 or T2. For comparison to a potential 'null' hypothesis (i.e. no selective attention), Figure 23 D shows results from a single Lobula Tangential Cell in the dragonfly (Evans et al. 2019). This neuron generates robust responses using spatial summation in order to encode wide-field optic flow, analogous to Lobula Plate Tangential Cells in Diptera (Hausen, 1982). We presented the same experimental paradigm, though with larger targets (1.5° x 10°) to elicit a response. In contrast to the results observed in CSTMD1, the optic flow neuron had a Selectivity Index around 0 (modulation at both frequencies of the paired-targets) indicative of neuronal spatial summation.

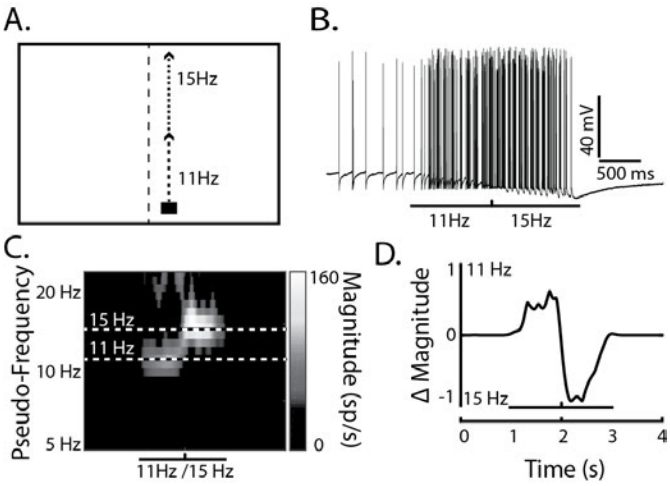

Figure 24: A simulation of an 'attentional switch' is encapsulated by the difference between the pseudo-frequency (scale) magnitudes over time. *A) Illustrated pictogram of a single target that changed frequency modulation halfway through the trial, simulating an attentional switch from one target to another. B) A single-trial example of CSTMD1's response to this switching stimulus. C) The CWT of the inverse ISI of the trial in B, reveals the switch that occurs halfway through the trial. The black-and-white dashed lines indicate the 11 Hz and 15 Hz frequency slices. D) A 'difference slice' (Delta Magnitude) is calculated by taking the difference between the wavelet slices at 11 and 15Hz across time.*

Target selection occasionally switches midway.

Not all of the paired-target trials were solely modulated by one of the target frequencies (Figure 23B, shared zone). What could account for this apparent shared modulation? There are two possible explanations. Firstly, that the neuron is excited by both stimuli at their respective frequencies and is not selecting a single target. That is, spatial summation similar to what is observed in the Lobula Tangential Cell (Figure 23D) and in primate V4 (Ghose and Maunsell, 2008). Secondly, a switch mid-way through the trial could result in significant modulation at both frequencies, as both targets are selected during the trial, though at discrete times.

To differentiate between these potential explanations, we first simulated a switch in response from f1 to f2 by presenting a single-target that changed frequency in the middle of the trial (Figure 24A). An example of the intracellular response to such a pseudo-switch stimulus is presented in Figure 24B. We then took a 'slice' from the Continuous Wavelet Transform at each frequency of interest (+/- 1 Hz) and subtracted these from one another (Figure 24C, dashed lines), thus producing a difference in magnitude between the two pseudo-frequencies over time (Figure 24D). This difference in magnitude provides a read-out through time of how much the modulation was determined by each target's frequency. A flat line near zero would indicate shared modulation distributed between the two frequencies.

We applied this 'difference slice' analysis to determine whether the paired-target responses with modulation at both frequencies (Figure 23B, shared or switch region) were due to spatial summation or switching. Figure 25A shows individual examples from six such trials, all of which exhibit discrete peaks and troughs across time. The traces indicate that these CSTMD1 responses are switching between targets, rather than being modulated by both target frequency tags simultaneously.

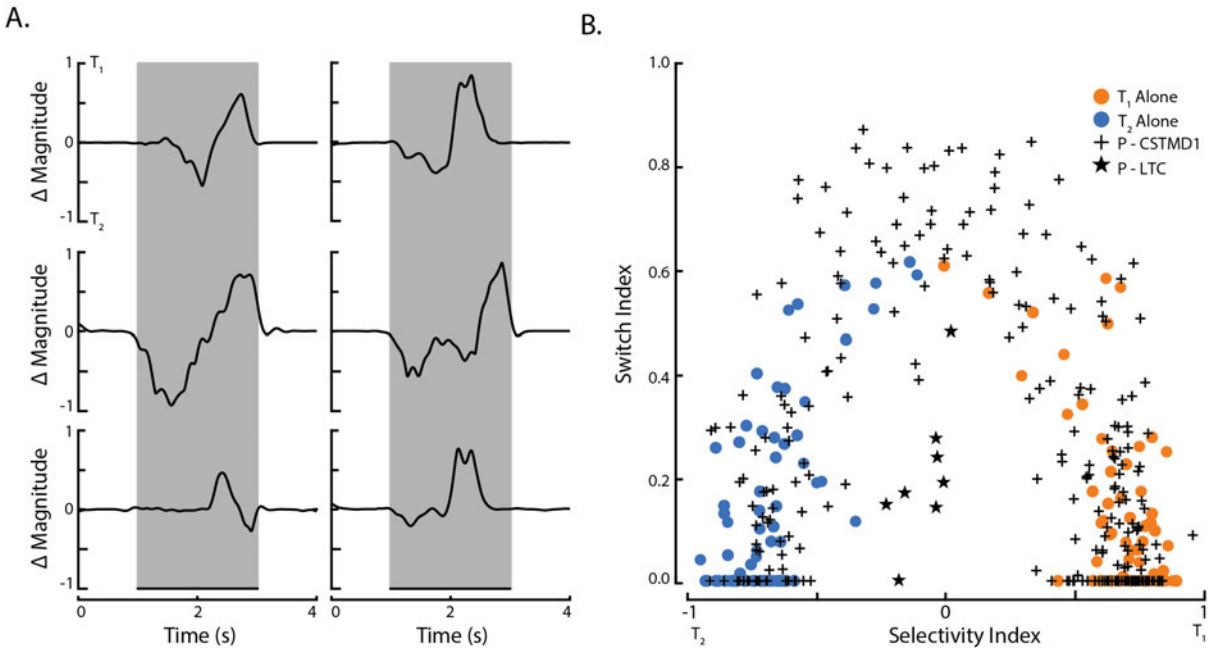

Figure 25: Shared modulation results from switches in selection over time. A) *Individual examples reveal high modulation for both targets, however only at different epochs of time.* ***B)*** *The 'Switch Index' and 'Selectivity Index' for all single target (orange and blue points) and paired-target (crosses) trials from figure 2B (total n = 447 trials across 13 dragonflies). When selectivity for paired-targets is low (middle abscissa, close to zero) then the Switch Index is high, indicating that responses switched between targets. In comparison, the optic flow neuron (stars) has low selectivity and low Switch, indicative of neuronal summation (modulation at both frequencies across points in time).*

To compare aggregate data, we calculated a 'Switch Index[1]' for each trial (Figure 25B). This index was calculated by determining the proportion of time the system selected either T1 or T2. To ensure that these selections were robust, we only considered a selection valid when either target was more than 5 spikes/s stronger than its counterpart. The time each target was selected was multiplied, thus if one of the targets was not selected, the Switch Index was zero. The Switch Index (normalized) is maximized when both targets are selected (not shared) for 50% of the trial. The Switch Index is low in single-target trials (orange or blue dots), since the time when the other target is selected is near zero. In paired-target trials the Switch Index is distributed between high and low values, representing either absolute selection of one target, or switches midway. In trials with a Selectivity Index around 0 (shared modulation), the Switch Index is uniformly high, indicating that the shared modulation results from switches in the selected target over the time course, rather than summation of responses to both targets. In comparison, paired-target trials in the control dragonfly Lobula Tangential Cell, show both a low Selectivity Index and low Switch Index, indicating genuine modulation at both frequencies

[1] Thesis Addendum: The Switch index was calculated as the proportion of time (t) in the trial spent tracking one target multiplied by the proportion spent tracking the other, defined in terms of Selectivity (S). Hence, Switch Index would be high when both values are high, but low when either value is low. The Equation is given as:

$$Switch\ Index = t_{S_{T1}>S_{T2}} * t_{S_{T2}>S_{T1}}$$

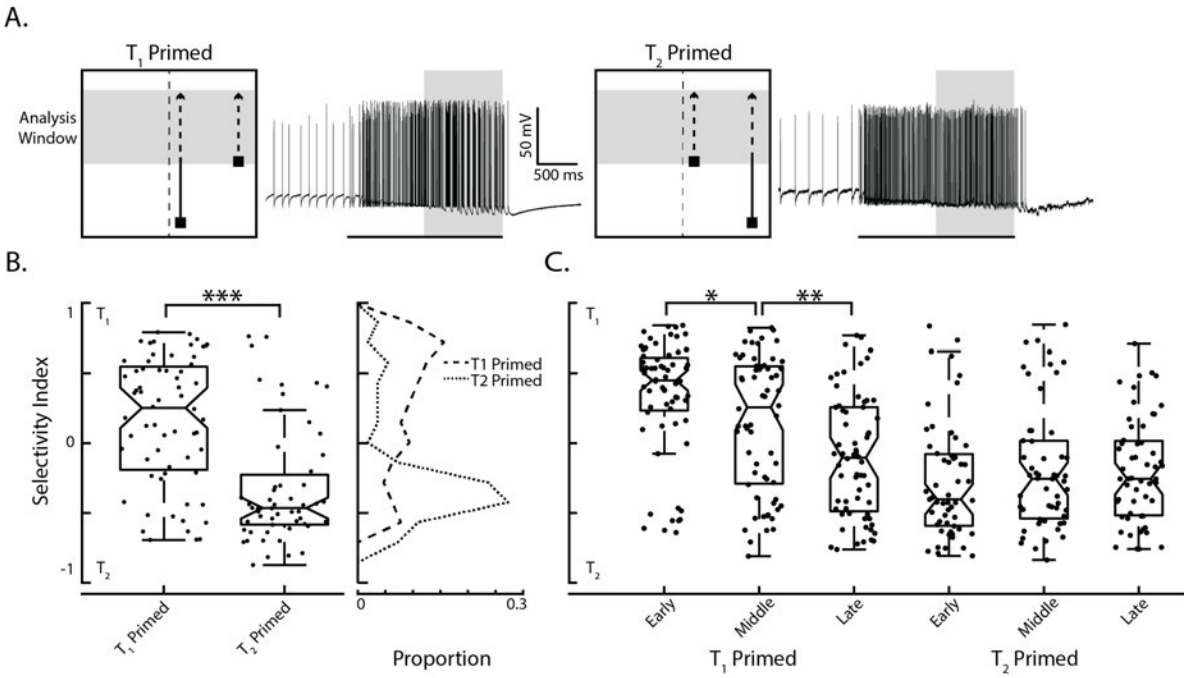

Figure 26: Priming with a preceding target biases selection towards the continuing trajectory. A) *Pictograms illustrate the biasing stimulus towards either spatial location T1 or T2, next to individual examples of CSTMD1 responses. The short-path target (distracter) appears at 1 second, when the preceding target reaches midway up the screen (the analysis window indicated with the grey shaded region).* ***B)*** *There is a significant difference in the Selectivity Index between T1 and T2 primed trials (n = 295 across 7 dragonflies), though priming is not effective in all trials. Frequency polygons reveal the distributions of the Selectivity Index for T1 primed (dashed line) and T2 primed (dotted line).* ***C)*** *The selective attentional capture from priming split over early, middle and late time windows. Over time, T1 primed selection shifts to T2, whilst T2 selection is retained over the three periods.*

(simultaneously) over time due to the spatial summation used in optic flow computations (Figure 25B, stars).

Selection can be biased with priming.

We then tested the ability of a priming stimulus to bias the selection of a spatially-associated target in a paired-target condition. In this experiment, a lone untagged primer was first presented for one second moving towards the trajectory of either spatial location T1 or T2 (Figure 26A). Note that here the frequency-tagged T1 and T2 pathways commence midway up the stimulus display, immediately after the single 'primer' target has moved along its trajectory. From our previous work, we expect CSTMD1 to predictively facilitate responses in front of the target's prior path (Nordström et al., 2011; Dunbier et al., 2012; Wiederman et al., 2017). We introduced a frequency-tagged distracter midway through the receptive field (horizontally offset by 20°) paired with a frequency-tagged target that continued along the primer's previous trajectory (Figure 26A). We calculated the Selectivity Index across the period (1 second) where both targets are presented together and reveal a significant ($P < 0.001$) biasing of selection towards the target that continues along the primed trajectory (Figure 26B). This selection may be due to 'predictive gain modulation', whereby a local spotlight of enhanced gain is generated ahead of a moving target, with suppression in the surround

(Wiederman et al., 2017). In our experiment, the continuing target is within the spotlight created by the preceding target, but the distracter appears within the suppressed surround.

In the human psychophysics literature, attentional capture is an effect whereby the presentation of an abrupt-onset stimulus (Yantis and Jonides, 1984) or a novel object (Franconeri et al., 2005) involuntarily captures attention (Remington et al., 1992), even when task-irrelevant. In order to test for a capture of CSTMD1's selection, we analysed the previous biased paired-target responses (Figure 26B) separated into three 400 ms periods (early, middle and late). We included 100 ms overlap between these periods because this duration was required for meaningful CWT analysis. If CSTMD1 responses displayed attentional capture, we hypothesise that the early period would be dominated by responses to the distracter stimulus, returning to the original path at later periods of time (as the distracter is assessed and ignored). Our results revealed the opposite effect (Figure 26C), with the early window exhibiting the strongest biasing effect, which can dissipate over time (via switches). This reveals that selection is not captured by abrupt-onset novel stimuli presented within CSTMD1's receptive field. Rather responses are 'locked' to the preceding target's predicted continuing trajectory and generally ignore a novel distracter that falls outside of this predicted location. Here we observed asymmetry in results from the T1 compared to T2 priming, which reflects the broader (noisier) distribution of values in the T1 primer condition when analysed over the entire analysis duration (Figure 26B). When primed to T1 (the target closer to the dragonflies' midline), the early window (Figure 26C) reflects this biasing to the continued path trajectory (though note the exceptions). However, in some cases over time (middle and late windows) selection can change towards the distracter location at T2. This results in significant changes in the Selectivity Index between these periods ($P < 0.001$). Visual inspection of the CWT analysis reveals that these are switches that occur at discrete points in time in the individual trials. In the T2 priming condition (the target located in the more peripheral location), the selection has locked on to the preceding target and maintains this selection throughout the rest of the trial, with no significant difference between the early, middle and late periods. Again, there are single-trial exceptions, however, these are distributed in either T1 or T2 selection.

Selection can lock-on to lower contrast targets.

In a traditional winner-takes-all network (Feldman and Ballard, 1982), the introduction of a higher contrast distracter during the presentation of a lower contrast target would result in a switch to the one with higher salience. However, how would the dragonfly feed in a swarm if often distracted by a novel, transiently more salient target? To determine whether CSTMD1 locks-on to the lower-salience stimuli, we presented primers of varying contrasts followed by introduction of a frequency-tagged distracter. We designed the lower contrast target to retain its lower saliency throughout the

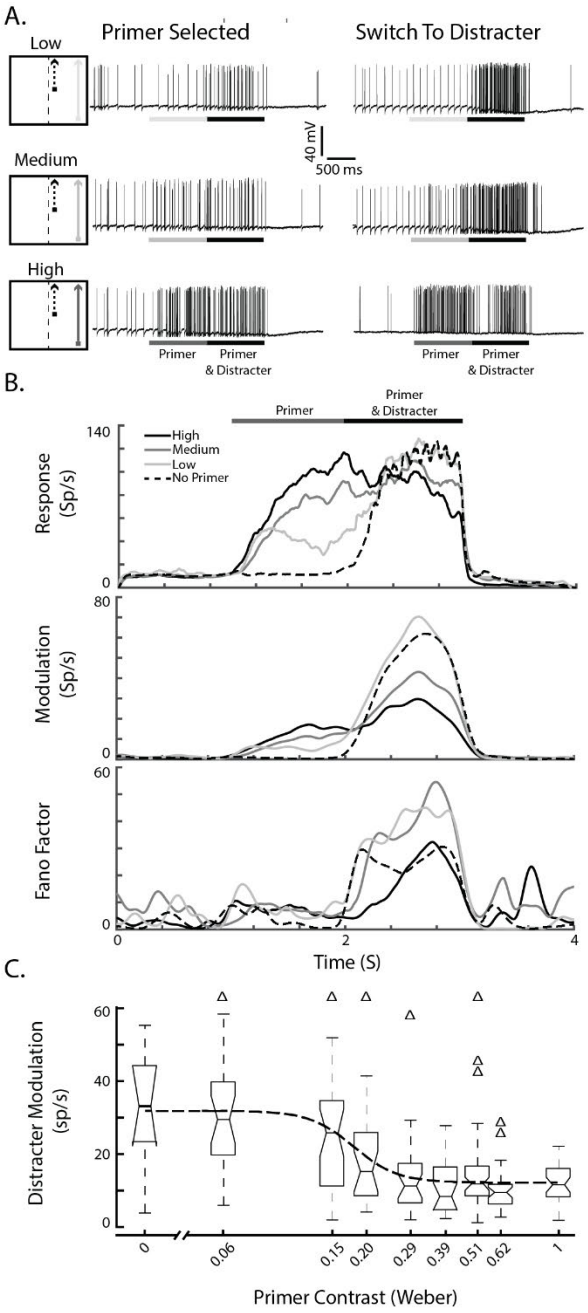

Figure 27: Selective attention in CSTMD1 can lock-on to a lower contrast target, ignoring the novel, high contrast distracter. *A) Stimulus pictograms and single-trial example traces from the same CSTMD1 for low, medium, and high contrast primer conditions. Left: responses when CSTMD1 locked-on to the primer and the presence of the distracter is ignored. Right: CSTMD1 responds to the distracter, once it is presented. B) Top: Average inverse interspike interval across all trials (n = 220 across 7 dragonflies), separated by primer condition (none, low, medium and high) Middle: Average modulation across all trials, separated by condition. In the primer & distracter period, rank ordering of the conditions is inverted compared to 6B Top. This shows a strong modulation in the low-contrast and no-primer conditions (i.e. distracter selection) and weak modulation in the high-contrast condition (primer selection). Bottom: a stronger Fano Factor reveals more variability of modulation, indicative of increased switching at lower primer contrasts. C) In an individual CSTMD1 recording, we assayed across a large range of primer contrasts, revealing a sigmoidal contrast sensitivity function (n = 217 in 1 dragonfly). Δ indicate outliers.*

course of the trial (i.e. no frequency-tagging), even during the period of the paired distracter. Hence the presence of any frequency modulation during the paired presentation would indicate the distracter being selected. Primers were presented at constant low (0.06), medium (0.15) or high (0.51) Weber contrast, pseudo-randomly located at spatial locations T1 or T2 (Figure 27A, primer at T2 location shown). The high contrast primer was set at 0.51 to be equiluminant with the average contrast (over time) of the frequency-tagged distracter. Figure 27A shows example responses of an individual CSTMD1 to these stimulus conditions, both when the lower contrast, primer trajectory retains selection and when selection switches to the high-contrast distracter. This shows that there can be trial-by-trial variability in which one of the targets was selected, either the continuing primer or the novel distracter.

Figure 27B shows the average spike activity across all trials within a primer contrast condition (Figure 27B, top). As expected, over the primer-alone period, the neuronal response increases with increased contrast (O'Carroll and Wiederman, 2014). When there is only the high contrast distracter (no-primer, dashed line) we observed the strong distracter response, subtly modulated by the frequency-tagging technique. For the low and medium contrast primer conditions, responses trend

towards the distracter response (as a proportion of trials). Interestingly, a high contrast primer sees a lower response over this duration, attributable to hyperpolarization observed from sustained firing.

We calculated the mean wavelet slice at the distracter frequency (Fd) across all trials for the same condition (Figure 27B, middle). This reveals the magnitude of frequency-modulation induced by the distracter, at each point in time. In the primer alone period, modulation increases with primer contrast due to more power in the noise component. Over the primer + distracter period the rank ordering of conditions is inverted in comparison to the primer only period in Figure 27B top. This inversion indicates that the conditions with the most aggregate power at Fd are the no-primer and low-contrast conditions, followed by the medium- and high-contrast conditions, respectively. This shows that the distracter is selected at a higher proportion of trials in no-primer and low-contrast primer conditions.

Due to the biasing effect of the primer (Figure 26), we expect more distracter modulation in the no-primer (dashed line) and low-contrast conditions, and the least when neuronal response locks-on to the high contrast primer, i.e. no distracter modulation (black line). We observe this effect: increased primer contrast is associated with less response in the frequency domain, indicating fewer responses to the distracter target. Statistically, we observed a significant reduction in distracter modulation in the medium ($P = 0.006$) and high ($P < 0.001$) contrast group, but not the low-contrast group ($P = 0.755$), compared to the no primer group.

These data are averaged and therefore do not show the amount of inter-trial variability associated with previously observed “rare” switching events between two equally salient targets (Wiederman and O’Carroll, 2013a). The Fano Factor of the modulation is a measure of the trial by trial neuronal variability (Figure 27B, bottom). The two conditions that exhibit the highest variability over the primer + distracter period are the low and medium contrast conditions. This indicates that low and medium contrast conditions have the highest rates of between-trial variability (i.e. on some trials the continuation of the primer is selected, while on other trials the distracter is selected) and within-trial variability (i.e., switching during the trial period.). In comparison, the no primer and high contrast primer conditions have less variability as either the continuing primer or distracter is selected, respectively. The variability in the high contrast condition rises over time (Figure 27B Bottom, solid black line), revealing an increase in the probability of switching targets over time. This is consistent with the finding that the effect of a primer diminishes over time (Figure 26C).

In one long CSTMD1 recording, we were able to assay across a large range of primer contrasts (Figure 27C). In this individual example, the sigmoidal function reveals that in a large proportion of trials, CSTMD1 locks-on to primer targets presented well below the average contrast of the introduced distracter (0.51).

Both the aggregate data and the individual example reveal that CSTMD1 frequently locks-on to lower contrast targets (medium, 0.16), selecting them even in the presence of the high contrast distracter (mean 0.51). The mechanism underling this neuronal selective attention thus cannot be a 'simple' winner-takes-all network unless evoking the competitive selection over sluggish temporal dynamics.

Intriguingly, responses to the low-contrast targets continuing along the primer trajectory are not associated with an increase in spike rate as would be expected by models of attention where low-contrast stimuli are attended by neuronally boosting the response in order to achieve competitive advantage against high-contrast distracters (Reynolds and Desimone, 2003). Instead, even when responding to low-contrast stimuli in the presence of a high-contrast distracter, CSTMD1 encodes the absolute strength of the attended target as if the distracter was not present (Figure 27A). This could be critically important in behaviour where a target is selected for pursuit amidst a swarm, where absolute rather than relative activity might underlie the closed-loop control system.

Modelling the neuronal processing underlying target responses

What mechanism best explains the measured data? To test this, we developed six algorithmic models. The six models included two models that assumed shared attention (including one with saturation), two models that applied selection and two models which applied selection with switching. For input to these models we collected the response modulation amplitude from the wavelet analysis for the single target trials (i.e. T1-only or T2-only) (Figure 23). From this we produced four lists (T1f1, T1f2, T2f1, T2f2) representing the response modulation amplitude at the target's flicker frequency and at the comparison frequency (i.e. no modulation). We binned these responses and fit a log-normal distribution to each target and frequency pair (T2 examples are shown in Figure 28A). We then infinitely sampled from these model distributions to generate an arbitrary number of synthetic target responses.

To simulate switching, we generated a 1 s time course of response modulation for testing all models, equivalent to taking a 1-dimensional slice from the CWT analysis (as in Figure 24). For realism, we added Gaussian white noise (5 spikes/s max width) and smoothed the data using a 0.2 s average filter. For switching models, this smoothing was done after calculating the switch. This produced waveforms qualitatively similar to those observed from taking a single-frequency slice of a CWT, including switch transitions (Figure 24C).

We sampled from the distribution 1000 times for each pairing (T1f1, T1f2, T2f1, T2f2). Each model used a combination of these to generate output responses for both f1 and f2. Basic Summation (BS) assumed that the output power at both f1 and f2 were the corresponding powers of the input target (i.e. T1f1 & T2f2). Saturating Summation (SS) summed like BS, then applied a soft saturation to

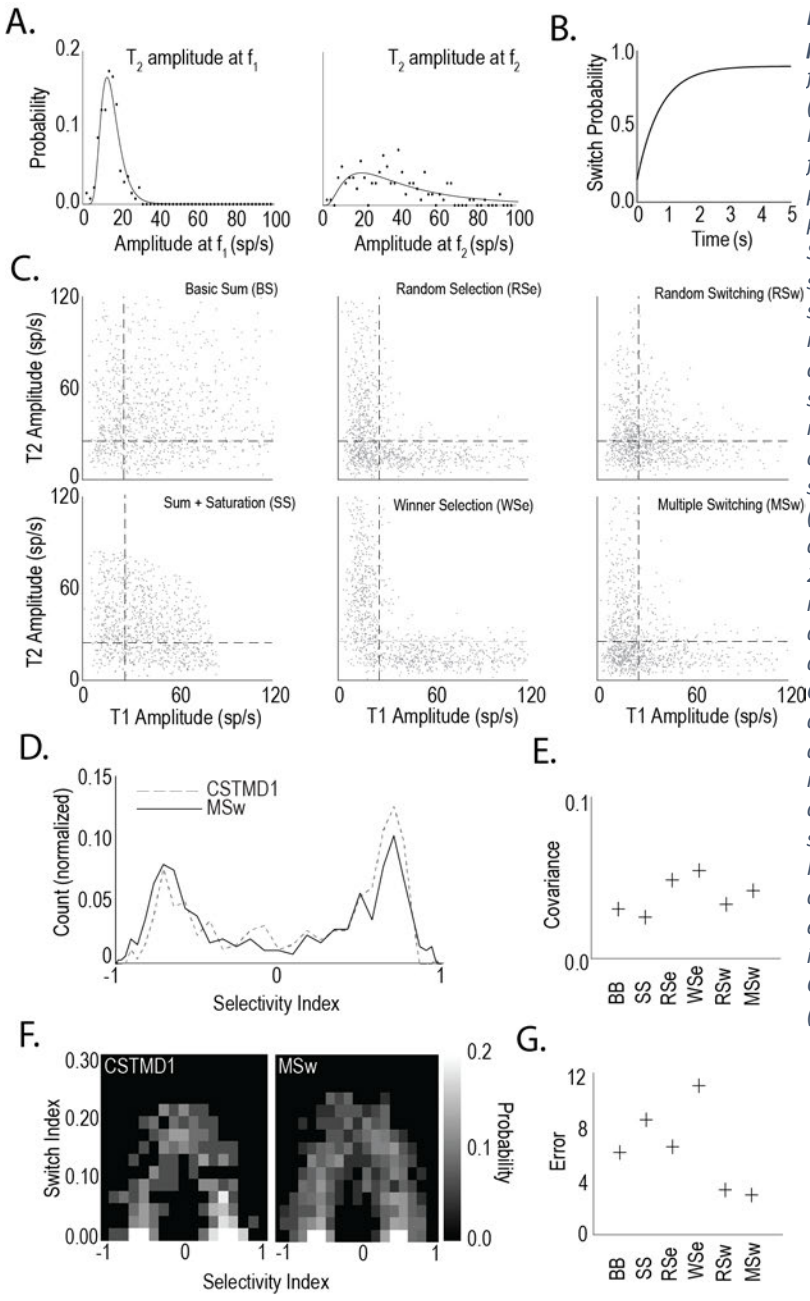

Figure 28: A switching model matches the physiological data. A) *Power distributions for frequency responses from T2 at f1 (left) and f2 (right) calculated from recorded trials. Modelled trial data were randomly selected from these power distributions representing the power contribution of each target.* ***B)*** *Switch probability as time progresses for Multiple Switching (MSw). Initially the likelihood of switching is low before rising to 90%. After a switch, the switch probability resets permitting more to occur.* ***C)*** *Example scatter plots for each of the six models tested: summation (top left), summation with saturation (bottom left), random selection (top middle), higher power always wins selection (bottom middle), random switching (top right), and multiple switch model (bottom right). These scatterplots can be compared to the physiological data in Figure 2B.* ***D)*** *Histogram of Selectivity Index for recorded CSTMD1 data and model output. Real data curves are cross correlated with model-derived curves to generate covariance score.* ***E)*** *Covariance of the six models against CSTMD1 data from the histogram analysis. Higher covariance (i.e. model WSe) is indicative of a more representative model.* ***F)*** *Two-dimensional histogram, that accounts for selectivity and switching, for CSTMD1 (left) and MSw model (right).* ***G)*** *Error calculated as RMS deviations (2D histograms) from the six models, each against recorded CSTMD1. Low values indicate the most representative model of both CSTMD1 selection (Figure 2B) and switching (Figure 4B).*

reduce the overall modulation power evenly between f1 and f2 (maximum power of 100 spikes/s). Random Selection (RSe) randomly selected either T1 or T2 and used that target's corresponding power for f1 and f2 (i.e. if T1 was selected the frequency responses would be T1f1 and T1f2). Winner Selection (WSe) selected a 'winner' target with the greatest modulated power, with the assumption that modulation was proportional to the target response (if T1f1 > T2f2, T1 would be selected and vice versa). Random Switching (RSw), randomly selected an initial target (as per RSe) however assumed that a switch occurred in a percentage of trials at some point during the trial's duration. Multiple Switching (MSw) assumed a more sophisticated switching rate, allowing the system to switch multiple times. The switch probability was defined by the following formula:

$$P(switch) = S - \tau e^{-t/\tau}$$

Where S represents the probability that a switch never occurs and τ represents the rate of increase of switching over time (Figure 28B).

The outputs of all six models are shown in Figure 28C. The summation model (BS) populates all four quadrants (including in the 'Shared or Switch' zone of Figure 23B). This combination of taking power from both targets together does not match the electrophysiological results (Figure 23B). Both selection models (RSe & WSe) adhere far closer to the distribution seen in Figure 23 except that the shared zone is too sparsely populated. The switching models qualitatively match the physiological data with a bias to T1/T2 only responses (the L shape) but with proportion of shared zone responses indicative of switching.

To assess each model quantitatively, we generated the frequency polygon (Figure 23,Figure 25) of the Selectivity Index values calculated from the model outputs. An example of the response of the MSw model (grey line) compared to the electrophysiological data (dotted line) is shown in Figure 28D. With cross-correlation, we compared each model's frequency polygon with CSTMD1's (derived from Figure 23C).

Via this metric, both selection models (RSe, WSe) provided the best match to the recorded data (Figure 7E). However, this selection metric ignores the switching behaviour inherent in the model. To test whether pure selection was sufficient to explain the data, we used the model outputs to calculate the 'Switch Index' (Figure 25) for each model's responses. We binned this data to generate a 2-dimensional histogram (Figure 28F). We repeated this process for the electrophysiological data and calculated the RMS error between them. As both switching models had free parameters (i.e. probability of switching) we optimized both these models against this RMS error. The RSw model was most successful with a 100% probability of a switch at a random time during the trial. The MSw model was optimal with a 90% switch probability and 0.75 s time constant. When accounting for both CSTMD1's selection and switching, the (MSw) model had the least error (Figure 28G).

Summation models (BS, SS) generated too many responses in the shared zone by increasing overall power, whilst Selection models (RSe, WSe) eliminated shared zone responses entirely. Switching models (RSw, MSw) provided the appropriate compromise, encapsulating the selection responses in upper-left and lower-right quadrants and generating some shared zone responses due to switching. Therefore, the parsimonious explanation for our observations is modelled with a process that selects a single target but is capable of switching one or more times during a trial.

Discussion

Our novel approach of analysing frequency-tagged, intracellular spike trains allowed us to verify the presence of selective attention in CSTMD1 (Wiederman and O'Carroll, 2013a), and build on this result by reliably identifying which target of a pair was selected at any moment in time.

Additionally, as frequency-tagging does not rely on an inhomogeneous receptive field to differentiate targets, this technique affords more freedom in experimental design and potential application to STMD neurons with either smaller or more homogeneous receptive fields. We leveraged this to design a set of experiments that probe the properties of selective attention in the context of low-contrast priming and abrupt-onset distracter presentation, thus moving beyond the capabilities of the technique presented in Wiederman & O'Carroll (2013).

Despite these advantages, on approximately 25% of trials regardless of stimulus conditions, levels of frequency modulation were below-noise, even with the stimulus generating spiking responses. Flickering targets located within the strongest parts of the receptive field may reach saturation during the low-contrast phase of the stimulus, resulting in a lack of modulation (i.e. clipping). Conversely, frequency-tagged targets presented in less sensitive regions of the receptive field may not elicit strong enough modulation over the carrier signal. Both CSTMD1's saturation and sensitivity may vary over time and between animals. In future experiments, these effects might be minimized by dynamically changing the stimulus waveform, decreased or increased to account for saturation or sensitivity respectively.

Although frequency tagging was used as an identifier, could the frequency itself interact with facilitatory or selective processing? Such a factor can play a role in other animal models, with honeybees preferencing 20-25 Hz visual flicker and avoiding 2-4 Hz (Van De Poll et al., 2015). Even a single luminance change is enough to break inattentional blindness in humans (Palmer et al., 2018). To minimise this possibility, we distributed the two tagging frequencies across two spatial locations (T_1 and T_2) as well as testing our entire experimental paradigm at two different frequency-tagged pairs. Throughout these experiments, we did not observe any effect of the frequency-tagging beyond our intended purpose as an identification technique.

Attention is a limited resource (Alvarez and Franconeri, 2007), therefore animals across species are motivated to guide the deployment of attention in an ethologically meaningful and efficient way. One guide is spatial or temporal cueing, often through inhibitory neural mechanisms (Römer et al., 2002; Ruthruff and Gaspelin, 2018). For example, Drosophila are more likely to orient towards cued locations of the receptive field when subsequently presented with multiple targets (Sareen et al., 2011). Female crickets prefer leading male auditory signals to signals arriving later (Snedden and Greenfield, 1998; Römer et al., 2002), suggesting an inherent bias towards 'locking on' to the first stimulus and ignoring those subsequent. This is similar to what we have observed in CSTMD1, with the priming by a preceding target biasing selection to those that continue along the projected trajectory. This property we termed 'predictive gain modulation' and our recent experiments reveal that even short duration primers can elicit robust predictive gain (Fabian et al.,

2019). The interactions between mechanisms underlying prediction and those of selective attention is a focus of our future research.

In CSTMD1, the effect of spatiotemporal cueing was so strong that even targets of lower visual salience can win over an abrupt-onset, high-contrast distracter. In attentional networks, saliency is a prominent attribute for guiding selection and seems to innately capture attention. This leads to a conundrum; if the most salient targets were to capture attention moment-to-moment, then the system might too often be distracted from any given task. For example, will the dragonfly ever feed if the prey of constantly varying contrast (i.e. moving against a cluttered background) are dynamically more or less salient than others in the swarm? Conversely, the onset of a novel salient stimulus may signal the necessity to attend to a new event or abandon the current task completely in favour of survival behaviour (e.g. an approaching bird).

Our results bear resemblance to behavioural results in Drosophila (Koenig et al., 2016a). Tethered flies in an arena were presented with a pair of vertical lines equally offset from the flies' midline. Flies made a decision to respond to either one line or the other by turning to bring it into the midline. In subsequent trials, these flies displayed a bias for turning towards the originally selected stimulus and ignoring the alternative. However, over time this bias was lost. The mean 'attention span' (time before the bias was lost) was 4 seconds in wild-type flies, but reduced to 1 second in mutants defective in selective attention. Active switching between competing stimuli may be indicative of endogenous drive by top-down control mechanisms (Miller et al., 2012). Van Swinderen (2007) found that, in Drosophila, a minimum amount of time must pass between the original selection of a target and switching to a new stimulus, and switching at all was reliant on short-term memory genes.

In human psychophysics, both abrupt-onset (Yantis and Jonides, 1984) and perceptually new objects (Franconeri et al., 2005) provoke attentional capture, a phenomenon where attention is automatically and involuntarily directed at a particular, often task irrelevant, feature (Remington et al., 1992). In our CSTMD1 recordings, we found no evidence for attentional capture. Instead, the earliest period of the paired-targets revealed the strongest bias to the previous primer trajectory, with the possibility of switching to the more novel distracter at a later time. Thus, rather than attending to a novel distracter, this system locked-on to the expected target trajectory. CSTMD1 predicts future target location, even following an occlusion, with an enhancement in front of the prior path and suppression in the surround (Wiederman et al., 2017; Fabian et al., 2019). During the initial window, the continuing target is facilitated (gain increase) by the preceding target and continuously moving into its self-generated spotlight of predictive gain modulation. However, the distracter appears within the suppressed surround (gain decrease) and therefore will not elicit attentional capture, similar to some recent findings in Psychophysics (Ruthruff and Gaspelin, 2018). By the middle period, the

distracter may itself have self-facilitated, enabling a more even competition for target selection and thus increasing the probability of a switch. Whether this self-facilitation occurs at both target locations before selection, or only at the single selected location is currently under investigation.

The possibility that non-selected stimuli also generate a spotlight of neuronal gain modulation is similar to proposed mechanisms underlying attention in primates (Reynolds and Desimone, 2003). Primate cortical cells are thought to be 'hard-wired' to respond to the highest contrast stimulus, a property that can be exploited by attentional systems (Schiller and Lee, 1991; De Weerd et al., 1999). Here the representation of stimuli is modulated by enhancing the effective contrast of the focus of attention (Martínez-Trujillo and Treue, 2002; Reynolds and Desimone, 2003). Through this enhancement, less salient and even non-preferred stimuli can come to dominate the response of neurons in V4 (Reynolds and Desimone, 2003), MT, and MST (Recanzone et al., 1997; Treue and Maunsell, 1999). However, it is important to note that, unlike in primates, CSTMD1 encodes the absolute strength of the selected stimulus – this includes encoding a low-contrast target with a low firing rate, even as that stimulus is selected despite the presence of a high-contrast distracter simultaneously presented within the receptive field (Figure 6A, middle left).

This neuronal enhancement observed in primates may be mechanistically similar to the predictive gain modulation observed in CSTMD1, where in response to a single target gain is increased ahead of the prior path and suppressed in the surround. In primates it is the presence of distracters that triggers this attentional enhancement (Treue and Maunsell, 1999; Reynolds et al., 2000; Reynolds and Desimone, 2003). However, in CSTMD1, facilitation (i.e. the local gain increase) enhances the neuronal response to even an individually presented single target (Dunbier et al., 2012; Wiederman et al., 2017). In the presence of distracters the facilitated strength of the selected target is retained as if the distracter did not exist.

How might the dragonfly brain utilize information represented by CSTMD1? In order for CSTMD1 to be behaviourally relevant for tracking targets through space, the spatial location of the selected target must be recovered from either CSTMD1, or a population of similarly tuned neurons. It should be noted that CSTMD1 is not necessarily the only neuron that inherits properties of both prediction and selection, presumably formulated in presynaptic networks. It is possible that the precise location of a target represented by an array of neurons with ambiguous responses, though with overlapping receptive fields, is calculated with divisive normalization (Evans et al., 2016). Although this has not been demonstrated in the dragonfly, normalization is a common neuronal computation that has been observed in a variety of brain systems and taxa (Carandini and Heeger, 2012).

The facilitation effect observed in CSTMD1 spreads ahead of a target along a straight trajectory (Wiederman et al., 2017), thus predicting that a tracked target will continue moving in a straight line relative to the dragonfly. This prediction matches ethological goals, where the dragonflies' main method of pursuit is an interception path (Mischiati et al., 2015) from behind and below the target (Olberg et al., 2007) perhaps even utilizing motion camouflage, where the target is kept in a stable position relative to the pursuing dragonfly (Mizutani et al., 2003). During predatory pursuit dragonflies fixate targets in a high-resolution optical fovea situated on the dorsal surface of the eye (Olberg et al., 2007; Mischiati et al., 2015). CSTMD1 responses are greatest for targets that move upwards and towards the periphery (away from the midline) thus may be involved in error signals driving movements that preserve the retinal position of the selected target in the optical fovea. Functional roles for CSTMD1 (and other STMD neurons) in such closed-loop pursuit scenarios still remain speculative, however we model these target-detection pathways in virtual-reality, computational simulations in order to elucidate these complex interactions (Bagheri et al., 2017a, 2017b).

The ability of a neuron to respond with the same strength to a target presented alone, or when selected in a pair, is likely to underlie the dragonfly's exceptional ability to hunt in swarms (Combes et al, 2012). Such neuronal processing may have evolved overcome the confusion effect by singling-out targeted prey amidst a swarm (Landeau and Terborgh, 1986). Behavioural studies in some dragonfly species, e.g *Libellula* adults (Combes et al., 2012) and nymphs (Jeschke and Tollrian, 2007), show that they are adept at hunting in swarms throughout life. Although not tested in *Hemicordulia*, this hawking dragonfly would also likely benefit from neuronal processing that reduces the confusion effect via selective attention, as they spend most of their adult life hunting and patrolling territory on the wing and can regularly be observed hunting amidst swarms or prey and conspecifics.

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Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron

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Abstract: The ability to pursue targets in visually cluttered and distraction-rich environments is critical for predators such as dragonflies. Previously, we identified CSTMD1, a dragonfly visual neuron likely involved in such target-detecting behaviour. CSTMD1 exhibits facilitated responses to targets moving along a continuous trajectory. Moreover, CSTMD1 competitively selects a single target out of a pair. Here, we conducted *in vivo*, intracellular recordings from CSTMD1 to examine the interplay between facilitation and selection, in response to the presentation of paired targets. We find that neuronal responses to both individual trajectories of simultaneous, paired targets are facilitated, rather than being constrained to the single, selected target. Additionally, switches in selection elicit a suppressive 'inhibition of return' which is likely an important attribute underlying target pursuit. However, binocular experiments reveal these results are constrained to paired targets within the same visual field, while selection of a target in one visual field establishes ocular dominance that prevents facilitation or response to contralaterally presented targets. These results reveal that the dragonfly brain preattentively represents more than one target trajectory, to balance between attentional flexibility and resistance against distraction.

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Benjamin H. Lancer		
Contribution to the Paper	Experiment & methodology conceptualisation and design; animal collection; electrophysiology; data curation, analysis & interpretation; figure generation; manuscript original draft, review, and editing.		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	11/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Bernard Evans		
Contribution to the Paper	Experiment & methodology conceptualisation and design; software development; animal collection; electrophysiology; data curation, analysis, interpretation, and validation; figure generation; manuscript review and editing.		
Signature		Date	11/10/21

Name of Co-Author	Joseph Fabian		
Contribution to the Paper	Experiment & methodology conceptualisation; electrophysiology (unpublished pilot data); data analysis & interpretation; manuscript review & editing.		
Signature		Date	6/10/21

Name of Co-Author	David O'Carroll		
Contribution to the Paper	Experiment & methodology conceptualisation; data interpretation; manuscript review & editing; supervision; funding acquisition.		
Signature		Date	29 Sept 2021

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Contribution to the Paper	Experiment & methodology conceptualisation; software development; data interpretation; manuscript review & editing; supervision; provision of resources; funding acquisition/		
Signature		Date	7/10/21

Thesis Context Statement

The core question and experiment in this paper was first undertaken by (now Dr.) Joseph Fabian, Ph.D. candidate at the time during the final year of his candidature, which was the first year of mine. Joseph's thesis (Fabian, 2017) had focussed on the gain enhancement (facilitation) observed as targets moved on a continuous trajectory, which left open an intriguing question: how does facilitation interact with selection? This question had also been mused by reviewers in the reviewer comments of Joseph's 2017 paper (Wiederman et al., 2017).

I was in position to adapt and extend on the experiment presented in Joseph's thesis to make use of both frequency tagging and cuing (from paper 1, Lancer et al., 2019), expecting only a short *eLife advance* follow-up to the 2017 paper before moving on with my other experimental plans. However, the dragonfly had other ideas.

What began as a short, one-experiment idea evolved heavily as additional experiments were added. This started with my decision to add in a cross-hemispheric version of the experiment that tested for interactions between facilitation and selection of targets across the visual midline, based on the small cross-hemispheric component of Joseph's 2017 paper (Wiederman et al., 2017) and intriguing results my colleague Bernard Evans was getting from targets embedded in full-screen naturalistic backgrounds (Evans et al., 2020). This addition, of course, required initial validation of both components (selection, facilitation) across the midline. In addition to increased experimental scope, the experimental results ran counter to *both* of my initial hypotheses, indicating that there was something significantly more complex going on. Ultimately, we found that while the target unselected from a pair does indeed generate a facilitatory hotspot (a speculation I first proposed in the discussion section of my 2019 paper, Chapter 3), the addition of a cue actively changes the results and reveals a scenario where a target that appears during ongoing tracking can be blocked from generating facilitation and exhibiting *Inhibition of Return* following an endogenously driven switch. Inhibition of return is a common finding within human and other primate attentional studies, describing attentional suppression of a previously selected target when attention switches away (to discourage switching back), but to our knowledge this is the first evidence of inhibition of return in an insect, or any invertebrate. Additionally, the results of the cross-hemispheric experiment also showed a curveball, exhibiting the generation of ocular dominance from selection in contrast to the interaction observed between same-hemifield targets. Overall, what started as a simple experiment for a short paper became very complex, very fast.

Introduction

Selective attention, the ability to respond to a selected subset of environmental stimuli, is important to many species across taxa and underlies a variety of behavioural tasks. The study of target selection and attention has largely focused on vertebrates, but there is mounting evidence that insects are capable of attention-like computations (De Bivort and van Swinderen, 2016; Nityananda, 2016). Adult dragonflies are predatory pursuit specialists(Lancer et al., 2020) that intercept prey mid-air with high success rates (Olberg et al., 2000; Combes et al., 2013), by flying along interception trajectories based on predictive internal models (Mischiati et al., 2015; Lin and Leonardo, 2017). We have identified a small-target, motion-sensitive, visual neuron in the dragonfly brain that exhibits selective attention via a winner-takes-all, competitive process when presented with paired targets, responding to the unselected target as if it did not exist (Wiederman and O'Carroll, 2013a; Lancer et al., 2019). This neuron, termed 'Centrifugal Small Target Motion Detector 1'(CSTMD1; Geurten et al., 2007), is then able to flexibly 'lock on' to an attended target even when challenged by an abrupt-onset, high-contrast distractor (Lancer et al., 2019) as well as dynamically 'switch' attention between targets of equivalent or varying contrast (Wiederman and O'Carroll, 2013a; Lancer et al., 2019).

CSTMD1 exhibits neuronal facilitation in response to a single target moving along a continuous path (Nordström et al., 2011; Dunbier et al., 2012). This facilitation manifests as a 'spotlight' of gain enhancement that spreads predictively ahead of the target's current trajectory and is concomitant with suppression of surround locations in the receptive field (Wiederman et al., 2017). This gain enhancement is thought to drive the neuronal response to saturation to render it less sensitive to transient changes in target saliency (Fabian et al., 2019). The interaction between attentional selection of a target and this facilitation mechanism for target trajectories is not yet known. One hypothesis is that facilitation subserves selection by boosting the signal of the attended target, resulting in a positive feedback loop similar to contrast-gain mechanisms observed in primate visual cortex (Hillyard et al., 1998; Reynolds et al., 2000; Martínez-Trujillo and Treue, 2002; Reynolds and Desimone, 2003). Alternatively, facilitation may precede selection, with even representations of unselected targets becoming enhanced. Studies in humans and other primates reveal simultaneous tracking of multiple, independent targets, where each target generates its own 'spotlight' of enhancement (McMains and Somers, 2004; Cavanagh and Alvarez, 2005; Störmer et al., 2013). These spotlights can form at non-contiguous, independent spatial locations at the level of extrastriate occipital pathways and V1 (Müller et al., 2003; McMains and Somers, 2004; Malinowski et al., 2007; Störmer et al., 2013). In the context of a dynamic scene where new opportunities and risks may become apparent over time, the ability to passively track multiple targets simultaneously may be desirable. However, it would remain critical to select only one target for the direction of action (Mysore and Kothari, 2020). Otherwise, the animal

could actuate an inaccurate 'average' action vector (Ottes et al., 1984; Lisberger and Ferrera, 1997; Ioannou et al., 2008, 2009; Nummela and Krauzlis, 2011).

Are more than one trajectory facilitated when one is selectively attended in the dragonfly target tracking system, or is it only the trajectory for the selected target that is predictively facilitated? Here, we test this directly by recording CSTMD1 spiking activity *in vivo* in response to the simultaneous presentation of a pair of equally salient, rival targets. We assess the facilitation state ahead of the trajectory of the 'ignored' target (i.e., the unattended target not represented in CSTMD1's spiking response). We show that when both targets are presented in the same visual hemifield, both are facilitated despite only one being selected. However, endogenous attentional switches between targets generate Inhibition of Return (IOR) that weakens representations on the trajectory of the previous selected target. In addition, we tested the extension of such mechanisms across the two sides of the insect brain when rival targets are presented in different visual hemispheres. We show that selection of a target in one hemisphere establishes ocular dominance and leads to long-lasting suppression of targets presented to the contralateral eye.

Results

Interaction between facilitation and target selection in the excitatory receptive field

To test for facilitation on unselected trajectories, we presented 'Paired Primer' Targets consisting of two 1.5° by 1.5° dark squares that moved upwards on the display at 50°/s, on rival trajectories (Figure 29A, T_1 and T_2) within CSTMD1's excitatory receptive field. Primer targets were frequency-tagged by modulating contrast (at different frequencies) in order to elicit a frequency-locked response (Lancer et al., 2019) from the selected target . This was used in subsequent analyses to determine the attended target. On any given trial, a 'Probe' target was presented as a continuation of the trajectory of *either* T_1 *or* T_2 (but never both). We interleaved control trials consisting of either a single 'Local Primer' (matched to the Probe trajectory) or 'Distant Primer' (unmatched to the Probe trajectory), 'Probe Alone' trials (no primer), and Paired Primer trials (Figure 29B). To measure facilitation, we counted spikes within a 100 ms window, 50 ms offset from the Probe target onset to account for neural delays (Figure 29B, dark green area). In the first set of experiments, we used short duration Primers ascending the receptive field at 50°/s for 400 ms (Figure 29C). As previously observed (Nordström et al., 2011; Dunbier et al., 2012), responses to a Probe that continued on the trajectory of a single Local Primer were significantly facilitated compared to the Probe Alone (Figure 29C, red, $p < 0.001$, $g = 2.36$ [1.99, 2.72]). At a 12° horizontal spacing between trajectory locations, we saw no significant effect on neuronal response to a Probe that appeared after a Distant Primer (Figure 29C, blue, $p = 0.08$, $g = 0.25$ [-0.03, 0.53]). Previously, surround suppression was observed at distances

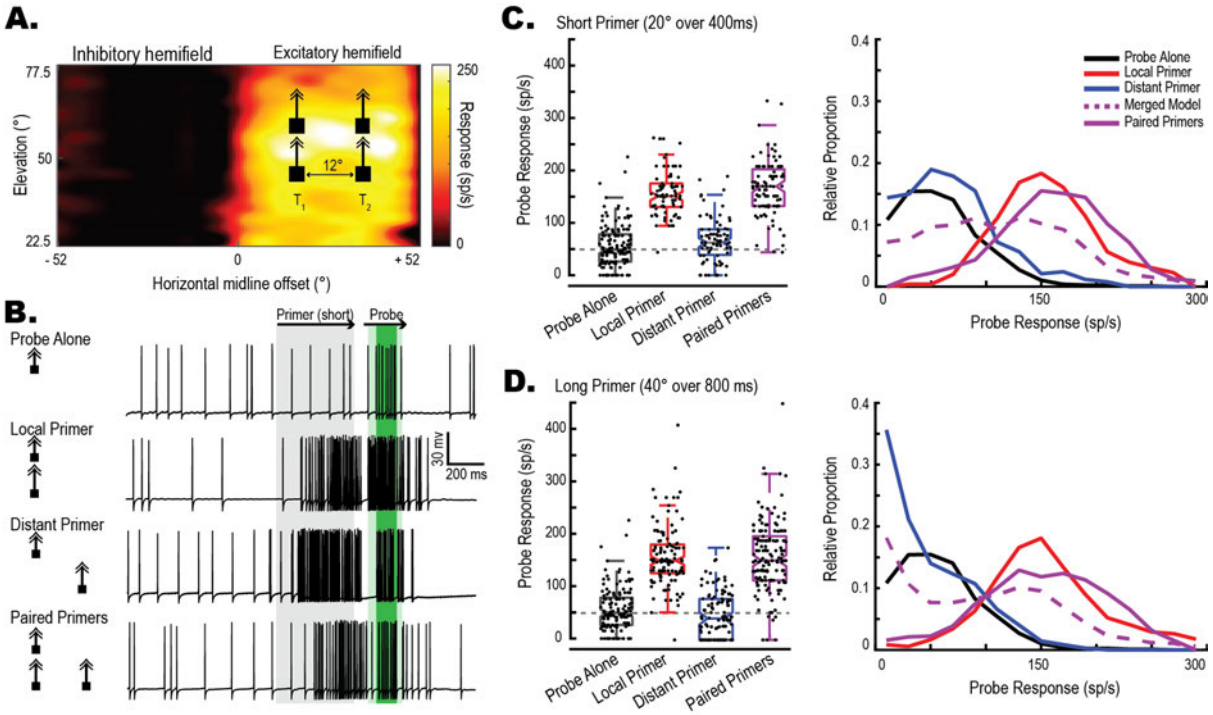


Figure 29: Facilitation is Generated on Unselected Trajectories. ***A)*** *CSTMD1's Receptive Field with schematic stimulus pictogram superimposed (not to scale). The receptive field was mapped with a single 2°x 2° target moving horizontally (left-to-right) at 80°/s. The receptive field consists of two distinct zones, the excitatory hemifield (contralateral to the recording site in the axon) and the inhibitory hemifield (ipsilateral to the recording site.)* ***B)*** *Left; Stimulus pictograms of four trial conditions (T_1 Probe locations shown; the same trials were also run using a T_2 Probe, i.e. mirrored). Top to bottom: Probe Alone, a 200 ms target Probe is presented alone. Local Primer, a Probe is spatiotemporally preceded by a facilitatory Primer on a matched trajectory. Distant Primer, the same Probe is preceded by a Primer on an unmatched trajectory (12° horizontal offset). 'Paired Primers', the same Probe is preceded by both a Local and Distant primer simultaneously. Right; Example spike trains drawn from the same neuron. Light green box indicates the 200 ms Probe period. Dark green box indicates the 100 ms analysis window.* ***C)*** *Results for the presentation of Short Primers lasting 400 ms. Left: Box-and-whisker plots with overlaid swarm plots showing Probe response to the varying conditions. Each dot represents an individual trial (389 total trials across 15 dragonflies). Spike rate was calculated from a 100 ms period 50 ms following the onset of the probe (B, dark green window). Right: Frequency polygons of the same data. Merged Model (purple, dashed) represents the combined Local (red) and Distant (blue) Primer distributions. The empirical Paired Primer distribution (purple, solid) more closely matches 'Local Primer' condition than a combination of local and distant primers, indicating overall facilitation.* ***D)*** *The experiment with Long Primers (800 ms) exhibits similar results (507 total trials across 15 dragonflies). Note 'Probe Alone' data is repeated from C.*

greater than 15° from the Primer trajectory (Wiederman et al., 2017). However, here our targets were likely placed in between the locally facilitated and these more distant suppressed regions.

When Paired Primers were presented in CSTMD1's excitatory receptive field, the neuron responded to just one target of the pair (Wiederman and O'Carroll, 2013a; Lancer et al., 2019). However, the dragonfly cannot know which of the Paired Primers would then be continuous with the follow-up Probe. Hence if only the selected Primer generates facilitation, we would expect the Probe to only be facilitated in a subset of the Paired Primer trials, leading to a broad distribution. This distribution would be equivalent to adding together those from the controls for single Distant and Local Primers (Figure 29C, dashed purple, 'Merged Model'). However, if the unselected Primer also generates neuronal facilitation, then the Probe should exhibit facilitation regardless of selection. We found that Probe response to Paired Primers (Figure 29C, purple) was significantly facilitated

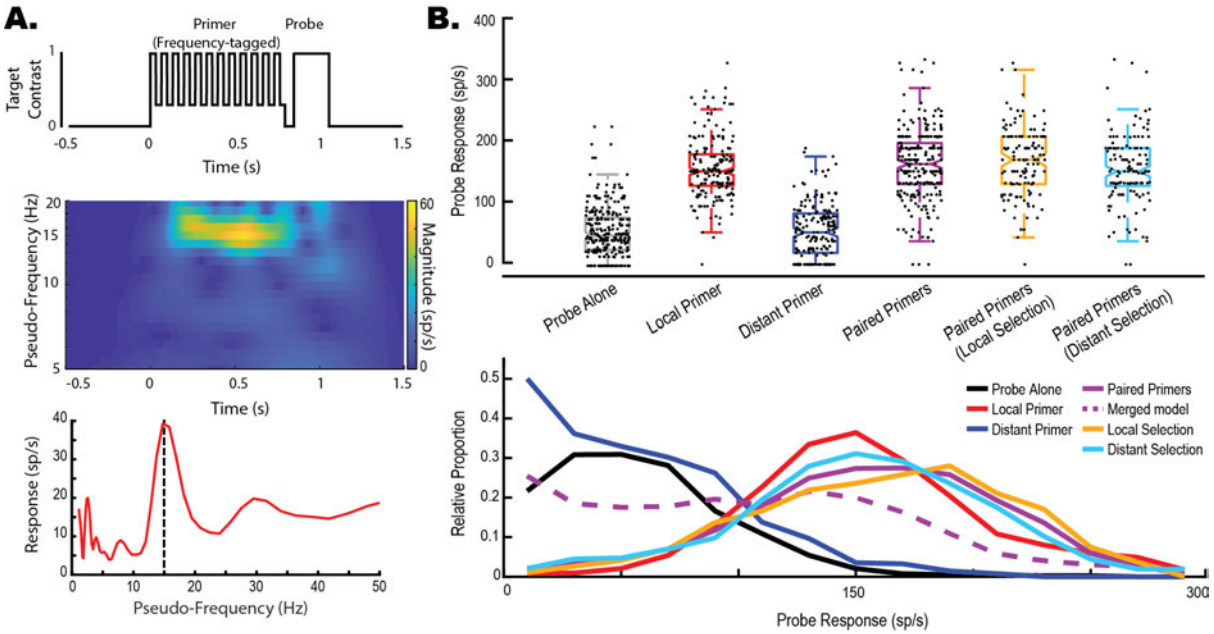


Figure 30: Frequency-Tagging Reveals Which Primer was Selected. A) *We applied frequency-tagging to Primers to determine which was selected on any individual trial. Top: An example of frequency-tagging with the modulation of one Primer contrast at 15 Hz before a Probe. Middle: Wavelet scalogram of the spike activity (Inverse Interspike Interval) in response to an example Primer target reveals a frequency-locked response at 15 Hz. Bottom:* Time-collapsed wavelet scalogram reveals a peak around 15 Hz. **B)** Top: *Box-and-whisker with overlaid swarm plots illustrating Probe response, for Long (800 ms) and Short (400 ms) Primers combined (896 total trials across 15 dragonflies). For comparison, Probe Alone, Local, Distant and Paired Primers are shown (i.e. same data as in Figure 1). Local Selection and Distant Selection box plots are the Paired Primers (purple) categorised by the Selectivity Index. We observe similar distributions between Local and Distant selection trials. Bottom: Frequency Polygons illustrating the distributions of each condition. Local Selection (gold) and Distant Selection (cyan) more closely match Local Primer (red) than the Merged Model (dashed purple) confirming that the Probe target is facilitated with either Local or Distant Selection.*

compared to both the Probe Alone (*p < 0.001, g = 2.36 [2.03, 2.70]*) and Distant Primer (*p < 0.001, g = 2.05 [1.69, 2.41]*) conditions, but not different from the Local Primer condition (*p = 0.123, g = 0.19 [-0.10, 0.49]*). Frequency Polygons (Figure 29C, right) show that Paired Primer responses (purple line) more closely matched the Local Primer (red line) than a theoretical equal combination of Local and Distant Primer responses ('Merged Model', dashed purple). We repeated this experiment with longer duration primers ("Long Primers") ascending the receptive field at 50°/s trajectory for 800 ms and observed similar results (Figure 29D), with Paired Primer responses facilitated in comparison to Probe Alone (*p < 0.001, g = 1.85 [1.57, 2.13]*) and Distant Primer (*p < 0.001, g = 1.98 [1.69, 2.28]*), but not different from the Local Primer (*p = 0.609, g = 0.02 [-0.26, 0.22]*). Thus, we observed neuronal facilitation even on the trajectory of non-selected targets during Paired Primer conditions.

Do selected and non-selected targets generate the same magnitude of facilitation? To identify which Primer was selected on any given trial, we utilized frequency-tagging as previously validated in CSTMD1 (Lancer et al., 2019). As illustrated in Figure 30A, each Primer target's contrast was modulated (Weber = 0.22 to 1) at a unique frequency (11, 15 Hz, square waveform), resulting in frequency-locked neuronal responses. This allowed us to identify the selected Primer in ~70% of Paired Primer trials using a Selectivity Index (Lancer et al., 2019; detailed description in *Methods*). As previously observed

(Lancer et al., 2019), in approximately 30% of trials frequency-tagging did not elicit sufficient modulation for us to confidently identify the selected target, so these trials were excluded from further analysis. Figure 30B shows the assignment for each Paired Primer trial. If the selection trajectory was 'matched' to the Probe, then it was categorized as 'Local Selection' (gold, 126 trials), or as 'Distant Selection' if unmatched (cyan, 128 trials). We found no statistically significant difference in CSTMD1's response to the Probe between Local Selection and Distant Selection trials (Figure 30B, gold vs cyan, *p = 0.052, g = 0.25 [0.006, 0.501]*), supporting the earlier result that similar facilitation was generated along both target trajectories, irrespective of target selection.

Do attentional cues influence facilitation generation?

We recently reported that presenting a spatiotemporally preceding 'Cue' on a single trajectory before paired targets biased selection towards that target (Lancer et al., 2019). Does the addition of such a Cue affect the generation of facilitation by Paired Primers? Figure 31A shows data where one Primer preceded the appearance of the second. This Cue was always matched to the subsequent Probe (either T_1 Cue and T_1 Probe; or T_2 Cue and T_2 Probe). In most trials (~80%) the Cue induced Local Selection (Figure 31A, gold), and the Probe then exhibited strongly facilitated responses compared to Probe Alone (*p < 0.001, g = 2.12 [1.79, 2.45]*), quantitatively similar to the facilitation induced by the single Local Primer condition (*p = 0.433, g = 0.009 [-0.26 0.28]*). However, we also saw Distant Selection in a smaller number of trials (25 trials, ~20%) presumably reflecting a switch in attention away from the Cue to the more novel target (Figure 31A, cyan). Here, we then observed a reduction in the Probe response compared to both Local Primer alone (*p = 0.009, g = 0.39 [-0.05, 0.82]*) and Local Selection (*p = 0.004, g = 0.40 [-0.04, 0.85]*). Nevertheless, while weaker than single local primers, the response was still much stronger compared with the unfacilitated Probe Alone (*p < 0.001, g = 1.64 [1.18, 2.11]*), indicating strong facilitation in at least a subset of trials.

What could account for the weaker facilitation in this case? In primate neurophysiology and human psychophysics, switching attention from one location to another is associated with a suppressive signal known as 'Inhibition of Return' (Klein, 2000) (IOR). Such inhibition prevents the attentional system from returning to previously assessed locations, allowing efficient visual search (Itti and Koch, 2001; MacInnes et al., 2014). In our data, selection returns to the originally cued trajectory as the selected Primer disappears and the single Probe appears. Therefore, we propose that although both targets of a presented pair generate facilitation, an attentional switch might generate an Inhibition of Return that supresses responses on the initially selected trajectory.

Figure 31B shows data when the Cue is unmatched to the Probe (either T_1 Cue and T_2 Probe; or T_2 Cue and T_1 Probe). While we observed overall facilitation of the probe response (i.e. compared to Probe Alone) for both Local Selection (*p < 0.001, g = 1.42 [0.90, 1.95]*) and Distant Selection (*p <*

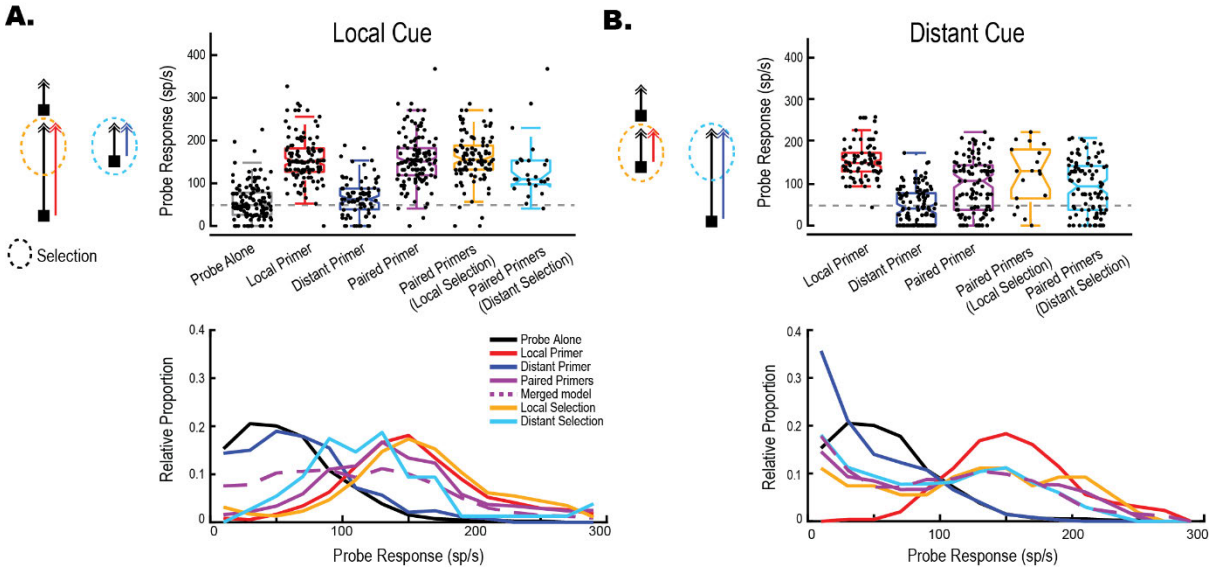


Figure 31: A Cue for Selection can Modulate Facilitation. *Left: Stimulus pictogram illustrating relevant trials. Right, Top: Boxplots illustrating the neuronal response to the Probe. The Paired Primer trials have been separated into Local and Distant selection based on the Selectivity Index. Right, Bottom: Frequency Polygons show Probe response distributions.* ***A)*** *Trials where the Cue was 'matched' to Probe location (Local). Total 122 Matched trials across 15 dragonflies, control data as from previous figures reproduced for comparison. Grey dashed line indicates the Probe Alone mean. In trials where the Cue is successful (Local Selection, gold) we observe facilitation. However, in trials where the Cue is ignored (Distant Selection, cyan) we observe suppression of the local path.* ***B)*** *Trials where the Cue was 'unmatched' to the Probe location (Distant). Total 111 unmatched trials across 15 dragonflies, control data as from previous figures reproduced for comparison. Grey dashed line indicates the Probe Alone mean (Distribution in A). Unmatched trials reveal Paired Primer responses similar to the broad distribution of the Merged Model (Dashed purple line), regardless of whether Paired Primers were categorized as Local Selection (gold) or Distant Selection (cyan).*

0.001, g = 0.70 [0.43, 0.98]), In the majority of trials (93 trials, ~83%; Fig 3B cyan) where the Cued, Distant Primer was selected, Probe responses were broadly distributed and elicited reduced overall facilitation compared to a simple local primer (*p < 0.001, g = 1.14 [0.81, 1.47]*). Intriguingly, some trials in this condition exhibited facilitation and others did not, matching the 'merged model' ($p = 0.632$, $g = 0.03$ [-0.21, 0.28]) combination of single Local and Distant Primers (Figure 31B frequency polygons, dashed purple). Even in trials where the Local Primer was selected from the Pair despite the distant Cue (18 trials, ~16%; Figure 31B gold), the distribution matched the 'merged model' ($p = 0.244$, g = 0.44 [-0.04, 0.93]) but with a small 'bump' of facilitation at the right tail. It is interesting that the Distant Cue condition is the only stimulus condition to exhibit such a broad distribution resembling the merged model. This reveals that facilitation generated by the introduction of a novel (i.e., not cued) target appearing during tracking of a cued target is an all-or-nothing effect. A feasible speculative explanation for this is that the attentional system may actively suppress *both* the response and the facilitation to targets appearing during ongoing tracking (I.e., when the system is already attending), in contrast to the facilitation of both the selected and unselected targets when the targets appear together. Such a an attentional suppression mechanism, sensitive to stimulus history, would result in stochastic trial-by-trial results due to natural variability in CSTMD1's response onset to a target and previously observed response 'delays' when presented with rival target pairs (Wiederman and O'Carroll, 2013a). This could lead to an 'all-or-nothing' outcome where a weak response occurs in

those trials where the novel target is supressed following prior establishment of attention by the cue and suppression mechanisms are engaged (I.e. the response as 'locked on' (Lancer et al., 2019), and stronger responses if the cue fails to establish strong attention before the rival target appears, such that pre-attentional facilitation occurs at both possible locations, as in un-cued trials (Figure 30). Prior results have shown that the effect of a cue is an overall bias towards the cued target (over many trials) rather than a trial-by-trial guarantee (Lancer et al., 2019), which would be explained by stochasticity in the time it takes for attention to 'lock on' and engage suppressive mechanisms. However, the biasing effect of a cue diminishes over 1000 ms (Lancer et al., 2019) suggesting that this suppression effect acts as a 'hurdle' to novel, transient distraction rather than complete suppression.

Target selection and facilitation in the binocular receptive field

CSTMD1's receptive field contains two discrete hemifields (Geurten et al., 2007), one excitatory and one inhibitory (Figure 29A). Our previous work found that when two targets are presented simultaneously in each visual hemifield, CSTMD1 responses were strongly supressed on average (Bolzon et al., 2009). However, this study was undertaken before the realization that CSTMD1 showed selective attention in individual trials for paired targets presented in the excitatory hemifield (Wiederman and O'Carroll, 2013a). We therefore again presented simultaneous target pairs consisting of one target in each of the inhibitory (T_i) and excitatory (T_e) hemifields, with individual examples shown in Fig. 4A (the analysis window shaded in green). Targets moved up the display monitor at 25°/s for 1 s. A subset of these trials included an additional 0.5 s Cue target to bias selection, as described earlier (Lancer et al., 2019).

Figure 32B shows inhibitory or excitatory responses to single targets (either Short or Long trajectories), dependent on the corresponding hemifield. In response to Paired targets without a Cue, ~80% of trials elicited suppression of spiking activity (< 25 sp/s), showing a preference for selecting the target in the inhibitory hemifield. This aggregate data reveals significant differences between the Paired Target conditions. Cueing for T_e elicited stronger responses than the uncued case (*$p = 0.008$, $g = 2.08$ [1.12 3.16]*)**,** revealing more frequent selection of the excitatory target if it precedes the other. Cueing T_i elicited weaker responses compared to the uncued case (*$p = 0.001$, $d = 1.11$ [0.27 2.01]*), with more frequent selection of the inhibitory target. In each individual neuron, we saw both inhibitory and excitatory responses to Paired Targets in individual trials with the corresponding Cue (Figure 32C T_i Cue: blue dots, T_e Cue: red dots). These data show that selection between Paired targets presented either side of the visual midline can be biased by a preceding target trajectory.

A mechanism that might underlie the biasing of a preceding Cue target is the generation of spatial facilitation at earlier levels of processing, i.e., prior to the synaptic sign inversion that gives rise

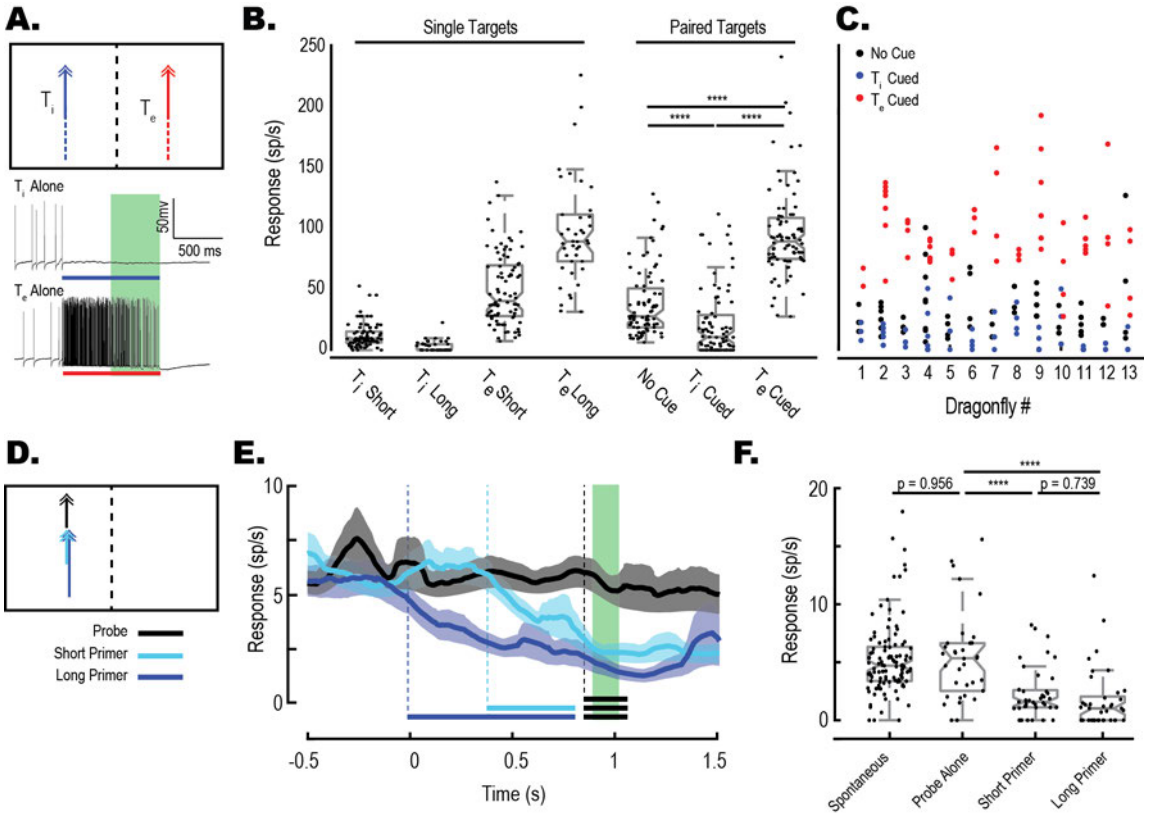


Figure 32: Target Selection and Facilitation in the Inhibitory Receptive Field. A) *Top: Targets vertically ascend a display within CSTMD1's excitatory or inhibitory receptive field, either individually or as a simultaneous pair. Targets are separated by 50° to avoid the 10° wide region of binocular overlap. Bottom; Example CSTMD1 responses to T_i (top) and T_e (bottom). T_i generates inhibition, T_e generates excitation. Target trajectories can be Short, Long or used as Cues, illustrated with dashed lines* ***B)*** *Boxplots with overlaid swarm plots illustrating the mean neuronal response over a 500 ms window (green shaded region in A). total 480 trials across 13 dragonflies. In Paired Target conditions, neuronal responses are broadly distributed, with some trials exhibiting inhibition and others exhibiting excitation. Compared to the Paired condition, a preceding T_i Cue shifts responses towards inhibition and a T_e Cue towards excitation.* ***C)*** *Responses in each of the 13 neurons to simultaneous paired target trials (black), T_i Cued trials (blue) and T_e Cued trials (red). The majority of neurons show inhibitory responses to Paired Target trials without cueing, however each neuron is able to respond to either the excitatory or inhibitory target with the appropriate Cue.* ***D)*** *Stimulus pictograms illustrating three conditions; Probe Alone (Black line only), Probe preceded by a short Primer (Black and Cyan line), and Probe preceded by a long Primer (Black and Blue). Trajectories are illustrative as they overlie in experiments* ***E)*** *Averaged response across all trials (shaded region is standard error, 110 trials across 11 dragonflies) reveals the time course of inhibition. Presentation of a target within the inhibitory receptive field drives neural activity below spontaneous rates (-0.5 to 0 s).* ***F)*** *Boxplots with overlaid swarm plots show that Probe response is reduced when paired with either a Short (400 ms) or Long (800 ms) primer. Spontaneous spike rate is also illustrated, and was measured over an equivalent time period before the beginning of each trial.*

to inhibition in one hemifield. Previous experiments on facilitation have largely been confined to CSTMD1's excitatory receptive field (Nordström et al., 2011; Dunbier et al., 2012; Fabian et al., 2019). However, in one experiment we observed that an inhibitory primer heading towards the excitatory hemifield still elicited facilitation across the visual midline (Wiederman et al., 2017). This revealed that facilitation information could transfer across brain hemispheres. Thus far, no studies have examined facilitation for trajectories constrained within the inhibitory hemifield.

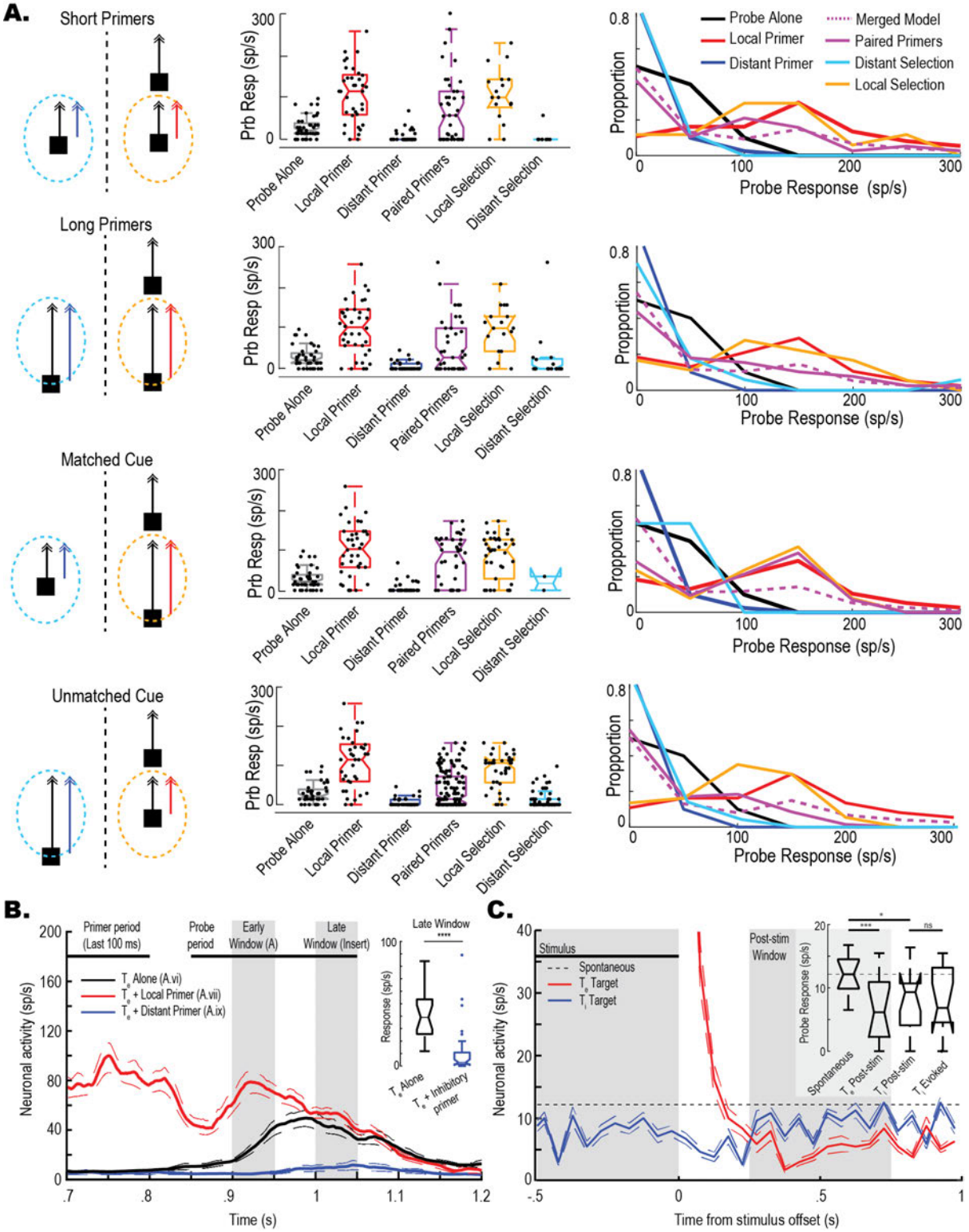
Do targets in the inhibitory hemifield generate neuronal facilitation similar to excitatory targets? We presented CSTMD1 with a Probe in the inhibitory region of the receptive field, either alone or following either a Short (400 ms) or Long (800 ms) Primer (Figure 32D) moved upwards along trajectories that remain confined to one hemifield. If inhibitory trajectories are also facilitated, we

would expect 'facilitation of inhibition' where spiking activity to a Probe continuing after an inhibitory Primer are driven even lower than the inhibitory responses to that Probe presented alone. We found that a brief (200 ms) Probe target did not generate significant inhibition relative to the spontaneous spike rate *(p = 0.956)*. However, the same probe elicited inhibitory responses when preceded by either a Short 400 ms (*p < 0.001, g = 1.10 [-1.60, -0.70]*) or Long 800 ms (*p < 0.001, g = 1.13 [-1.65, -0.64]*) Primer, on the same trajectory (Figure 32F). This decrease in spike rate is likely the result of an increased inhibitory drive from presynaptic excitatory facilitation, thus we refer to it as a 'facilitation of inhibition', rather than long-term depression. In line with several earlier studies suggesting that facilitation is largely complete within 400ms of stimulus onset (Nordström et al., 2011; Dunbier et al., 2012; Wiederman et al., 2017; Fabian et al., 2019), we found no difference in the amount of increased inhibition elicited by the Short or Long Primer conditions (Figure 32 E, F, *p = 0.739*).

To examine how facilitation and selection interact across hemispheres, we then presented both paired or cued paired Primers on both inhibitory (T_i) and excitatory (T_e) target trajectories, analogous to the experiments presented in Figures 27 and 29. We observed facilitation of an excitatory-hemifield Probe following selection of the matched primer from uncued Paired Primer presentations (Figure 33A, gold conditions; *Short Primers p < 0.001, g = 1.88 [1.23, 2.57]; Long Primers p < 0.001, g = 1.70 [1.08, 2.36]*). We also observed facilitation of the excitatory Probe irrespective of whether the Paired Primer presentation followed a Matched (*p < 0.001, g = 1.23 [0.75, 1.72])* or Unmatched *(p < 0.001, g = 1.57 [1.07, 2.10]*) Cue. These results show that when selected, an excitatory hemifield Primer can generate facilitation despite the existence of a simultaneous inhibitory-hemifield stimulus. However, when the inhibitory-hemifield Primer was selected from a pair (Figure 33A, cyan conditions), we observed a sharp suppression of the subsequent response to a T_e Probe, compared to Probe Alone presentations (Short Primers p = 0.011, g = -0.82 [-1.61, -0.05]; Long Primers p = 0.011, g = 0.09 [-0.66, 0.47]). Intriguingly, we also observed suppression of T_e Probe responses following T_i Primer alone trials (Figure 33A, Blue; Short Primers *p = 0.001, d = 1.07 [-1.54, -0.61]; Long primers p <0.001, d = -1.12 [-1.60, -0.65]),* indicating that this suppression is not the result of a selection process. Instead, these data show that responses to an inhibitory target suppress CSTMD1's ability to respond to a subsequently presented excitatory primer, even after a 50 ms pause.

Long-lasting cross-hemispheric inhibition establishes ocular dominance.

To examine this long-lasting inhibition further, we analysed conditions in which the excitatory Probe interacted with either excitatory or inhibitory single Primers (Figure 33A). We observed that T_e Probes elicit early facilitated responses when preceded by Local Primer (Figure 33B red line), compared to the Probe Alone (black Line). The T_e Probe Alone already begins to facilitate within 100



ms once its path commences, as expected from prior findings (Fabian et al., 2019). When the T_e Probe is preceded by a Distant (T_i) Primer it is significantly inhibited compared to the T_e Probe Alone in both an early window (*50 ms post Probe onset, as noted above)* and a late (150 + ms post Probe onset) analysis window (*$p < 0.001$, $g = 1.77$ [1.15, 2.42]*). This is despite the late analysis window being set sufficiently after the time required for the T_e probe in unprimed trials (black line) to reach a similar degree of facilitation to the fully primed condition induced by the Local Primer. Thus, we observe

Figure 33: Target Selection establishes Ocular Dominance. *Previous Page* ***A)*** *Left: Stimulus pictograms illustrating relevant trials. Middle: Boxplots illustrating the neuronal response to the probe. Right: Frequency Polygons illustrating the Probe Response distributions. The 'Local Selection' condition (gold) is consistently enhanced compared to Probe Alone (black) indicating facilitation, however both Distant Primer (Blue) and Distant Selection (cyan) conditions are inhibited. Total 351 trials across 12 neurons.* ***B)*** *Average inverse ISI plots reveal the time course of the neuronal response to an excitatory (Te) Probe. Total 195 trials across 12 neurons. Te Probes elicit early facilitated responses when preceded by a Te Primer (red line) compared to when alone (black line). However, even the Te Probe alone is able to reach a facilitated state after approximately 100 ms. When preceded by a Ti Primer the Te Probe is significantly inhibited (blue line) as in A, but this inhibition remains even in a late analysis window 150 ms after the onset of the Probe (Insert, boxplots), well within the time necessary to self-generate facilitation independently of prior priming. This observed suppression indicates cross-hemispheric inhibition evoked by a Primer in the inhibitory visual field lasts for at least 200 ms, although some individual trials are able to break through this suppression (insert, blue outliers).* ***C)*** *Averaged PTSH reveals post-stimulus inhibition occurs for both excitatory and inhibitory stimuli. Data reanalysed from Figure 4 (fig 4C, second and fourth from top); 160 total trials across 12 dragonflies. Following the stimulus offset (time = 0) of either an excitatory (red line) or inhibitory (blue line) small moving target, CSTMD1's spike rate is supressed for an extended period (at least 1 s) in comparison to the spontaneous firing rate (black dotted line). Insert: Boxplots illustrating the average per-cell response across conditions. Paired-samples t-tests were based on average neuronal responses (12 neurons).*

profound long-lasting inhibition evoked by a Primer in the inhibitory hemifield (lasting at least 200 ms), masking our ability to examine facilitation of a subsequent T_e Probe. It is not clear whether following in Inhibitory Primer, there is no facilitation at the Excitatory Probe, or whether local facilitation still exists but is masked by this long-lasting inhibition. In either case, target trajectories in the opposite hemifield to the selected stimulus do not generate net facilitation in CSTMD1's response.

To further examine post-stimulus excitation or post-stimulus inhibition, we re-analysed the single-target trials presented in Figure 32B, focusing on a time window after the stimulus offset (500 ms window starting 250 ms after target disappearance). CSTMD1's responses exhibited significant post-excitatory inhibition following the offset of a T_e target (Figure 33C; T_e post-stimulus window vs Spontaneous, $p < 0.001$, $g = 0.54$ [-0.27, 1.37]). Such post-excitatory inhibition is a common property of spiking neurons, typically associated with the build-up of slow potassium currents during excitation resulting in sustained hyperpolarization. What occurs following the target placed within the inhibitory hemifield? Many neurons exhibit a similar 'post-inhibitory rebound' of enhanced sensitivity or spontaneous firing following an inhibitory signal, contributing to a Motion After Effect in visual circuits (Harris et al., 2000). However, in CSTMD1 we observed long-lasting inhibition following a T_i target (Figure 33C; T_i post-stimulus window vs Spontaneous, $p = 0.019$, $g = 1.52$ [0.64, 2.49]), which matched the suppression generated by the presence of a target (T_i stimulus window (-0.5 to 0 s) vs post-stimulus window (0.25 to 0.75s), $p = 0.917$). We did not observe increased sensitivity following the disappearance of an inhibitory stimulus. As noted above, we observed the opposite, where a normally excitatory target was unable to generate a response despite appearing 50 ms following the offset of an inhibitory target (Figure 33A, 'Distant Primer' Condition). In contrast, an excitatory target is able to re-engage spike generating mechanisms if it appears during the post-excitatory rebound of another excitatory target (Figure 33A, 'Local Primer' condition), although it remains moderately inhibited if the second target does not appear within the facilitated region of the first (Dunbier et al., 2012). Thus, CSTMD1 responds to stimulus offset with robust inhibition, regardless of the valence of the stimulus.

Discussion

We have shown that when a pair of targets occupy the excitatory receptive field, each target generates its own spotlight of spatial facilitation, thus encoding the trajectory of both. One of these is selected for representation in CSTMD1's spiking response, suggesting that computations underlying target facilitation precede competitive selection in the dragonfly attentional network. As facilitation has been observed in other small target motion detectors (STMDs) within the lobula complex (presumed to be upstream of CSTMD1) (Wiederman et al., 2017), it is likely that this is a property of this earlier STMD pathway. These results resemble primate studies showing simultaneous tracking of multiple independent targets (Pylyshyn and Storm, 1988), where each target generates an independent 'spotlight' of enhancement(McMains and Somers, 2004; Cavanagh and Alvarez, 2005; Störmer et al., 2013). However, in contrast to our findings, a primate's ability to track multiple targets is independently divided between left and right hemispheres (Alvarez and Cavanagh, 2005). For humans, it is easier to track one target presented to each eye than it is to track a pair of targets in the same eye (Störmer et al., 2014) and attentional modulation is attenuated for multiple targets presented in the same hemifield (Störmer et al., 2014; Walter et al., 2014).

We have also shown that targets presented in CSTMD1's binocular (inhibitory) receptive field elicit both 'facilitation-of-inhibition,' a facilitation-like enhancement of inhibition over time, and selective responses, whereby either an inhibitory or excitatory target can be selected. Examination of CSTMD1's morphology reveals a dendritic arbour corresponding to the excitatory hemifield (in the midbrain) as well as a possible input/output arborisation (on the other side of the midbrain) corresponding to the inhibitory hemifield (Geurten et al., 2007). However, any specifics of the neuronal architecture and behavioural functionality associated with this interplay between inhibitory and excitatory interactions in CSTMD1's response is not yet known. We have previously speculated that CSTMD1 is involved in signalling pursuit error when a target drifts away from the visual midline (Lancer et al., 2019). Strong Inhibitory interactions between each hemispheric CSTMD1 would ensure error signals are being generated for only one target at a time, and enable the dragonfly to quickly reorient during an active pursuit when a target crosses the visual midline from the excitatory receptive field of one CSTMD1 to the other. Critically, targets moving upwards and towards the periphery elicit the strongest facilitation (Wiederman et al., 2017), while prior research shows targets presented in the inhibitory hemisphere elicit the strongest inhibition when moving upwards or towards the periphery (Bolzon et al., 2009), presumably due to facilitation-of-inhibition via the contralateral

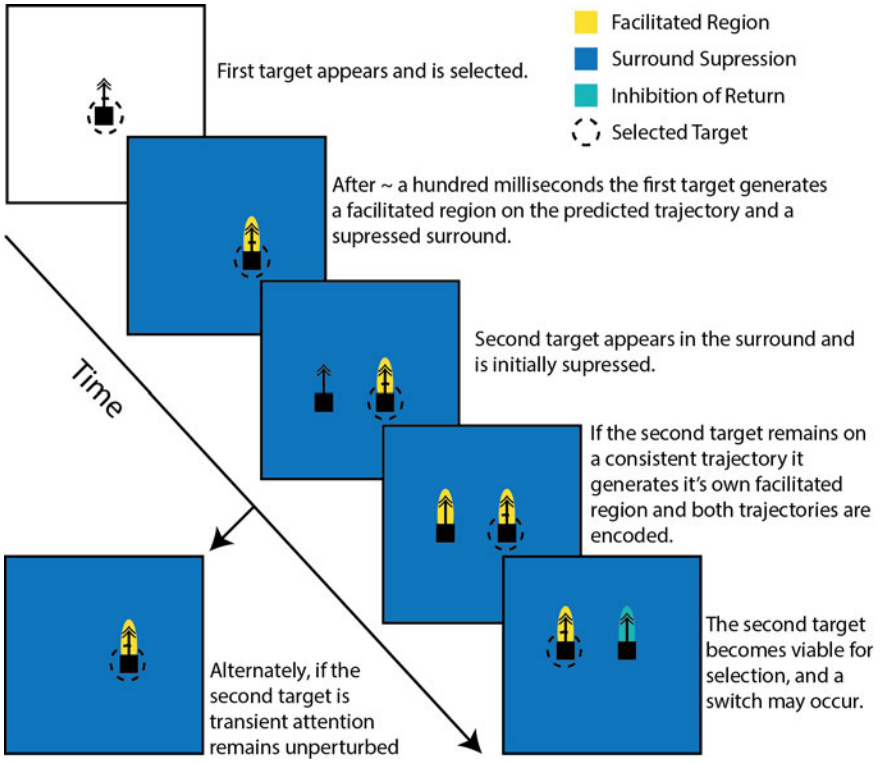


Figure 34: Schematic Depicting the Proposed Interaction Between Predictive Gain Modulation and Target Selection. *Predictive Gain Modulation may act as a gatekeeping mechanism for selection by supressing transient stimuli that appear in the suppressed surround, but passing consistent stimuli that may be of potential behavioural relevance. This ensures the attention system does not get captured by transiently highly-salient distractions, but is still afforded the flexibility to track and respond to novel stimuli.*

CSTMD1. These inhibitory effects are reduced when targets are directed towards the midline – i.e., when the predicted path crosses from one hemisphere to the other (Wiederman et al., 2017).

Our earlier work proposed that facilitation enhances the encoding of a target in variable visual conditions by increasing contrast sensitivity, inducing directional selectivity, and driving the neuron to saturation (Wiederman et al., 2017; Fabian et al., 2019), allowing CSTMD1 to be both feature invariant and highly tuned depending on the situation. Thus, once a target is facilitated, it is able to generate a robust neuronal signal even during a high-speed pursuit where the target's local contrast against the immediate background may be highly variable due to background clutter. Additionally, the predictive encoding of a target's future position (Wiederman et al., 2017) may be involved in predictive behaviours performed by dragonflies before and during pursuit (Olberg et al., 2000, 2007; Mischiati et al., 2015; Lin and Leonardo, 2017). However, we have shown that facilitation is generated even for non-selected and inhibitory targets. Why should such distractors be similarly enhanced? We suggest that facilitation may also act as a 'gatekeeping' mechanism for selective attention (Figure 34). By initially supressing targets in the surround, Predictive Gain Modulation ensures attention is not recruited by abruptly appearing, transient highly-salient stimuli (Lancer et al., 2019). However, if a stimulus remains on a consistent trajectory generating its own facilitatory spotlight, it can become a viable target for stimulus selection. Therefore, the various components of Predictive Gain Modulation (spatial facilitation, induced directional selectivity, suppressive surround) work together to gatekeep attention by blocking distracting, transient and inconsistent stimuli, whilst enhancing stimuli on consistent trajectories, likely to represent potential prey, predators, or conspecifics.

This generation of facilitation on unselected paths can account for the diminishing effect of cueing previously observed (Lancer et al., 2019). A cued target benefits from facilitation generated by the cue, while a non-cued target appears within the supressed surround, thus biasing selection to targets continuing on the cue trajectory. Previously, we observed that the strength of this biasing diminishes over time and that endogenous switches in attentional selection increase in probability as both targets remain on continuous trajectories (Lancer et al., 2019). Here we have shown that non-selected targets generate facilitation and that switching results in a suppressive Inhibition-Of-Return (IOR), allowing the system to identify and reliably switch to novel targets that appear during ongoing tracking. Such IOR has not, to our knowledge, been previously described from an invertebrate, but is an important component of computational models of winner-take-all visual attention (Itti and Koch, 2001) that has been extensively studied in primate behaviour and neurophysiology (Klein, 2000; MacInnes et al., 2014).

The described neurophysiology is consistent with dragonfly behaviour in predator-prey interactions. Dragonflies are broadly generalist predators who forage on a variety of prey (Baird and May, 1997; May and Baird, 2002; Combes et al., 2013), achieving high capture-success rates on preferred prey species (Olberg et al., 2000; Combes et al., 2013), even amidst high-density swarms (Combes et al., 2012). During target pursuits (predatory or conspecific) dragonflies can exhibit extremes of aerobatic performance (Combes et al., 2012, 2013; Bomphrey et al., 2016; Lohmann et al., 2019; Nakata et al., 2020; Rüppell and Hilfert-Rüppell, 2020), outcompeting the majority of their prey on basic measures of flight performance (Combes et al., 2013). However, 3D video analysis of flight trajectories suggests some prey actively manoeuvre, and are often able to evade dragonflies several times before the predator either captures them or gives up (Combes et al., 2012, 2013). Additionally, conspecific pursuits much longer in duration and involve significant weaving, turns, and role reversals (Lohmann et al., 2019). Pursuit success in these scenarios is significantly lower than when targets either cannot or do not manoeuvre (Combes et al., 2012, 2013). In addition to potential biomechanical limitations, reduced capture success for erratically moving targets may be related to the limits of neuronal facilitation in the STMD system. Spatial facilitation in CSTMD1 encodes targets moving on a consistent, straight trajectory and, although CSTMD1 is not direction selective, spatial facilitation establishes temporary direction selectivity (Wiederman et al., 2017). Thus, prey or conspecifics moving on erratic pathways are likely to repetitively slip outside of the spotlight that attentional facilitation has generated and into the suppressed surround, impairing the neuronal response and possibly contributing to reduced capture success (Combes et al., 2012). This so-called ‘protean movement’ behaviour pattern is exhibited by many prey species (Humphries and Driver, 1970; Jones et al., 2011; Richardson et al., 2018) is displayed across taxa (Bilecenoğlu, 2005; Briffa,

2013; Eifler and Eifler, 2014; Herbert-Read et al., 2017), including in insects (Humphries and Driver, 1970; Yager et al., 1990; Domenici et al., 2008; Combes et al., 2012; Hügel and Goerlitz, 2019), with some species even exhibiting protean movement pre-emptively (Humphries and Driver, 1970; Combes et al., 2012) without knowledge of a nearby predator ('protean Insurance'). Intriguingly, 'protean' behaviour appears to be a successful anti-predator defence even if the movements are technically predictable (Richardson et al., 2018), such as the spiralling take-off observed in *chironomid* midges (Humphries and Driver, 1970), so long as the prey avoids a straight trajectory that is ideal for generating neuronal facilitation. The effect of protean movement on target tracking in CSTMD1 is currently under investigation.

The dragonfly target tracking system has balanced two mutually opposing demands of attention: ignoring distractions, and flexibly responding to novel stimuli, by utilizing Predictive Gain Modulation as a gate-keeping mechanism. We have shown that when a pair of targets is presented in the excitatory receptive field, both targets generate a spotlight of facilitatory gain enhancement as they move along a linear trajectory, despite only one being attentionally selected for active representation in the spiking response of CSTMD1. This implies that target facilitation computations occur upstream of target selection in the STMD network. In addition to potential roles for ensuring robust responses in dynamically varying environments (Fabian et al., 2019) and driving target trajectory predictions (Wiederman et al., 2017), we suggest that facilitation of non-selected targets represents a strategy for low-level, passive target tracking of potentially important stimuli without negatively influencing encoding of the single target ultimately selected for the direction of behaviour.

Methods

Experiment Preparation.

We recorded from 33 wild-caught male *Hemicordulia sp.* Dragonflies were immobilized to an articulating magnetic stand with a 1:1 wax-rosin mixture. The head was tilted forward to allow access to the back of the head capsule and a small hole dissected in the chitin over the lobula complex of the left optic lobe. We pulled aluminosilicate electrodes (Harvard Apparatus) using a Sutter Instruments P-97 and filled them with a 2M KCL solution. Electrodes were inserted into the brain with the perineuronal sheath in-tact (aside from the puncture zone) using a piezo-electric stepper for a typical resistance of 40-140 MΩ. Intracellular responses were digitized at 10 kHz for offline analysis.

Experimental Design and Visual Stimuli.

Visual stimuli were presented on a high-definition LCD monitor (refresh rate: 165 Hz) using a custom-built presentation and data acquisition suite based on MATLAB (RRID: SCR_001622) and

Psychtoolbox (RRID: SCR_002881. Available at: www.psychtoolbox.org). The animal was placed 20 cm from the monitor and centred on the visual midline in order to minimize off-axis artefacts.

Depending on the trial condition, either single or paired targets (1.5° by 1.5° squares) were presented at 12° or 50° horizontal separation ascending the visual display at 50°/s. Within CSTMD1's excitatory receptive field, we define Target-1 (T_1) as nearer to the midline and Target-2 (T_2) as more peripheral. When presented in each visual hemifield (25° equidistant from the visual midline), we refer to Target-Inhibitory (T_i) and Target-Excitatory (T_e), with respect to the excitatory and inhibitory regions of the receptive field. In experiments testing facilitation, target trajectories were vertically divided into 3 sections: 'Probe', 'Primer', and 'Cue,' leading to 6 possible trajectory segments; 3 vertical locations on 2 contiguous trajectories. For selective attention experiments not addressing facilitation, we refer more simply to a 'Target' on a described trajectory (Bolzon et al., 2009; Wiederman and O'Carroll, 2013a). To limit habituation, experimental trials were randomly interleaved with at least a 12 s rest period.

To measure the degree of facilitation, a 200 ms Probe target (Weber contrast = 1) was presented either alone (Probe Alone control conditions) or following one Primer target. For Probes presented within the excitatory receptive field, we counted spikes within a 100 ms window, 50 ms offset from the Probe onset to account for response delays.

To generate facilitation of the Probe target, Primer targets were presented for 400 ms. Primer targets were either; absent for a Probe Alone, no-facilitation condition; matched to the Probe trajectory in the 'Local Primer' condition; on a horizontally offset ('unmatched') trajectory in the 'Distant Primer' condition; or presented simultaneously at both matched and unmatched trajectories in a 'Paired Primers' condition. On a subset of trials (50%) we introduced an attentional cue (400 ms), which biased selection towards either the T_1 or T_2 Primer (Lancer et al., 2019). In all trials, selection was determined by a Selectivity metric (see below), not assumed on the basis of cue locations.

Analysis of Selective Attention.

To allow identification of which Primer was selected during paired trials, we used frequency-tagging as previously described (Lancer et al., 2019). Briefly, each target's contrast was modulated (0.22 to 1) at a unique frequency (11, 15 Hz) with a square waveform, resulting in frequency-locked responses. This allowed us to read-out the selected Primer on ~70% of Paired Primer trials. On the remaining 30% of trials, frequency-tagging did not elicit sufficient modulation, likely due to neuronal habituation or saturation. These trials where selection could not be identified were excluded from further analysis. We used continuous Wavelet Transforms (Analytic Morse wavelet, gamma = 3) to extract pseudo-frequency information from the Inverse interspike interval (ISI), which represents the instantaneous spike rate of the neuronal response (Lancer et al., 2019). At frequencies corresponding

to T_1 and T_2 target contrast modulation, we average wavelet output across time to yield a measure of responsiveness to each frequency ($T1_r$, $T2_r$). Selectivity was defined as:

$$Selectivity\ (S) = {T1_r^2}\Big/{\sqrt{T1_r^2 + T2_r^2} - \sqrt{T1_r^2 - T2_r^2}}$$

Yielding a single-value metric ranging between +1 (selection of T_1) and -1 (selection of T_2), where ~0 would be indicative of a switch in attention during the trial.(Lancer et al., 2019) We applied a selectivity threshold of ± 0.3 to discard trials with unreliable target identification.

For a robust wavelet measure, we required at least 400 ms of continuous data. As CSTMD1 is known to 'switch' selected targets in less than 400 ms (Wiederman and O'Carroll, 2013a; Lancer et al., 2019) and that biasing effects diminish over time (Lancer et al., 2019), we additionally weighted Selectivity closer to the Probe onset.

$$Weighted\ Selectivity = \sum_t S_t * 2 * t/n$$

Where t = time point and n = total time points.

Statistical Analysis.

As selective attention is evident on a trial-by-trial basis, any given trial is independent and averaging across the trials (technical replicates) would mask the observation. To ensure statistical robustness, we repeated the experiment across several dragonflies. We use 'n' to denote the number of trials and additionally report the number of dragonflies.

All data analysis was conducted in MATLAB R2019a (RRID: SCR_001622), including the Wavelet Toolbox. We report exact P except when < 0.001. For all datasets, statistical outliers > 5 * the standard deviation have been removed (3 trials total). We additionally report Hedge's g [95% confidence interval] as a measure of effect size. Unless otherwise stated all tests are nonparametric (Mann-Whitney), two-tailed and corrected for multiple comparisons (Bonferroni-holm correction).

Acknowledgements

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Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Thesis Context Statement

There is an excellent literature on dragonfly behaviour available, and several recent studies have addressed the question of what exactly makes a stimulus attractive to a dragonfly (e.g., Olberg et al., 2005; Combes et al., 2013; Duong et al., 2017, and; Lin and Leonardo, 2017). This question had been addressed behaviourally, usually by presenting a series of targets to an unrestrained, perched dragonfly either in the field or lab environment and observing which targets elicit take-off and pursuit. However, little of this had been done from the neural side – and if CSTMD1 is indeed involved in a target-tracking system to guide pursuit as had been tacitly assumed, then the behavioural choices observed by dragonflies should be reflected in CSTMD1's selection between targets. On the one hand it seems obvious, but on the other hand it seemed an excellent opportunity to validate claims about CSTMD1's (presumed) relevance to pursuits. We found that selection preferences for CSTMD1 presented with paired, rival targets was broadly matched to the tuning profile of the neuron responding to one target. That is, as long as targets remained within CSTMD1's normal tuning range, there was little preference between them – similar to behavioural results, where as long as a target is within a broad range for pursuit initiation, it is highly likely the target will be pursued.

In particular, I was interested in determining if cases of failed behavioural pursuits could (in part) be attributed to the limitations of neuronal target tracking in CSTMD1. Does CSTMD1 have difficulty tracking the same kinds of targets behaving dragonflies have difficulty pursuing? Many insects engage in evasive manoeuvres while being pursed and evidence across labs has shown that pursuing dragonflies have more trouble with prey that behaves evasively than those that don't (as do primates, and humans). Is this difficulty purely due to flight biomechanics, or is an 'evasive' flight path more difficult to neuronally represent and track? Although this manuscript does not completely address the question (much less causally answer it), we found that CSTMD1 does indeed perform worse when targets act evasively and repetitively 'slip-out of the facilitation hotspot,' showing fundamental limitations in neuronal processing and representation could be a limiting factor in behaviour.

Overall the intent of this manuscript was to begin explicit bridge-building between neurophysiological and behavioural literature in dragonfly predation, but there is still a long way to go. Nonetheless we have built on the existing selection attention literature by probing the exogenous stimulus properties that drive selection in this model system, and seen how limitations in target-tracking ability influence selection.

Introduction

Successful behaviour requires selective allocation of attention according to behavioural goals and environmental demands, but deciding what to pay attention to at any given moment is a non-trivial task. In order to support such decision making, many systems rely on rule-like heuristics to drive selection between multiple alternatives, where some stimuli are granted innate 'salience' to make them attractive targets for attentional allocation. For example: colour, motion, and size all reliably capture attention for humans (Wolfe and Horowitz, 2004). These innate saliences represent heuristics that allow attention to be quickly and efficiently guided to stimuli most likely to be ethologically relevant to the animal, and usually highlight properties of relevance to an animal's behaviour and survival.

Although focussed on one target, behavioural studies in dragonflies have investigated the heuristics used when making a go/no-go pursuit decision. Work across labs and species has found that a target's angular size and speed are the import important factors for a dragonfly's' decision to initiate pursuit from a perch (Olberg et al., 2005; Combes et al., 2013; Duong et al., 2017; Lin and Leonardo, 2017). In contrast to size and velocity, colour has not been found to influence pursuit decisions in dragonflies (Rashed et al., 2005; Duong et al., 2017), but does play an important role for damselflies (Schröder et al., 2018). Following pursuit initiation, dragonflies fixate prey in the dorsal acute zone of their compound eye, (positioning themselves behind and below the target) (Olberg et al., 2007; Mischiati et al., 2015), fixating the prey at a variable distance where it makes up roughly 1 degree of visual space (Horridge, 1978; Lin and Leonardo, 2017).

Are these behavioural preferences reflected in the dragonfly's visual neurophysiology? We have identified an efferent visual neuron in the dragonfly (*Hemicordulia sp.*) Optic Lobe and midbrain (Geurten et al., 2007) thought to be involved in target pursuit (Lancer et al., 2020), named Centrifugal Small-Target Motion Detector 1 (CSTMD1). CSTMD1 is tuned for the movement of small (1-3° angular size) targets (O'Carroll, 1993; Geurten et al., 2007), matching the properties of targets likely to give rise to a behavioural pursuit (Labhart and Nilsson, 1995; Combes et al., 2013; Lin and Leonardo, 2017). CSTMD1 also exhibits winner-takes-all selective attention when presented with a pair of equal targets (Wiederman and O'Carroll, 2013), including the ability to 'lock on' to a selected stimulus and ignore attentional capture by an abrupt-onset distractor, even if the initial target was weak (Lancer et al., 2019), as well as the ability to respond robustly to targets embedded in highly cluttered environments (Wiederman and O'Carroll, 2011; Evans et al., 2020).

However, it is currently unknown what target properties drive selection in CSTMD1. Here, we use a 'Two-Target Choice' paradigm to probe CSTMD1's target selection preferences when presented with paired targets that vary along one parameter (Contrast, size, velocity, direction of motion, and path

complexity). We find that selection preferences broadly match established neurophysiology and behavioural results seen in other species.

Methods

Animals and Preparation

We recorded from a total of 41 wild caught *Hemicordulia sp.* dragonflies. Dragonflies were stored in a dark fridge at 7°C for up to 10 days before experimentation. For the experiment, dragonflies were allowed to warm to room temperature (Ambient temperature during preparation & experiment = 26°C) and were restrained to an articulating magnetic stand using a 1:1 wax-rosin mixture using a heated dental probe (~85°C). A small (1^2 mm) piece of the posterior head capsule was removed to provide visual and physical access to the brain. We inserted an aluminosilicate probe filled with a 2m KCL salt solution into the brain using a piezo-electric stepper.

Visual Stimuli

Visual stimuli were presented on a high-definition LCD monitor (Refresh Rate: 165 Hz) using a custom software suite based in MATLAB and Psychtoolbox). The animal was placed 20 cm from the monitor and cantered on the visual midline in order to minimise off-axis artefacts.

Two-Target Choice Experimental Paradigm

To probe selection choices in CSTMD1's response, we presented paired moving targets differing in parameters across multiple trials in order to determine which parameters attracted CSTMD1's selection at higher proportions. Each pair consisted of a designated 'Target' which had fixed parameters (frequency tagged at 15hz, weber contrast 0.22 – 1; moving at 50°/s; 1.5°x1.5° size) paired with a 'distractor' that varied in one parameter (Contrast, size, velocity. Direction of motion) at a time. The spatial locations (whether the 'target' was on the left and 'distractor' on the right or vice versa) was randomized between trials. Additionally, in a 'Zigzag' experiment, we paired a standard 'Target' (as above) with a 'Distractor' that moved on a complex 'Zigzag' pattern with variable angle and frequency.

Data Analysis

Data was saved to the data acquisition computer at 10 kHz for offline analysis in MATLAB, including the Wavelet Toolbox. As selective attention is evident on a trial-by-trial basis and the same stimulus can result in selection of a different target on a probabilistic basis, each trial is considered an independent observation. To ensure statistical robustness we repeated experiment across several dragonflies. We report exact P except when < 0.001, and report both the number of individual trials as well as the number of dragonflies.

Results

CSTMD1 selection prefers dark targets.

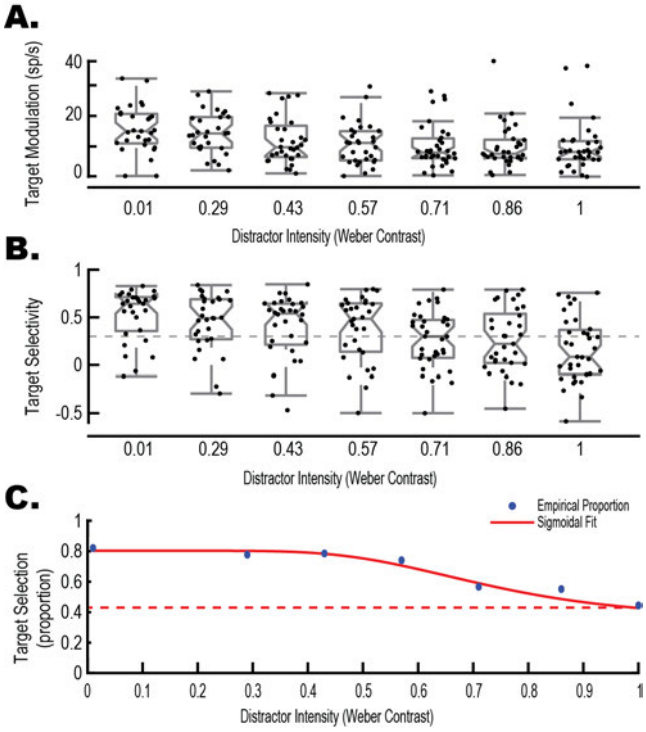

Figure 35: CSTMD1 prefers dark targets. A) *Modulation at the frequency of the frequency-tagged target during Two-Target Choice trials. As Distractor Intensity increases, average modulation at the target frequency decreases (although some individual trials of high modulation remain). n= 228 across 11 dragonflies.* ***B)*** *Target Selectivity of the same data in A. Individual trials with Selectivity > 0.3 are taken to have selected the 'target', whilst reduced selectivity indicates selection of the (non-frequency-tagged) distractor.* ***C)*** *Proportion of trials where the target was selected is decreased with increasing distractor intensity. Dashed line indicates the selection proportion at Distractor intensity = 1.*

We first tested CSTMD1's selection preference for targets of different contrasts. Although we have already shown that when a low-contrast target is primed, CSTMD1 can maintain selection of that low-contrast target in the presence of a high-contrast distractor (Lancer et al., 2019), we predicted that when both targets were presented simultaneously CSTMD1 would be biased towards the darker target. We presented paired targets in a 'Two Target Choice' paradigm consisting of one frequency tagged 'Target' (15 Hz, weber contrast = .22 – 1, μ = 51) and one distractor of variable contrast (weber contrast = 0.01, 0.29, 0.43, 0.57, 0.71, 0.86, 1). Data is presented in Figure 35. As the Distractor could not be frequency tagged, the presence of any frequency modulation during the Two-Target Choice presentation would indicate selection of the Target. We observed a reduction in average target modulation as Distractor Intensity increased (Figure 35A), indicating reduced selection of the Target at higher Distractor Intensities. We modified the Selectivity Index (Chapter 3: A Target-Detecting Visual Neuron in the Dragonfly Locks on to Selectively Attended Targets) to compare the Target modulation frequency against a 'noise' comparison frequency (11Hz) in order to determine Selectivity for each trial (Figure 35B). We applied a Selectivity threshold of 0.3 in line with previous experiments (Chapter 3: A Target-Detecting Visual Neuron in the Dragonfly Locks on to Selectively Attended Targets), and found reduced selection of the Target with increasing Distractor intensity (*ANOVA; df = 6, F = 5.32, p > 0.001*), indicating increased selection preference for the Distractor with increasing Distractor intensity. We then calculated the proportion of trials in which the Target was selected of all trials in a condition (Figure 35C). We found that even with Distractor Intensity = 0.01, the proportion of trials in which the Target was selected was only 0.81. However, frequency-tagging is known to be effective on only ~75% of trials so we would expect an approximately

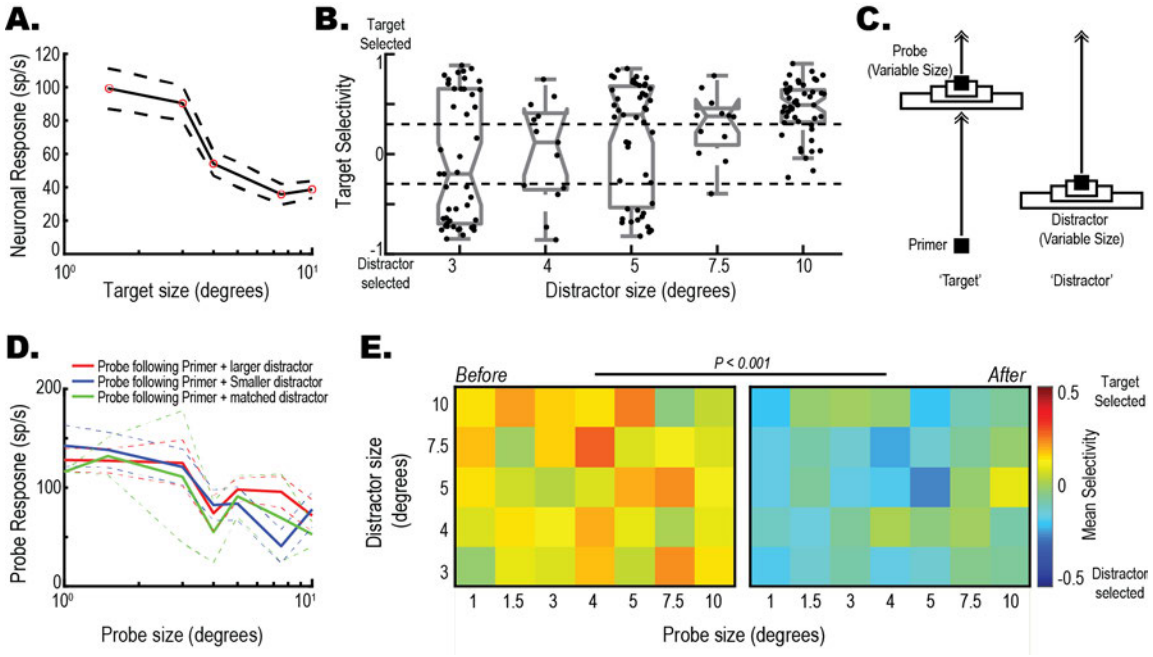

Figure 36: CSTMD1 prefers small targets. A) *5-point size tuning for CSTMD1 (n = 134 trials across 16 dragonflies). CSTMD1 responds best to small (1.5 – 3°) targets with reduced response to targets > 3 degrees.* ***B)*** *Two-Target Choice experiment, n = 117 trials across 16 dragonflies. CSTMD1 exhibits no selection preference between the Target (1.5x1.5°) and a distractor within CSTMD1s usual response range at 3°, but consistently prefers the target as the distractor size increases. intriguingly, CSTMD1 still sometimes selects the 5° despite it being outside CSTMD1s normal tuning range (A).* ***C)*** *Stimulus pictogram for the 'Size Change' experiment. A primer target is presented for 700 ms, during which a distractor of variable size appears (t=200). At t=700 the primer target is replaced with a probe that is also of variable size.* ***D)*** *Size tuning curves for the response of the Probe target in C. n = 139 trials across 16 dragonflies. We observed no significant differences between curves based on the size of the distractors. All three curves are shifted upwards compared to A due to the facilitatory effects of the primer.* ***E)*** *Selection before and after the size change. N = 407 trials across 16 dragonflies. Each square represents the mean Selectivity for trials of that condition. Before the size change, the target was overall more likely to be selected as evidenced by predominance of warmer colours. However, following the size change attention was likely to switch to the Distractor as evidenced by the overall cooler colours.*

25% false negative rate for identifying target selection. Although it is impossible in principle to determine the difference between a false negative for Target Selection and genuine Distractor Selection, the false negative rate is not expected to change with distractor intensity. We observed decreased proportion of Target selection with increased distractor Intensity, especially at distractor intensities > 0.71, higher than the mean contrast of the frequency-tagged target (μ_{weber} = 51). We therefore show that CSTMD1 prefers to select darker higher contrast dark targets.

CSTMD1 shows no selection preference between targets within its size range.

Target angular size has been shown to be important for a dragonfly's decision to initiate a behavioural pursuit (Olberg et al., 2005; Combes et al., 2013; Duong et al., 2017; Lin and Leonardo, 2017). We tested CSTMD1's selection preference for paired, rival targets of different sizes (Figure 36). CSTMD1 responded robustly to targets < 5° (Figure 36A) and was able to flexibly select distractors < 5° when paired with a standard (1.5°) target. We observed an overall increase in Target Selection as Distractor size increased (*ANOVA; df = 4, F = 6.33, p > 0.001*). While CSTMD1 showed no size preference in the 3° and 5° distractor size conditions, the smaller 1.5° was slightly preferred in the 5°

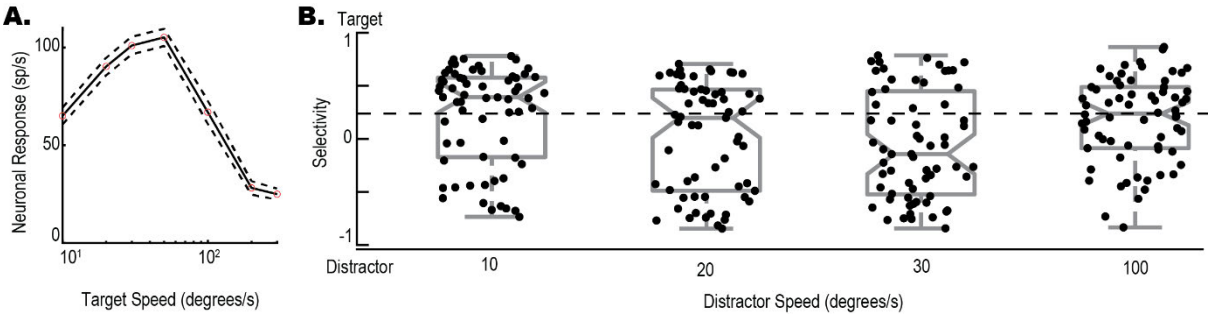

Figure 37: CSTMD1 prefers fast targets. A) *7-point Velocity Tuning curve for CSTMD1. n = 333 trials across 11 dragonflies.* ***B)*** *Two-Target Choice experiment, n = 264 across 11 dragonflies. CSTMD1 exhibits a selection preference for the Target (50°/s) when paired with an extremely slow (10°/s) or fast (100°/s) target, but not for targets within the middle of the range.*

distractor size condition, and highly preferred at higher (7.5° & 10°) distractor sizes (Figure 36B). These data suggest CSTMD1's selection shows no selection preference between small targets already within CSTMD1's size tuning range (1-3°), but stimuli outside of this range are ignored as potential targets.

In primates, attentional selection has been shown to alter neuronal tuning curves (Spitzer et al., 1988; Martinez-Trujillo and Treue, 2004; David et al., 2008). In a second set of experiments (Figure 36C), we tested this for the dragonfly by adding an additional Probe Target of variable size to the end of our original target, which was started earlier in order to capture attention (Lancer et al., 2019). For trials where the Target was selected, we analysed size response tuning curves across conditions where the distractor was larger, smaller, or matched to the Primer target size. We expected that the size tuning curve would be shifted away from the distractor – i.e., the size tuning curve would be shifted leftwards (towards smaller sizes) in the presence of a larger distractor, and rightwards (towards larger sizes) in the presence of a smaller distractor. However, we observed no differences between size tuning curves across all conditions (Figure 36D).

We additionally sought to determine if a change in size would trigger a switch in attention, using a variant of the experiment presented in Figure 36C where the Distractor continued to the end of the trial (Figure 36E). We analysed Selectivity over 400 ms immediately before (Figure 36E left) and after (Figure 36E right) the Target size change. We observed a significant difference between Mean Selectivity before and after the size change ($P > 0.001$), indicating that changing the size of the actively tracked Target triggered a switch in attention towards the distractor.

CSTMD1 shows no selection preference between targets within its velocity range.

Target angular velocity has also been shown to be important for a dragonfly's decision to initiate a behavioural pursuit (Olberg et al., 2005; Combes et al., 2013; Duong et al., 2017; Lin and Leonardo, 2017). We sought to test this in CSTMD1 by presenting paired, rival stimuli at different velocities including one 'target' moving at 50°/s and one distractor, moving at 10, 20, 30, or 100°/s (Figure 37). We found an overall significant effect of distractor velocity on selectivity (*ANOVA; df = 3, F = 5.28, p = 0.001*). CSTMD1 preferentially selected the target moving at 50°/s when paired with an

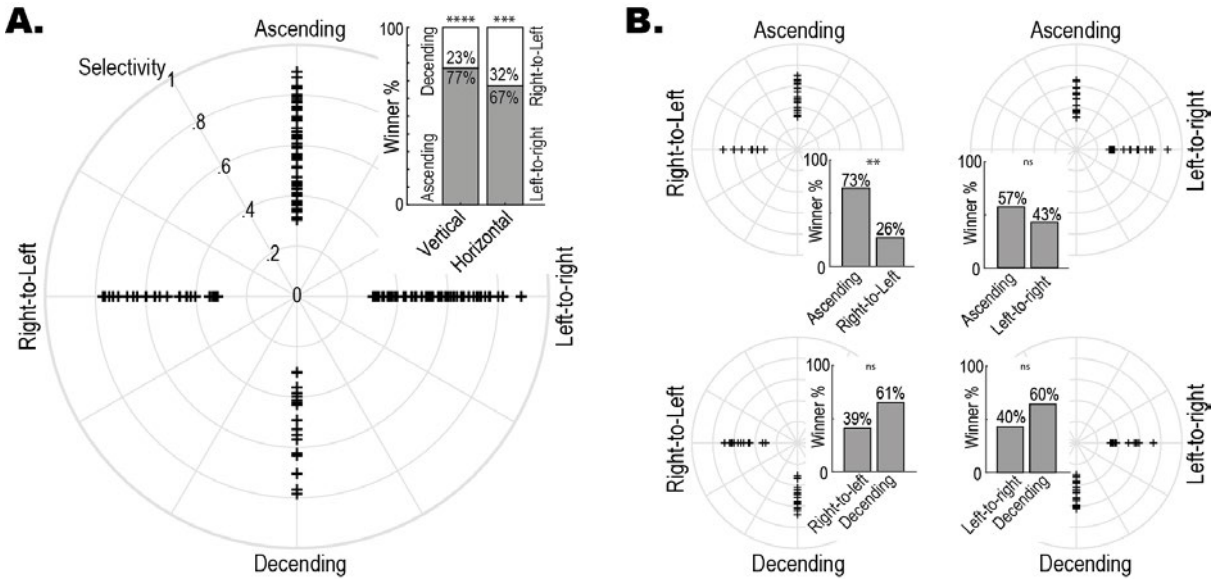

Figure 38: CSTMD1 prefers targets moving upwards and to the right. A) *Two-target choice experiment where two rival targets 'jousted' either horizontally or vertically. Ascending targets were preferenced against descending targets on vertical joust trials, while targets moving rightwards (left-to-right) were preferenced on horizontal joust trials. n = 174 trials across 10 dragonflies.* ***B)*** *Variant two-target choice experiment where two rival targets moved orthogonally. From top left clockwise: Ascending vs right-to-left; ascending vs. left-to-right; descending vs left-to-right, and; descending vs right to left. Of these, the only significant selection preference observed was for the ascending target paired against a rival right-to-left target. n = 131 trials across 7 dragonflies.*

extremely slow distractor (10°/s) or extremely fast (100°/s), although in these scenarios CSTMD1 still selectively responded to the slower and faster targets on some trials. However, CSTMD1 showed no significant selection bias when the target was paired with a 20 or 30°/s distractor, indicating that, as with size, there is limited selection preference between targets already within CSTMD1's tuning response range.

CSTMD1 prefers targets moving upwards and to the right.

CSTMD1 responds robustly to targets moving within the receptive field regardless of the direction of motion, although with a slight increase in firing rate for targets moving upwards and to the right (I.e. laterally, away from the midline) (Wiederman et al., 2017). To test for any direction preference in target selection, we presented paired, rival targets moving in different directions in a two-target choice experiment (Figure 38). We first tested targets 'jousting' in opposite directions, in either a 'Vertical' condition (One target ascending, one target descending) or a 'Horizontal' condition (one target moving left-to-right, one target moving right-to-left). Targets were frequency tagged at unique frequencies (11, 15Hz) but otherwise identical (weber contrast 0.22 – 1; moving at 50°/s; 1.5°x1.5° size). All targets remained within the excitatory receptive field for the duration of the experiment. We report the binomial probabilities.

We found that CSTMD1 significantly preferred the ascending target ($p < 0.001$) in the 'vertical' joust condition and the left-to-right moving target ($p < 0.001$) in the 'horizontal' joust condition (Figure 38A). It is important to note that the 'left-to-right' moving target in this experiment began at the visual

midline and moved peripherally, so this preference may reflect a preference for 'peripherally' moving targets rather than 'rightward' moving targets, and without recording from the contralateral CSTMD1 and the opposite visual hemifield it is impossible to disambiguate this.

We next presented these targets moving orthogonally to each other for a total of four possible combinations (Figure 38B). Here, we found CSTMD1 significantly preferenced the ascending target *(p = 0.005)* when it was paired with a target moving right-to-left (i.e. towards the midline), however we did not observe any statistically significant preferences across any other conditions (ascending vs. left to right *p = 0.095*; descending vs. right-to-left *p = 0.065*, descending vs. left-to-right = 0.067).

Overall, these results indicate a strong selection preference for vertically ascending targets, as well as a preference for targets moving left-to-right from the midline towards the peripheries.

'Zig-Zag' movement disrupts Neuronal Encoding of target trajectory in CSTMD1.

Pursuits are rarely conducted in a straight line. In conspecific engagements, dragonflies actively employ an underdamped pursuit strategy that involves rapidly alternating from side to side around the target (Lohmann et al., 2019), and during predatory pursuits many prey species actively manoeuvre during flight, reducing dragonflies' capture success probability (Combes et al., 2012, 2013). The spatial facilitation observed in CSTMD1 enhances targets moving on a consistent, straight trajectory and establishes temporary direction selectivity (Dunbier et al., 2012). Thus, prey moving on erratic pathways are likely to repetitively slip outside of the facilitatory spotlight their neuronal representation has generated and into the suppressed surround, impairing the neuronal response and possibly contributing to reduced capture success (Combes et al., 2012).

To test this, we presented targets on a 'Zig-Zag' trajectory where targets ascended the receptive field by alternating diagonal movements (Figure 39). We varied two properties of the 'Zig-Zag trajectory,' the 'angle,' denoted θ, at which the target changed directions during the Zig-Zag trajectory, and the distance between direction changes, denoted *d* (Figure 39A). Intriguingly, we found that the neuronal response to a zig-zagging target was *increased* compared to a straight target control for 45° (*p < 0.001*) and 90° (*p < 0.001*) angles, but *decreased* for an angle of 135° (*p < 0.001*) as the target moved outside of the predictive facilitation region (Figure 39C).

In order to test if CSTMD1 is able to 'lock on' to a zigzagging stimulus, on a subset of trials we introduced a novel target ascending a straight trajectory (weber contrast 0.22 – 1; moving at 50°/s; 1.5°x1.5° size), which began 200 ms following onset of the Zigzag Distractor. We found that increased Zigzag Angle (θ) was associated with increased Selection of the Straight-trajectory target across all Distances (*d*), but the effect was more pronounced at shorter distances (Figure 39C). We found the

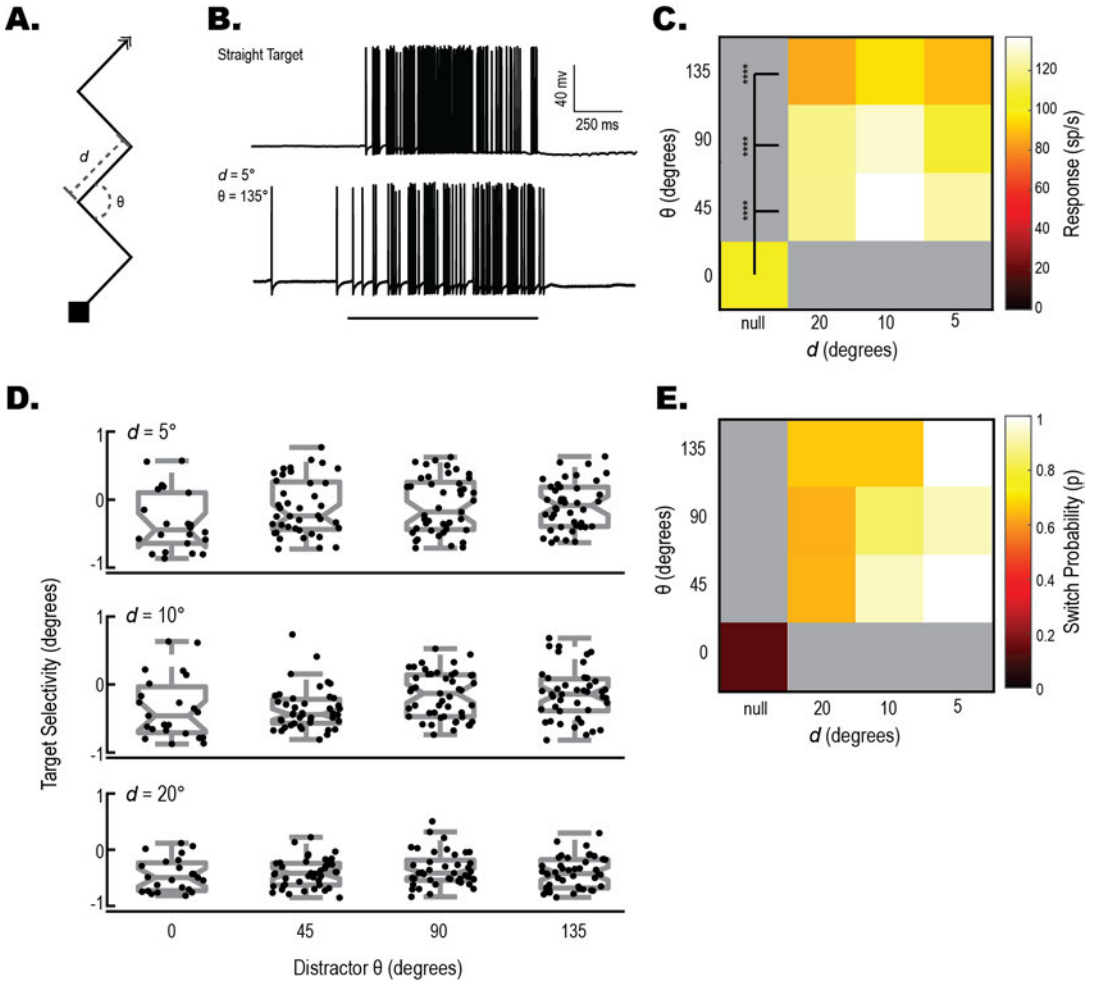

Figure 39: Zigzag movement disrupts neural encoding of target trajectory. A) *An example zigzag trajectory. Theta (ϑ) denotes the angle at which the zigzag trajectory changes. Distance (d) denotes the distance in visual degrees between zigzag changes.* ***B)*** *Example spike-train responses to an ascending straight target (top) and a target on a zigzag trajectory (bottom; d = 5°, ϑ = 135°).* ***C)*** *Mean neuronal response to a single target across zigzag conditions. n = 649 trials across 16 dragonflies.* ***D)*** *Two-target choice experiment with a rival straight and primed zigzag distractor. n = 522 trials across 16 dragonflies.* ***E)*** *Switch Probability across zigzag conditions.*

probability of switching to the linear target was greatly increased with decreased Zigzag distance (Figure 39C). Overall, these results show complex Zigzagging trajectories disrupt neuronal encoding in CSTMD1, and CSTMD1 prefers to track linearly moving targets.

Discussion

Overall, we observe selection preferences in CSTMD1 that broadly match established neurophysiology. CSTMD1 exhibits preferred selection for ascending, darker targets size and velocity matched to CSTMD1s response-tuning preferences. Despite this general trend, we observed individual examples where the overall non-preferred stimulus was selected on an individual trial, indicating that these selection preferences are largely a probabilistic bias rather than a hard boundary.

In the contrast domain, we observed a sigmoidal reversal from majority selection of the Target to majority selection of the Distractor when the Distractor Contrast became higher than the average

contrast of the frequency-tagged target, suggesting CSTMD1 preferentially selects darker targets, however we also observed individual trials of lighter target selection.

Behavioural experiments with both real and artificial prey have shown that dragonflies reliably initiate pursuit when target angular size and velocity are within a certain range (Lin and Leonardo, 2017), with little preference variability within that range. We have observed similar findings in CSTMD1, where for both size and velocity CSTMD1 showed a significant preference for the well-tuned target when paired with an out-of-tune Distractor, but no significant preferences when the target was paired with an in-tune Distractor. This suggests that the target selection system implements a broad assessment of target suitability, without fine-grained comparison of rival targets within the suitability range. Such a heuristic strategy could be beneficial when making rapid behavioural decisions. By limiting pursuit to targets within an angular size and velocity range (which can act as a proxy for distance estimation), the dragonfly can ensure it only pursues targets within a reachable distance within a desired time. One interesting, unresolved question in dragonfly behaviour is weather the presence of competitors reduces the distance at which a perching dragonfly will pursue play in order to avoid territory loss.

Intriguingly, we also observed that a sudden change in size of the tracked target was associated with increased probability of switching to the consistent distractor. This unexpected finding raises important questions for target pursuits, which in both territorial and predatory contexts involve rapid manoeuvring likely to alter stimulus properties (Lohmann et al., 2019). The effects of target parameter changes on both dragonfly pursuit performance and response characteristics of CSTMD1 and other dragonfly visual circuitry is an important direction for future study.

Behavioural research has shown that although dragonflies will pursue a target heading in any direction, perching dragonflies undertake a series of preparatory movements before take-off that align the dragonfly body axis with the prey's direction of flight (Lin and Leonardo, 2017). Once in aerial pursuit, dragonflies attempt to keep the prey at a fixed retinal location and maintain relative positions (Olberg et al., 2007; Mischiati et al., 2015). Due to the angular position of the head required for electrophysiological recording, a target 'ascending' the stimulus presentation monitor begins in the anterior surface of the eye (at the bottom of the monitor) and moves posteriorly (ascends to the top of the monitor) across the dorsal acute zone, where CSTMD1's receptive field is located. Thus, from the point of view of the dragonfly, an 'ascending' target appears to recede towards, above, and behind the dragonfly, a pattern of retinal stimulation consistent with a dragonfly approaching a target for capture.

We observed a significant preference for selecting such a target, when paired with a rival going in the opposite direction (Beginning behind the dragonfly and moving ahead). Additionally, we

observed a selection preference for horizontal targets moving away from the midline when paired with a rival moving towards the midline. These results match the weak direction selectivity observed in CSTMD1, where targets ascending of moving towards the peripheries elicited a slightly higher spike rate response than targets descending or moving towards the midline (Wiederman et al., 2017). Intriguingly, we previously observed a similar effect in spatial facilitation in CSTMD1 (Wiederman et al., 2017). CSTMD1's response to a target on a continuous trajectory is enhanced (Nordström et al., 2011; Dunbier et al., 2012), concomitant with surround suppression of the receptive field > ~15° away from the facilitated target (Wiederman et al., 2017). Such 'Predictive Gain Modulation' is thought to drive the neuron to saturation in order to limit the impact of stimulus variability during pursuit (Fabian et al., 2019), and plays a role in distractor filtering during rival stimulus presentations (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron. We have observed that this spatial facilitation occurs ahead of only targets either ascending or travelling rightward (peripherally, from the midline), matching our target preference results. Spatial facilitation may therefore provide a positive feedback loop that enhances the responses to targets travelling in these directions, making them more likely to win competitive interactions involved in selective attention.

Finally, we examined the effect of a 'zigzag' target trajectory on CSTMD1's selection and response. Unexpectedly, we observed that acute angular changes (Zigzag θ = 45°, 90°) elicited *stronger* overall responses than a target on a straight, consistent trajectory. Although a 45° and 90° change in direction is still within the expanded 'spotlight' of facilitatory gain enhancement (Wiederman et al., 2017), facilitation alone cannot account for these results as we expect the straight target to have elicited stronger facilitation (Wiederman et al., 2017). This implies an as-of-yet unidentified enhancement of targets moving on an acute-but-continuous trajectory. In contrast, we observed a significant decrease in neuronal response when the zigzag θ was 135°, on the edge of the gain enhancement spotlight (Wiederman et al., 2017). This suggests that an obtusely moving target may compromise the neurons response by repetitively moving outside the facilitation window and into the supressed surround, potentially accounting for reduced capture-success for erratically manoeuvring prey (Combes et al., 2012, 2013). How does this affect target selection? We observed increasing zigzag θ was associated with increased attentional switching towards a straight-trajectory target, especially for shorter inter-angle distances on the zigzag path. This indicates CSTMD1 has a strong selection preference for targets moving in a straight line, presumably representing prey that is easier to catch.

Overall, we have shown that target Selection in CSTMD1 is well-matched to behavioural target selection by dragonflies, and neurophysiological tuning properties of CSTMD1. By selecting a broad range of stimuli, the dragonfly target selection system ensures robust operation during complex

pursuits with rapidly changing visual input and supports highly efficient generalist predation. None-the-less, obtuse manoeuvring by targets appears able to disrupt optimal target tracking within the dragonfly visual system and may account for reduced capture success rates observed in such pursuits.

Chapter 6: Performance of a Dragonfly Target-Tracking Neuron in Swarm Conditions

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Contribution to the Paper	Experiment & methodology conceptualisation and design; animal collection; electrophysiology; data analysis & interpretation; figure generation; manuscript original draft, review, and editing.
Overall percentage (%)	75%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 11/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Methodology conceptualisation; software development; data interpretation; manuscript review; supervision; provision of resources; funding acquisition.
Signature	Date: 7/10/21

Thesis Context Statement

The ability to select between two targets is an exciting property, but as my co-supervisor David O'Carroll liked to point out, there is a big difference between 'two' and 'multiple.' Previously, all experiments on selection in CSTMD1 (including my own) had focused on pairs of rival targets so investigating the limits of selection was an obvious next step. Additionally, I was very intrigued by two behavioural papers showing dragonflies seemed to be robust against the confusion effect, a reduction in predatory success rates normally seen with increasing prey density (Jeschke and Tollrian, 2007; Combes et al., 2013). I was therefore very interested in assessing how CSTMD1 would respond to a 'swarm' of targets. The first step was to confirm CSTMD1 was able to select among >2 targets, which was achievable with the simple addition of a third frequency tagged target. However, it was clear this approach could not be scaled up very well – adding a third frequency tagged target reduced our overall ability to detect the frequency-tagged signal from ~70% to less then 35% of trials. Instead, we fell back on design that we had previously used in contrast-related experiments, where one 'target' was frequency tagged and identifiably by a signature in the frequency domain, but other targets were untagged. The broad interpretation here is that in the absence of a positive frequency tagging signature, we can assume attention was not directed at the frequency-tagged target. The biggest issue with this experimental design is that *a priori* it becomes increasingly unlikely the frequency-tagged target would be selected from a 'swarm,' so in order to bias selection towards the target we cued it in line with previous experiments.

We showed that in 'Swarm Alone' trials (with no tagged target), CSTMD1 was able to respond with spike rates 'consistent with' the selection of a target, but as density increased the overall response declined due to target-target interactions like lateral inhibition. With the introduction of a cued, tagged target we were able to show that CSTMD1 was able to reliably 'lock-on' and track the tagged target despite the appearance of the swarm.

Many predators overcome swarming prey by targeting 'odd' individuals which are easier to track and pursue (Perhaps due to odd colouration or spatial isolation). I therefore wondered if an 'odd' target would 'pop-out' from a swarm similar to 'pop-out' attention observed in humans. Surprisingly however, 'odd' targets were almost completely ignored, suggesting oddity itself is not a salient feature for the dragonfly target selection system.

The conceptualization of this set of experiments was my own, driven by my interest in answering questions about how CSTMD1 would behave under more challenging conditions likely to be experienced during real behaviour. However, I am indebted to both Bernard Evans and Steven

Wiederman for verifying my experimental plans and suggesting minor tweaks and changes, as well as to Bernard Evans for help creating the 'Swarm' stimulus.

Introduction

Prey species across taxa have evolved the tendency to congregate in large groups, herds among mammals, flocks among birds, schools among fish, and swarms among insects. Grouping behaviour is thought to confer protection against predation via several (non-exclusive) mechanisms (Miller, 1922), including the dilution effect whereby any individual is simply less likely to be targeted by a predator (Brighton et al., 2020), the vigilance effect whereby increased group size increases the chance of a predator being spotted (Treisman, 1975; Treherne and Foster, 1981), and; the confusion effect, a reduced capture-success rate experienced by predators hunting groups of prey (Jeschke and Tollrian, 2007; Schradin, 2019). Of these benefits, the confusion effect is the only mechanism that resolves from a property intrinsic to the predator themselves, namely, sensory information processing capacity. Some authors have argued that confusion can be attributed to the degradation of prey position information in dense-prey environments (Krakauer, 1995; Tosh et al., 2006; Ioannou et al., 2008), attributing confusion to the oversaturation of the information capacity of a predators sensory mapping rather than a breakdown in 'cognitive ' mechanisms (Tosh et al., 2006; Ruxton et al., 2007; Jones et al., 2011).

If confusion is the result of a perceptual bottleneck in low-level neuronal networks, attention is an alleviator rather than a drive of the confusion effect, and may have arisen as an evolutionary response to overcoming confusion (Krakauer, 1995; Tosh et al., 2006). Confusion is diminished when a predator is able to isolate individual prey, either due to a unique feature (Landeau and Terborgh, 1986), spatial exclusion (Milinski, 1977), or a predictable prey trajectory (Jones et al., 2011), processes which aid in attentional tracking. *In silico* evolutionary modelling of predators and prey found that predators evolved a more 'focused' visual system in response to prey swarming in order to limit distraction (Olson et al., 2016). This model altered a simulated predators' 'focus' by altering the view angle, but real animals may make use of neuronal processes such as selective attention to 'focus' sensory processing on one, or a subset, of stimuli whilst maintaining the broad optical architecture required for a wide field of view useful for prey discovery (Ioannou et al., 2009).

Dragonflies are aerial pursuit predators that specialise in hunting amidst swarms (Edman and Haeger, 1974; Corbet, 1999; May and Baird, 2002; Combes et al., 2012; Lancer et al., 2020). In one study, increased prey density actually increased the prey-capture success rate (Combes et al., 2012). Although this increase was attributed to reduced fight-space under swarm conditions restricting the evasive manoeuvrability of the prey (Combes et al., 2012), it is notable that the dragonfly was able to take advantage of such an effect without falling to the confusion effect.

Dragonflies possess a pair of large compound eyes with discrete 'zones' specialised for different tasks (Lancer et al., 2020). In the majority of species, the dorsal side of the compound eyes are 'fused' together to form a continuous, almost flat plane of high acuity vision in which targets are fixated during predatory flights (Olberg et al., 2000; Mischiati et al., 2015). These optical specialisations give dragonflies both high-acuity (relative to other compound eyes) vision in a focal 'foveal' area and a broad scope for prey detection.

We have identified a visual neuron in the dragonfly optic lobe and midbrain which is tuned for the detection of small moving targets (Geurten et al., 2007) and thought to be involved in target tracking for pursuit (Lancer et al., 2019). This visual neuron is named 'Centrifugal Small Target Motion Detector 1', or CSTMD1. CSTMD1 exhibits a 'winner-takes-all' like selective attention when presented with a pair of targets, where the neuronal response matches one of the paired rival targets as if that target were presented alone (Wiederman and O'Carroll, 2013a; Lancer et al., 2019). This selective attention is able to be biased by the presentation of a spatially preceding primer and even 'locks on' to a selected target in the face of an abruptly-appearing high contrast distractor (Lancer et al., 2019).

However, previous studied have only considered the presentation of two, paired, rival targets. Here, we address how CSTMD1 responds to >2 Targets using *in vivo* intracellular recording. We find that CSTMD1 is overall inhibited by the presence of high-density 'swarms' of targets but is still able to select and respond to an individual target amidst a swarm.

Methods

Animals and Preparation

We recorded from a total of 20 wild caught *Hemicordulia sp.* Experimental preparation was as in Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron.

Visual Stimuli

Visual stimuli were presented as in Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron.

Swarm Stimulus

To probe CSTMD1's response to 'swarm-like' stimuli, we presented a background of multiple hexagonally arranged moving targets ("Swarm Distractors") separated by *x* degrees (6, 12, 18, 24, 30; Figure 40A, top left). All Swarm Distractors were sized 1.5°x1.5°, moving at 50°/s (except in 'velocity' experiments) and ascending the visual field (except in 'direction' experiments.) In addition, in a subset of experiments we presented a single frequency tagged 'Target' (15Hz, weber contrast 0.22 – 1; 1.5°x1.5° size), which was primed for 200 ms prior to the Distractor Swarm appearance. In 'Oddity'

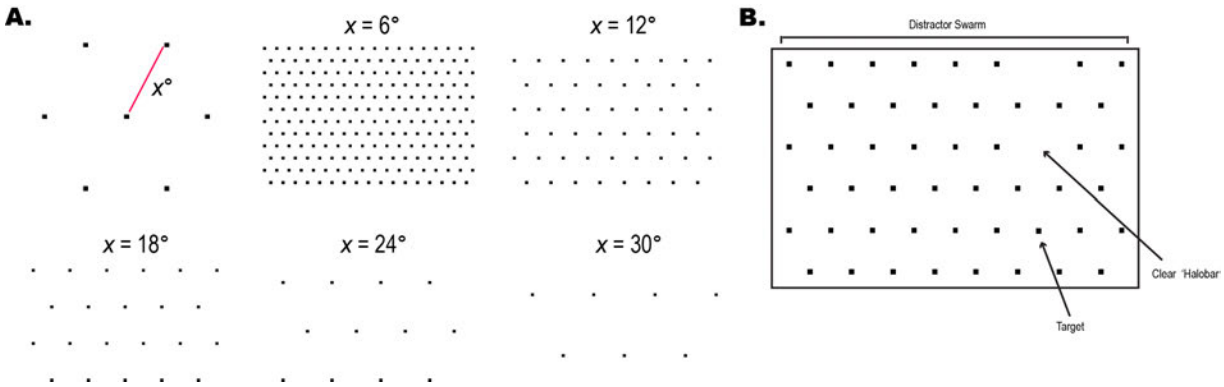

Figure 40: 'Swarm' Stimulus. A) *Pictograms illustrating the Swarm Distractor Field (not to scale on the page). Top Left: Swarm Separation was measured as the distance between each hexagonally arranged target.* ***B)*** *Illustration of the Target and Halobar within a swarm distractor field. The Target is prevented from moving directly behind or within 3° of another target to prevent simple stimulus-stimulus interactions.*

experiments, the Target was not primed but instead moved in either an 'odd' direction (ascending vs descending) or velocity (50°/s vs. 100°/s), as compared to the Distractor Swarm.

In order to ensure any effects were due to swarming and not attributable to local interactions around the target, trials that included a 'Target' also included a white 'Halobar' behind the target. The Halobar was a rectangular 'track' for the target to follow which was the same colour and intensity of the background that extended 6° in width and the full height of the monitor, which blocked out any competing distractor targets from being presented too close or on the path of the target, in order to prevent the target from moving directly behind or adjacent to a Swarm Distractor (Evans et al., 2020) and prevent local inhibition (Geurten et al., 2007)

Data Analysis

Data analysis as in Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron.

Results

CSTMD1 can select one target from a triplet.

The ability to select between multiple (> 2) targets is an important aspect of an attentional system, and a non-trivial extension of simple selection between paired targets (Mysore and Kothari, 2020). To test for selection between > 2 targets, we replicated our original selective attention experiment using three targets (T_1, T_2, T_3), each frequency-tagged at a unique frequency (11, 15, 33 Hz). Targets were presented with 12° horizontal spacing and ascended the receptive field at 50°/s. As expected, we observed strong frequency-modulation in CSTMD1's response matched to the unique tag frequency of one of the targets in a three-target presentation on a trial-by-trial basis, indicating CSTMD1 was able to select and respond to one of the three presented targets uniquely (Figure 41, red, blue, and green dots). Additionally, on a smaller number of trials we observed 'shared power'

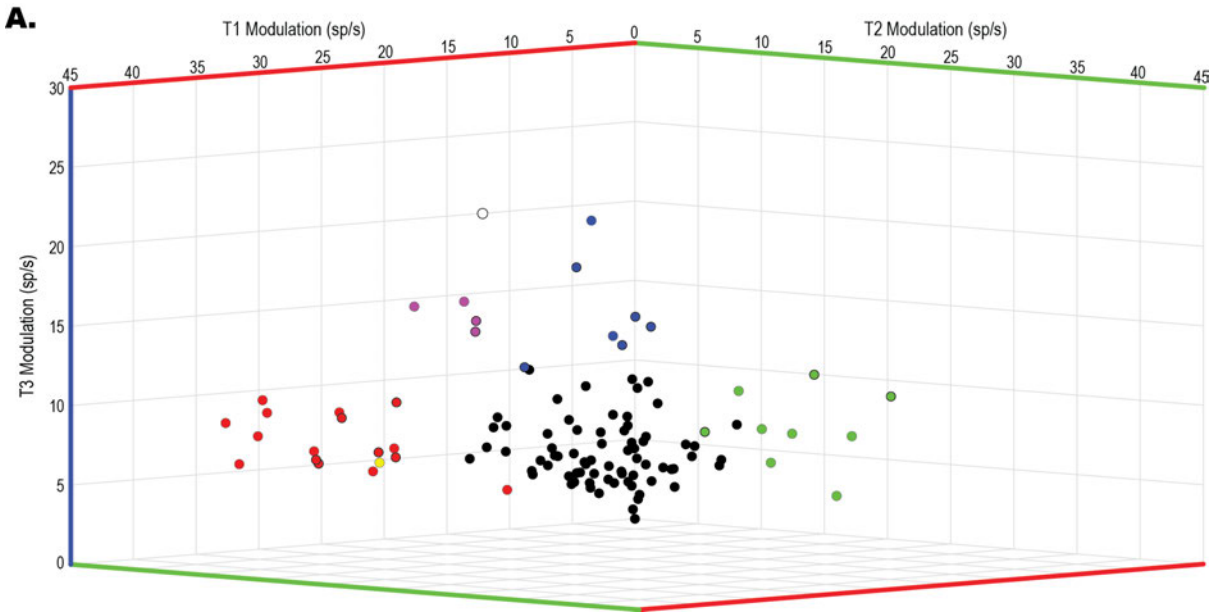

Figure 41: CSTMD1 attends one of three targets. *3D scatterplot illustrating Modulation at the frequency tag of each target in each 3-target trial (dots). 110 trials across 7 dragonflies. Each target was assigned a colour (T1 = Red, T2 = Green, T3 = Blue), which determined the RGB colour channel for each trial. For each trial, the RGB channel was set to 1 if the modulation at that channel was above a noise threshold and set to 0 otherwise. Thus, a red dot indicates the modulation at T1 was above the threshold, but modulation for neither T2 nor T3 was. A white dot indicates modulation for each of the three targets was above the noise threshold, and a black dot indicates the noise for each target was below the noise threshold. We observe individual trials of selection for all three targets T1, T2 and T3 as well as some switches (Purple, Yellow, and White dots). However, in the majority of trials (~65%) CSTMD1 failed to show a modulated response above noise for any target, despite a robust spiking response (<50 sp/s).*

between multiple targets (Figure 41, purple, yellow, and white dots), consistent with a switch in attention during the trial (Lancer et al., 2019). However, the frequency tagging technique failed to generate a significant frequency-locked response on over half of the presented trials (65.45%; Figure 41, black dots). In earlier uses of frequency tagging with only two targets at a time we observed a failure rate of 25-30% (Lancer et al., 2019), due possibly to neuronal habituation or saturation resulting in 'clipping' of the modulated signal. Here, we observe that adding a third target with a unique frequency results in increased failures and reduces overall modulation (Compare sp/s modulation in Figure 41, capping at ~30 sp/s to Figure 23B, capping at ~100 sp/s).

Although these results show CSTMD1 is able select a single target from a presented triplet, it is clear that additional frequency-tagged targets reduce the efficacy of frequency-tagging as an identification technique.

CSTMD1 Selects and Responds to individual targets within swarm-like stimuli.

To test CSTMD1's response to 'swarm-like' conditions involving many targets, we presented a 'swarm' stimulus made up of a repetitive hexagonal array of targets variably spaced apart (Figure 40). As CSTMD1 has a two-component receptive field (RF) with an excitatory half on one side of the midline (contralateral to our recording in the axon) and an inhibitory half on the other side of the midline, we presented two variants of the swarm stimulus (Figure 42A); one variant with swarming targets

exclusively in the excitatory receptive field (approx. 50°*50°), and one variant with swarming targets across the entire receptive field (approx. 50*100°).

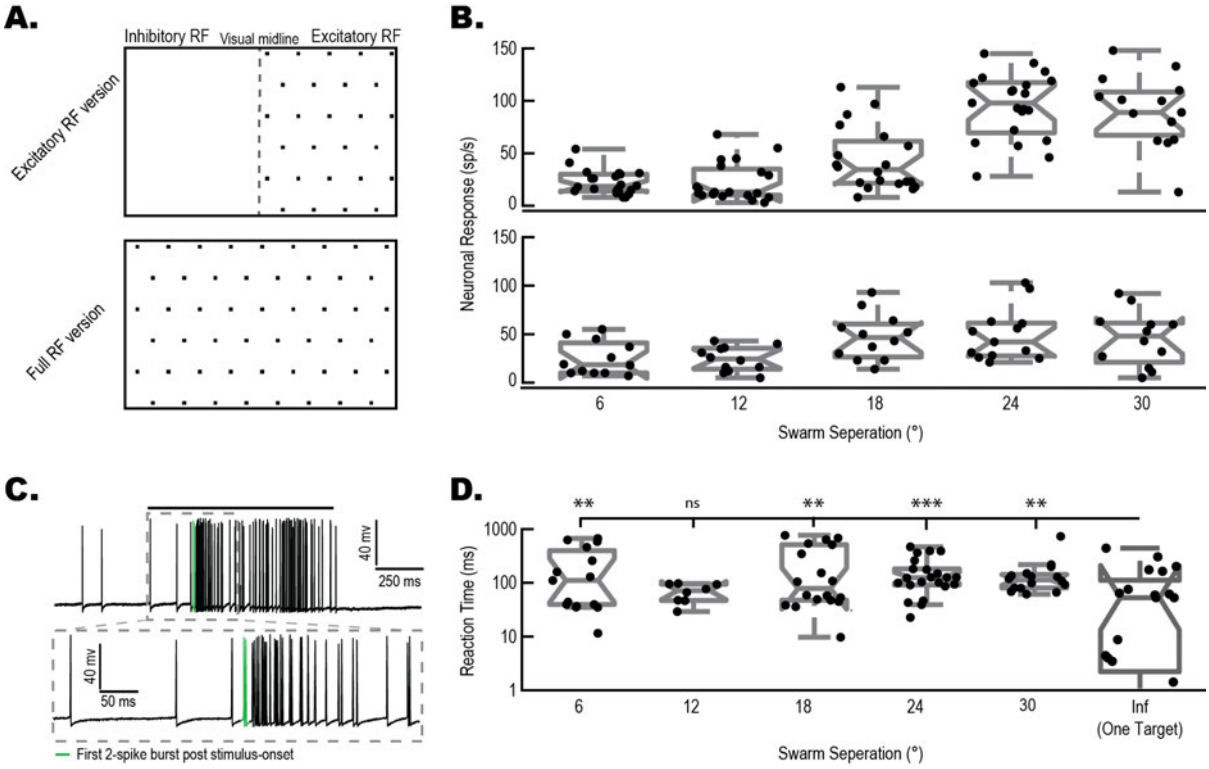

Figure 42: CSTMD1 responds robustly to swarm-like stimuli. A) *Stimulus pictograms illustrating swarm stimuli (not to scale) in the excitatory receptive field (top) and across the full receptive field (bottom).* ***B)*** *Neuronal responses to presentations of different Swarm Separations. Top: Swarm presented in the excitatory receptive field only. n = 98 total trials across 11 dragonflies. Bottom: Swarm in both the excitatory and inhibitory receptive fields. n = 61 total trials across 7 dragonflies. We observe individual examples of 'target-like responses' (>50 sp/s) in each swarm separation condition, suggesting that CSTMD1 is able to select and respond to a target within a swarm. However, we observe an overall decrease in response with reduced swarm separation.* ***C)*** *Top: example spike train response to an excitatory swarming stimulus. To measure 'reaction time we calculated the time between stimulus onset and the first two-spike 'burst' in the response spike train (Green). Bottom: a zoomed in version of Top.* ***D)*** *Neuronal reaction time across conditions was stable.*

We found that CSTMD1 exhibited robust target-like responses to the sparsest swarm-stimuli, suggesting the attentional system was able to select and respond to one of the targets present within the swarm (Figure 42B). However, decreasing Swarm Separation reduced CSTMD1's overall spiking response (1s analysis window) in both the excitatory-RF (*ANOVA; df = 4, F = 37.82, p > 0.001*) and full-RF (*ANOVA; df = 4, F = 3.68, p > 0.009*) experimental variants, beginning at swarm separation = 12-18° (Figure 42B). Although full-RF data are more challenging to interpret as either a high spiking response or a low spiking response could indicate target selection (i.e., selection of a target in the excitatory or inhibitory receptive field, respectively.)

In order to measure CSTMD1's 'neuronal reaction time' to stimulus presentation, we took advantage of recently described spike-bursting behaviour in CSTMD1s stimulus-evoked response (Fabian and Wiederman, 2021). While CSTMD1 exhibits a relatively high spontaneous firing rate at rest (12-25 sp/s), spike 'bursts' (defined as grouped successive spikes within < 5 ms of each other,

followed by a period of quiescence) occur only in stimulus evoked responses. Therefore, to quantify the 'neuronal reaction time we calculated the time between stimulus onset and the first two-spike burst in CSTMD1's response (Figure 42C). As spike-bursting can only be detected in excitatory responses, we excluded Full-RF trials from Neuronal Reaction Time analysis.

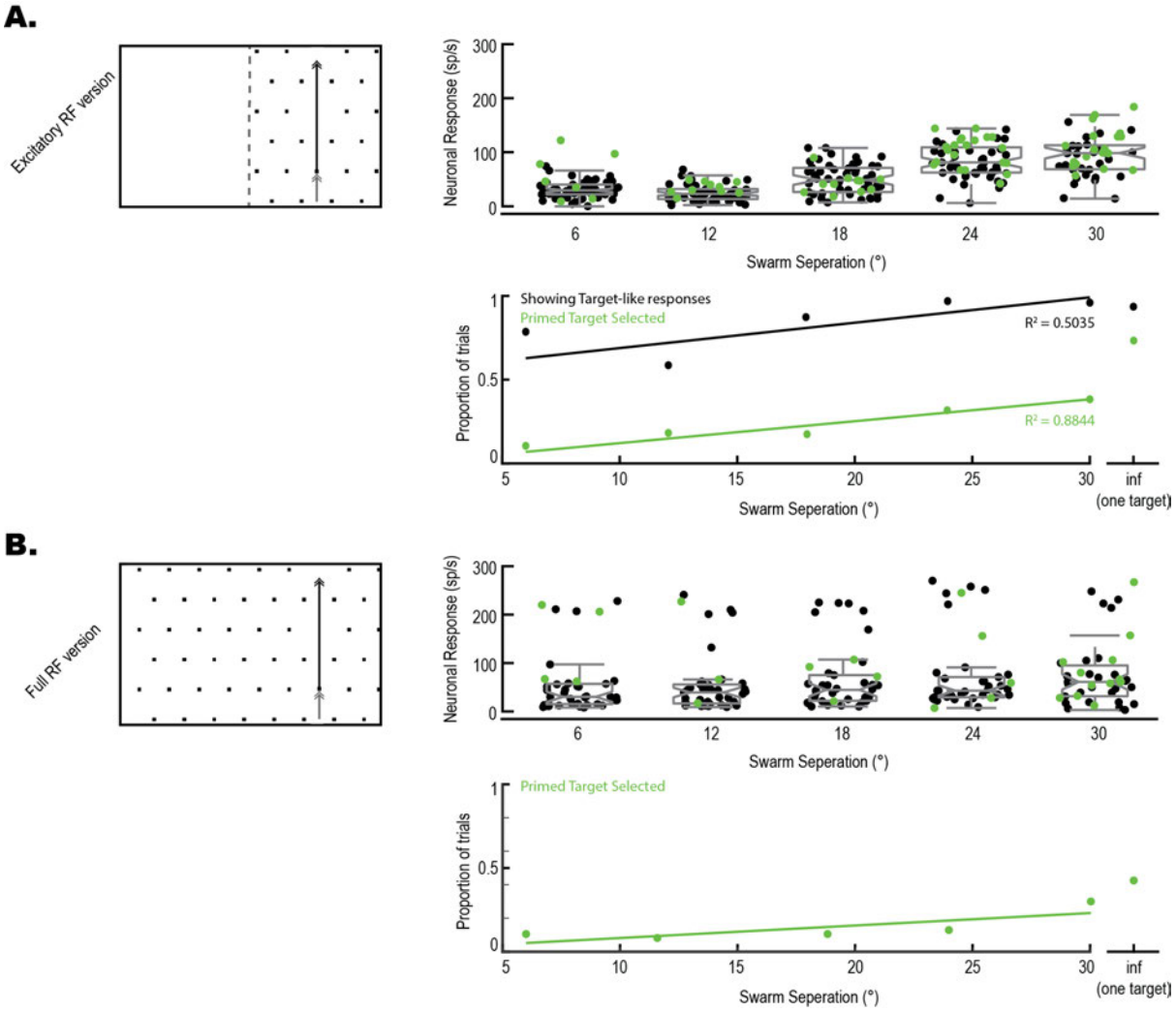

Figure 43: CSTMD1 can track a target amidst a swarm. A) *Excitatory RF version. Left, Stimulus pictogram. Right, top, Neuronal response to Target + Swarm stimuli across Swarm Separations. Green dots indicate trials where measured Selectivity indicated Selection of the Target. We observe a decrease in overall response with decreased Swarm Separation, but individual's examples are consistent with Target selection or target-like response across all Swarm Separation conditions. Right, bottom: Proportion of trials showing a target-like response (black) and selection of the primed Target (green) across Swarm Separations. n = 305 total trials across 11 dragonflies.* ***B)*** *Full RF variant of the experiment gives qualitatively similar results as A. n = 192 total trials across 7 dragonflies.*

Intriguingly, we saw no differences between CSTMD1s neuronal reaction time responding to swarm stimuli of different swarm separations (*ANOVA; df = 4, F = 0.83, p = 0.512*), with an overall mean reaction time of ~100 ms, and an average lower IQR reaction time of ~30 ms, although there were individual trial examples where CSTMD1 experienced a long delay (<500 ms, or more than half the trial) before responding. We also observed individual trials with very short (~10 ms) response

times, around that of a photoreceptor, indicating the presence of some outliers probably due to rare spontaneous two-spike bursts (Fabian and Wiederman, 2021).

Overall, we observed small-but-significant delays in CSTMD1s response to a swarm compared to the response to a single target ($p < 0.001$, Hedges'g = 1.32 [0.79, 1.85]). These results suggest the appearance of multiple targets causes a short delay in CSTMD1's response time, but the finding that response time did not increase with increasing swarm separation suggests a parallel search function (Itti and Koch, 2001), similar to that sometimes observed in primates (Wolfe, 2020) and bumblebees (Nityananda and Pattrick, 2013).

To confirm that CSTMD1 is indeed able to select and respond to an *individual* target amidst a swarm, in a subset of trials we included an additional frequency tagged 'Target' (15Hz, weber contrast 0.22 – 1) that ascended the centre of the excitatory receptive field on a background-matched 'halo bar' to prevent inhibition from leading Swarm Distractors on the same vertical trajectory (Geurten et al., 2007; Evans et al., 2020). Aside from frequency tagging, the 'Target' and 'Swarm Distractors' were otherwise visually identical. In order to facilitate 'locking on' and determine if CSTMD1 could track a target within the context of swarm-like distraction, we primed the Target for 200 ms before the onset of the Distractor swarm. Data from this experiment is presented in Figure 43.

Simillar to observations in swarm-alone trials, we observed a reduction in overall response as Swarm Seperation decreased for the excitatory-RF variant (*ANOVA; df = 4, F = 80.82, p < 0.001*), but intriguingly not in the full-RF variant (*ANOVA; df = 4, F = 0.91, p = 0.4568*). However, despite this reduction we observed many individual trials exhibiting robust target-like responses. To determine if the 'Target' was selected, we used the Selectivity Matric from Chapter 3: A Target-Detecting Visual Neuron in the Dragonfly Locks on to Selectively Attended Targets and applied a selectivity threshold of 0.3 in order to classify a trial as 'Primed Target Selected.' We observed numerous such trials across all Swarm Separation conditions, suggesting CSTMD1 is able to select and respond to an individual target amidst a swarm. We calculated the relative proportion of 'Target Like response' (>50 sp/s) or 'Primer Target Selected' responses and found that the probability of selecting the primed, frequency-tagged target decreased with decreasing Swarm Separation, indicating that the attentional system is not completely immune to increased distraction.

CSTMD1 Ignores 'odd' Targets.

The confusion effect in predation refers to the reduction in a predator's capture success rate with increasing density of potential targets (Jeschke and Tollrian, 2007; Ioannou et al., 2009; Schradin, 2019). One strategy predators may use to overcome the confusion effect is the targeting of an 'odd' target that 'sticks out' from the rest of the group, which has been called the 'Oddity Effect' (Landeau and Terborgh, 1986; Schradin, 2019). Targeted 'oddity' can come in the form of visual distinctiveness

(Landeau and Terborgh, 1986) or spatial distinctiveness (Milinski, 1977). Does CSTMD1 preferentially select odd targets in order to overcome confusion induced by a swarm?

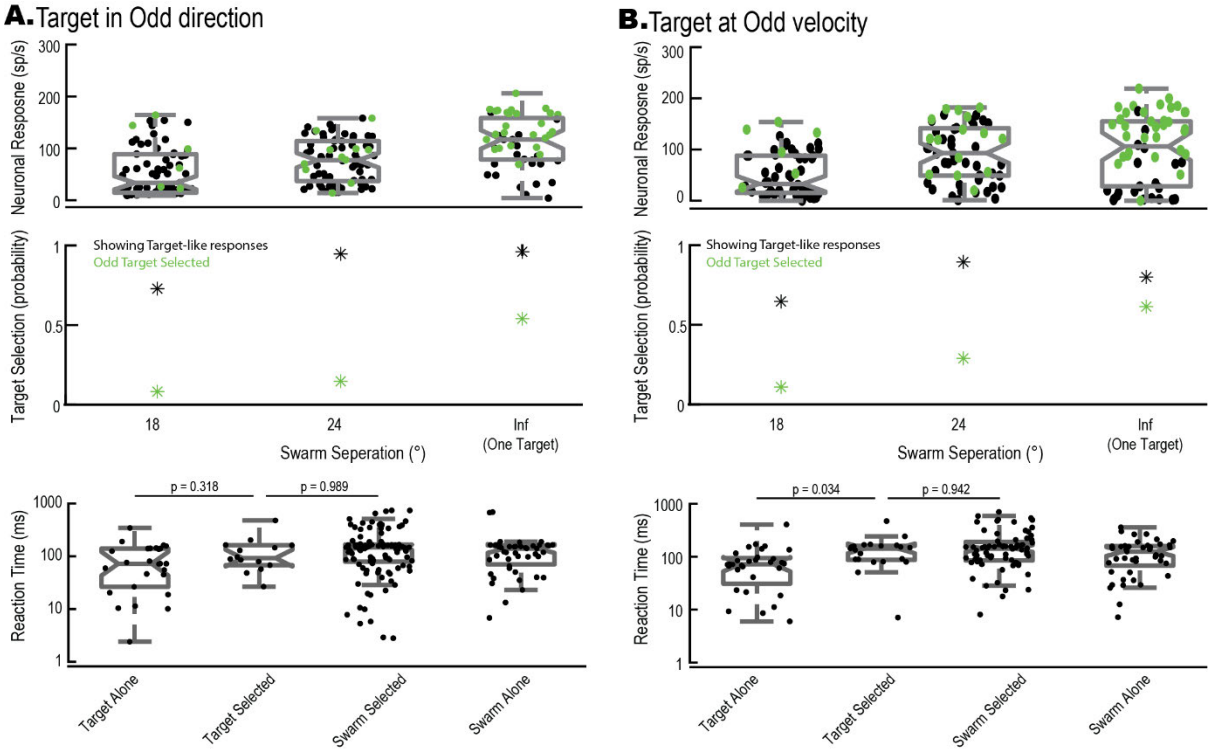

Figure 44: CSTMD1 Ignores Odd Targets. A) *Top: Neuronal response to a Swarm and Target moving counter-directionally. Green dots indicate selection of the 'odd' target. Middle: Proportion of trials showing Target-like responses (Black) and positive selection of the odd target, measured via Selectivity (Green). We observe Target-like responses in a high proportion of trials, but only a small proportion of trials indicate selection of the odd target. Bottom: We observed no difference in neuronal reaction time between trials where the Target was selected and trials where a Swarm Distractor was selected (Swarm Separations pooled). n = 195 total trials across 7 dragonflies.* ***B)*** *A variant of the experiment where the Target and Swarm moved at different speeds (50°/s vs 100°/s) shows qualitatively similar results. n = 165 total trials across 7 dragonflies.*

To test this, we presented Distractor Swarms at two Swarm Separations (18°, 24°) paired with a frequency tagged 'Target' (15Hz, weber contrast 0.22 – 1) that moved 'oddly' compared to the swarm on either one of two dimensions, direction (ascending vs. descending) or velocity (50°/s vs 100°/s). Importantly, in these experiments and in contrast to the previously described experiments, the odd target was *not* primed and had the same onset as the rest of the swarm. The results are presented in Figure 44.

We found that while in the majority of trials in all conditions CSTMD1 exhibited a robust target-like response consistent with the selection of an individual target, only in a small minority of trials was the 'odd' target selected for response (Figure 44A & B, middle). Contrary to our expectations, these data indicate that CSTMD1 largely ignored the odd target in favour of selecting a Swarm Distractor. One possible explanation for this is that CSTMD1 may prioritize selection of a swarm target in this experiment as the Swarm Distractors are of higher contrast average contrast (weber = 1) to the frequency-tagged Target (weber contrast 0.22 – 1, $\mu = 0.51$) with modulated contrast, consistent with

earlier experiments showing CSTMD1 prefers higher-contrast targets (Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron).

Do odd targets 'pop out'? Research in human psychophysics and other primates shows that an odd stimulus can 'pop out' of the visual field and automatically attract attention (Wolfe, 2020), with similar observations in bumblebees (Nityananda and Pattrick, 2013). However, we observed no difference in neuronal reaction time when the off target was selected compared to when a Swarm Distractor was selected in either the Direction ($p = 0.989$) or velocity ($p = 0.949$), suggesting the target did not 'pop out.'

Discussion

We have shown that the dragonfly attentional system is able to select and respond to a single target out of a 'swarm', likely an important function for a predator hunting amidst highly cluttered, distraction-rich environments (Edman and Haeger, 1974; Jeschke and Tollrian, 2007; Combes et al., 2012).

Despite the ability to select a single target from a swarm, we also observed an overall reduction in neuronal response with decreased Swarm Separation beginning at around > 18°. This observation suggests inhibitory mechanisms such as Lateral Inhibition or suppression from the Optic-flow system shape CSTMD1s response to Swarming stimuli. Previously, CSTMD1 has been shown to be able to respond to targets embedded in optic flow stimulation (Wiederman and O'Carroll, 2011; Evans et al., 2020) suggesting that optic flow information does not play a suppressive role in CSTMD1's response, unlike in some other target-detection neurons (Nicholas et al., 2018). Previous experiments in CSTMD1 have presented two targets on the same trajectory, with one target 'lagging' behind the other by a specified distance (Geurten et al., 2007). These experiments found that CSTMD1's overall response was suppressed when the trailing target was < 15° behind the leading target, and completely inhibited when targets were spaced only 5° apart. Although such inhibitory mechanisms could account reduced responsiveness to swarms with low target separation, out data shows that in many individual trials CSTMD1s response is still consistent with the selection of one target.

In contrast to our original hypothesis that CSTMD1 would preferentially select the 'odd' target from a swarm despite the lack of cueing, we found that a target moving in a different direction or at a different velocity to the 'Swarm Distractors' was selected with low probability, indicating that target 'oddity' does not generate saliency in the dragonfly target detection system.

Although we observed Neuronal Reaction Time to Swarm-like stimuli was slightly reduced compared to a single target presented alone, we found no evidence for increasing Neuronal Reaction Time with decreased Swarm Separation, suggesting a parallel search process where the dragonfly target selection system is able to efficiently select and respond to a target within a swarm.

Dragonflies display remarkable prey-capture success rates above 90% (Olberg et al., 2000; Combes et al., 2013), and exhibit the ability to hunt amidst high-density swarms without significant reductions in predatory success (Combes et al., 2012). The ability of the dragonfly target tracking system to select and respond to individual targets within a swarm may aid these predators during predatory pursuits among swarming distractors.

Chapter 7: General Conclusions

Selective Attention in CSTMD1

Prior to this work, there was only limited research into selective attention in CSTMD1. Early papers had shown CSTMD1 was able to respond remarkably well to individual targets embedded in cluttered scenes (Nordström et al., 2006; Wiederman and O'Carroll, 2011) demonstrating a kind of 'selection' between target and non-target-like (wide-field optic flow) stimuli. Early studies involving multiple 'target' stimuli before the identification of selective attention averaged neuronal responses across many trials, leading to results that 'masked' attentional effects (Geurten et al., 2007; Bolzon et al., 2009), that at least in one case resulted in the mis-attribution of attentional affects to long-range, average inhibition (Bolzon et al., 2009).

The original selective attention paper published in 2013 was the first to demonstrate differential, selective responses to paired targets on an individual trial-by-trial basis (Wiederman and O'Carroll, 2013a). This was originally demonstrated in a clever way; by taking advantage of the inhomogeneous receptive field, my supervisors Steven Wiederman & David O'Carroll were able to identify unique response time courses ('Fingerprints') for targets moving on different trajectories. With the presentation of paired targets, they found that CSTMD1s response was closely matched to one of these individual fingerprints, rather than a summation or other combination (Wiederman and O'Carroll, 2013a). This 'fingerprint' method was enough to show selective attention, but unfortunately relying on response fingerprints to identify the selected target precludes any experimental design likely to change the neuronal response, such as priming/cueing and facilitation, or altering other stimulus parameters such as size, velocity, or contrast. However, even at this early-stage selection in CSTMD1 exhibited the interesting property of encoding the *absolute* strength of the selected stimulus. That is, CSTMD1 selects and responds to one target as if that was the only presented target, in comparison to 'weighted' attention observed in many other neuronal systems. CSTMD1 remains, to my knowledge, the only single-neuron model of winner-takes all visuospatial selective attention currently identified.

In order to move behind the initial demonstration of selective attention we developed 'frequency tagging' (Lancer et al., 2019), based on similar techniques used in human EEG research (Norcia et al., 2015) and local field potentials in insects (Paulk et al., 2014; Van De Poll et al., 2015; Cohen et al., 2016, 2018). Frequency tagging allowed us to identify the target selected for neuronal response independently of other stimulus properties, opening up the possible experimental space for more detailed investigation of selective attention in CSTMD1, which has been the broad focus of this thesis.

Overall, we have shown target selection in CSTMD1 is significantly more complex than a traditional winner-takes-all algorithm. In addition to the basic property of selection, CSTMD1 exhibits (contributions of this thesis bolded);

- Absolute encoding of the selected stimulus (Wiederman and O'Carroll, 2013a; Lancer et al., 2019).
- Endogenously driven 'switches' in attentional selection (Wiederman and O'Carroll, 2013a; Lancer et al., 2019).
- **Sensitivity to spatiotemporal priming/cueing** (Lancer et al., 2019).
- The ability to **'lock on' to a selected target, even when challenged by an abruptly appearing high-contrast distractor** (Lancer et al., 2019), likely **driven by spatial facilitation** (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron)
- **Immunity to attentional capture** (Lancer et al., 2019)
- **Inhibition of Return** to previously selected stimuli following an endogenous switch (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron, previously only described in vertebrates), to our knowledge observed for the first time in an invertebrate.
- Predictive facilitation of targets on continuous trajectories (Nordström et al., 2011; Dunbier et al., 2012) **that is Preattentive** (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron) and combined with surround suppression (Wiederman et al., 2017).
- **Selective attention and spatial facilitation for targets across the midline**, within the cell's Inhibitory receptive field (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron).
- **Establishment of ocular dominance with target selection** (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron).
- **Selection preferences broadly matched with behavioural demands** (Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron).
- **Selection for a target amidst multi-target swarm-like conditions** (Chapter 6: Performance of a Dragonfly Target-Tracking Neuron in Swarm Conditions).

These properties combine to make the attentional system driving CSTMD1's selective responses an intriguing insect model for several fundamental neuronal computations; selection, representation, tracking, and prediction of target stimuli. We believe these properties underlie the

dragonflies exceptional predatory success (Olberg et al., 2000; Combes et al., 2013), predictive behaviours (Mischiati et al., 2015; Lin and Leonardo, 2017) and resistance to confusion by high-density swarms (Combes et al., 2012).

How Dragonflies balance avoiding distraction and responding to novelty

Attention to some stimuli necessarily implies *inattention* to other stimuli. For example, birds foraging in a cluttered environment are prone to miss potential food that does not 'match' the current target, even food that is easier to acquire or more nutritious (Plaisted and Mackintosh, 1995; Dukas and Kamil, 2000; Tosh et al., 2007). In a dynamically changing environment where novel opportunities and threats can appear in real-time, too 'narrow' attention can therefore be detrimental. On the other hand, too 'fickle' attention can result in frequency distraction and attentional switches, reducing overall behavioural efficiency (Milinski, 1990; Krause and Godin, 1996; Dukas and Kamil, 2000; Dukas, 2002). Therefore, the optimal allocation of attention requires a balance between two opposing demands: the ability to avoid detrimental distraction, and the ability to flexibly respond to novel opportunities or threats. How has the dragonfly balanced these demands?

We have proposed that the preattentive generation of facilitatory gain enhancement on even unselected target trajectories acts as a kind of 'gatekeeper' to the attentional system (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron), thereby allowing the system to ignore transiently highly-salient distractors and avoid attentional capture (Lancer et al., 2019), whilst retaining the possibility of flexibly switching to novel targets that remain consistent enough to generate facilitatory hotspots (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron), as observed in the 'ZigZag' experiment where attention switched reliably from a zigzagging-target that generated poor facilitation to a straight target ideal for facilitation generation, despite priming of the zigzag path (Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron). Such a system strikes a balance between robustness-against-distraction and responsiveness-to-novelty that may allow the dragonfly to reliably track targets during predatory pursuits, even amidst cluttered environments (Nordström et al., 2006; Wiederman and O'Carroll, 2011) and among distracting swarms (Combes et al., 2013; Chapter 6: Performance of a Dragonfly Target-Tracking Neuron in Swarm Conditions).

Are dragonflies specialised for hunting amidst swarms?

The confusion effect is a reduction in pursuit success experienced by predators hunting amidst swarms of prey (Schradin, 2019), and appears to be a perceptual consequence of having too many stimuli within the predators' visual field (Ruxton et al., 2007; Jones et al., 2011) leading to overwhelming of the predator's visual system (Krakauer, 1995; Tosh et al., 2006; Ioannou et al., 2008).

Krakauer (1995) argues that when faced with potential confusion resulting from a swarm of prey, a predator has a choice of two options in attempt to overcome the confusion effect. First, predators may develop (over evolutionary time) the cognitive neurobiology to overcome the perceptual bottleneck imposed by prey density, or second, they may disrupt the prey group in order to relieve the perceptual bottleneck by isolating the individual (Krakauer, 1995). Most predators opt for the second option. For example, predatory fish species that feed on schooling prey often disrupt the structure of the school into smaller chunks (Major, 1978; Schmitt and Strand, 1982), and hawks hunting amidst swarming bats exiting a cave prefer to pursue lone bats despite overall higher hunting efficiency when targeting the main bat column, due to increased opportunity to catch a target (Brighton et al., 2021). The 'oddity effect' refers to a predators ability to focus on a target that is somehow different ('Odd') compared to other targets, providing relief from the confusion effect (Schradin, 2019). The oddity effect can take many forms, including preference for stray or spatially distinct prey (Milinski, 1977; Brighton et al., 2021), visually distinct targets (Landeau and Terborgh, 1986) or targets moving on a predictable trajectory (Jones et al., 2011). Thus, the most effective strategy to avoid confusion is the ability to single out an individual target and 'lock on.'

The specific behavioural strategies used by dragonflies when hunting amidst swarms have not yet been studied in detail, but dragonflies are known to hunt amongst swarms during crepuscular feeding flights swarms (Edman and Haeger, 1974; Parr, 1983; Corbet, 1999; May and Baird, 2002; Combes et al., 2012), and evidence thus far suggests that some dragonflies may be immune or highly resistant to confusion (Jeschke and Tollrian, 2007; Combes et al., 2012).

Could dragonflies have taken Krakauer's first option in response to the evolutionary pressure of swarming prey? Although some authors view the confusion effect as a breakdown of cognitive attention mechanisms (Schradin, 2019), other authors have argued confusion results from lower level representations and attention acts as an *alleviator* (Tosh et al., 2006). Selective attention in CSTMD1 exhibits several properties that may be advantageous for alleviating confusion, such as *absolute* representation of a selected *single* target (Wiederman and O'Carroll, 2013a; Lancer et al., 2019). In contrast, in most studied models of selective attention the unattended stimulus still exerts some influence on the overall neuronal population response (Treue and Maunsell, 1996, 1999; Luck et al., 1997; Reynolds and Desimone, 2003), so-called 'weighted attention.' Selection of a single target amidst multiple options is then achieved via a 'biased competition' process, where the selected representation is artificially enhanced (Hillyard et al., 1998; Treue and Martínez Trujillo, 1999; Reynolds et al., 2000; Martínez-Trujillo and Treue, 2002; Reynolds and Desimone, 2003), resulting in reduced perceptual accuracy (Mehrpour et al., 2020) which may account for reduced behavioural accuracy when targeting a single target amidst distractors (Ottes et al., 1984; Lisberger and Ferrera,

1997; Ioannou et al., 2008, 2009; Nummela and Krauzlis, 2011). In contrast, absolute representation of a *single* target in CSTMD1 may have evolved to allow dragonflies to specialise in hunting amidst swarms. Examining the interactions between neural representations of targets, selection, and behavioural success in swarming conditions is an exciting avenue for future research in dragonflies and other animal models.

Limitations of Frequency Tagging

Frequency tagging has been one of the experimental keystones of this work but is not without issue. I was very fortunate that the main study neuron I was focused on, CSTMD1, exhibited all the properties required for frequency tagging to work: contrast-dependant rate encoding (O'Carroll and Wiederman, 2014), rapid onset and offset response kinematics, and a large enough overall spike rate to carry a frequency-locked signal without prematurely 'clipping.' However despite the success of Frequency Tagging within CSTMD1, even in our earliest frequency tagged experiments we observed failure to produce a frequency-modulated response on approximately 30% of trials (Lancer et al., 2019), which only increased with the addition of more frequency-tagged targets (Chapter 6: Performance of a Dragonfly Target-Tracking Neuron in Swarm Conditions). These trial-by-trial failures are likely due to due stochastically higher responses or more excitable cells saturating the neuronal response and leading to occasional clipping. One possible solution future frequency tagging experiments may implement is a reduced modulation range: we modulated targets at a weber contrast of 0.21 to 1, producing a large range of modulation that drove the neuron to a maximal contrast response. Reducing the peak of the contrast signal to > 1 may leave cells with more dynamic range with which to generate a response. However, this may come at the cost of reducing the signal-to-noise error in the frequency domain, so care is advised. Alternately, changing other highly salient stimulus properties (size, velocity) so that targets are not matched to CSTMD1's peak tuning response may result in reduced overall response and increased dynamic range to carry modulation, but this has the potential to further interfere with experimental design and selection (Chapter 5: Target Properties that Drive Selection in a Dragonfly Target-Tracking Neuron). The major limitation of frequency tagging is the requirement for rapid response kinetics and a high spike rate, rendering the technique inappropriate for neurons unable to 'keep up' (personal observations).

We found that we required at least 400 ms of continuous neuronal response in order to conduct meaningful wavelet analysis and resolve a signal from a frequency-tagged neuronal response. Since CSTMD1 is thought to be able to switch targets in less than 400 ms, (Wiederman and O'Carroll, 2013a; Lancer et al., 2019), the exact time-point of a switch in attention is unable to be resolved. This requirement presents an interesting balancing act. Longer (potentially slower) stimuli are likely to

generate a better frequency tagged signal for more a more reliable analysis, but also provide more opportunity for target switching and facilitation generation (Lancer et al., 2019; Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron). Such endogenous switches can lead to 'dud' trials if the experimental question required selection of a primed target, however, may also open new opportunities to study the effects of endogenous switching (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron).

Opportunities for Future Work

There are three major directions of opportunity for future biological work relating to Selective Attention in the dragonfly visual system: Physiological intervention, extracellular recording techniques, behavioural interactions. Additionally, modelling of physiological data via algorithmic or network models provides an excellent next step into understanding data currently collected.

Selective Attention During Behaviour

There is a large literature on predatory behaviour in dragonflies (See *Introduction: Dragonfly Behaviour*, p. 30 or Lancer et al. (2020) for a review), however aside from a small number of studies focussed on predatory success during swarm-hunting (Jeschke and Tollrian, 2007; Combes et al., 2012) questions of selective *attention* have not yet been studied. It would be highly beneficial to compare behavioural and neurophysiological performance within a single species, in order to establish direct links between behaviour and neurophysiology. Although it is not yet possible to record from the brains of freely moving dragonflies, such approaches have been used in other insects (Paulk et al., 2013; Guo et al., 2014; Martin et al., 2015).

One important behavioural question relates to the attentional switching observed in CSTMD1 (Wiederman and O'Carroll, 2013a; Lancer et al., 2019). Does switching occur in dragonfly behaviour? To my knowledge, no study thus-far has looked at switching during pursuits. One hypothesis is that attention switching may only occur during pre-take-off, as dragonflies assess potential targets before making a pursuit decision. Dragonflies are known to make a rapid head saccade to detected targets followed by smooth-pursuit tracking until a behavioural decision is made (Lin and Leonardo, 2017). Attentional switching observed in CSTMD1 may be related to a 'rejection' of the current target, and fixation of the next target for assessment. However, attentional switching may become less common during pursuit, where it is possible selective mechanisms could be 'ramped up' to ensure distraction less tracking during an active pursuit. In such a scheme, attentional switches may be artificially more common in the intracellular neuronal recordings presented in this thesis as any attempt to pursue a selected target is prevented by experimental restraints. Alternatively, attentional switches may feature at all stages of a pursuit. Such behavioural experiments could be profitably undertaken in

perching dragonflies as in other behaviour literature, using either head angle or interception path as an index of 'target selection' (Mischiati et al., 2015; Lin and Leonardo, 2017). However, it would be ideal to combine such behavioural experiments with either *Hemicordulia* sp. or with new electrophysiological characterisation of switching in the perching dragonfly used for behavioural results.

Selective attention in Pre-Synaptic STMDs

CSTMD1 is an efferent midbrain projection neuron that sends a large axon into the Optic Lobes, making it *relatively* easy to obtain lengthy intracellular recordings from. However, CSTMD1 is thought to be the output integrator of a broader presynaptic network of STMDs involved in target selection and representation. Some of these cells have been shown to exhibit complex target-tracking properties, such as facilitation in lower-order STMDs (Wiederman et al., 2017) and selective attention in BSTMD2 (Evans et al., 2020) and (hoverfly) STMDs (Bekkouche, 2021).

Investigation into the Selective Attention properties of lower-order STMDs has to potential to reveal a great deal about the neural circuitry underlining target selection in dragonflies. In particular, one hypothesis is that the absolute encoding and unitary choice properties observed in CSTMD1 are common properties of the 'output' of a selection network. If this is so, we would expect to find 'weighted attention' more reminiscent of studies in primate early visual cortex from lower order STMDs. Another interesting question regards the consistency of target selection across multiple neurons. If SF-STMDs exhibit selective attention (either absolute or weighted), is the target selected at lower levels consistently the same as that selected by CSTMD1? The use of extracellular probes techniques to target smaller STMDs less amenable to stable, long-lasting intracellular recording is an exciting avenue of future research.

Physiological Intervention

Anaesthesia

One interesting next-step in investigating selective attention and the functional role of CSTMD1 involves anaesthetic knock-out. Isoflurane is a widely used inhalant anaesthetic that has been shown to abolish behaviour in *Drosophila* (Kottler et al., 2013) in association with a dose-dependent reduction of brain activity (van Swinderen, 2006) with minimal long-term side effects (MacMillan et al., 2017). intriguingly, Isoflurane has differential effects in the central brain verses the peripheries. Under isoflurane, frequency-tagged response to whole-screen flicker in Local Field Potentials is weaker in the central brain compared to the peripheries (Cohen et al., 2016). In addition, brain oscillations associated with feedback activity from the central complex to peripheral areas was knocked out at lower isoflurane dosages than oscillations associated with feedforward information

flow (Cohen et al., 2018), suggesting that endogenous feedback modulation can be disrupted while sparing feed-forward perceptual processing at a carefully chosen dosage. Taken together this provides strong evidence that isoflurane anaesthetics disturb the insect nervous system in a dose-dependent manner, affecting higher-order feedback into sensory neuropil at lower dosage than it affects feedforward sensory propagation.

Although isoflurane is likely to silence CSTMD1, which is a midbrain feedback neuron, recent observations of selective attention in ascending Lobula STMDs (Evans et al., 2020; Bekkouche, 2021) suggest an interesting avenue for potential work. In particular, understanding how midbrain input influences complex aspects of selective attention, such as switching between targets and the ability to 'lock on' in the context of an abruptly appearing, high contrast distractor (Lancer et al., 2019). One hypothesis is that in the absence of midbrain modulation, the optic lobe target selection network may function as a simple winner-takes-all network that selects the exogenously strongest stimulus at any moment in time, similar to observations in *drosophila* (Xi et al., 2008). However, given that Preattentive facilitation thought to underlie priming and cueing (Chapter 4: Preattentive Facilitation and Inhibition of Return in a Dragonfly Target-Tracking Neuron) has been observed in lower-order optic lobe SF-STMDs (Wiederman et al., 2017) this may not be the case. Alternately, midbrain input may underlie switching behaviour, allowing the attentional system to lock on 'by default' and only change targets via an endogenous command.

It is currently unknown what complex selection properties (priming, switching, locking) are present in earlier SF- and LF-STMDs and what traits are unique to CSTMD1, but the application of isoflurane anaesthesia presents an exciting opportunity to find out.

Octopamine

Octopamine is an endogenous neurohormone found in insects, thought to be involved in behavioural activation (Orchard et al., 1993; Roeder, 1999; Suver et al., 2012) and motion encoding (Suver et al., 2012; Arenz et al., 2017; Staedele et al., 2020), and can be broadly thought of as an insect homologue to mammalian adrenaline. Since Octopamine or the Octopamine agonist chlordimeform (CDM) can be used to experimentally mimic a behaviour-like state, Octopamine provides an intriguingly possibility to bridge between electrophysiology and behavioural studies, especially if placed parallel to behavioural results. As with anaesthesia, assessing the effects of Octopamine on CSTMD1's ability to lock-on and switch attention provides an interesting avenue of research linking neural activity and behaviour. In line with the hypothesis expressed above (Selective attention during behaviour), if attentional switches are associated with rejection of the current target, octopaminergic signalling during active pursuit may supress the target selection system's ability to reject and switch targets, ensuring consistent target tracking during active pursuit behaviour.

Appendix: The Visual Neuroecology of Anisoptera

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Highlights

- Dragonflies are highly successful aerial predators that rely almost exclusively on vision to drive behaviour.
- Regional specialisations of the dragonfly eye assist in different behaviours.
- The neuronal target tracking system is finely tuned for predicting the location of small targets in both background visual clutter and swarming conditions filled with distractor s.
- Once a target has been observed, the dragonfly may implement a number of distinct pursuit strategies for target capture.

Abstract

Dragonflies belong to the oldest known lineage of flying animals, found across the globe around streams, ponds and forests. They are insect predators, specialising in ambush attack as aquatic larvae and rapid pursuit as adults. Dragonfly adults hunt amidst swarms in conditions that confuse many predatory species and exhibit capture rates above 90%. Underlying the performance of such a remarkable predator is a finely tuned visual system capable of tracking targets amidst distractors and background clutter. The dragonfly performs a complex repertoire of flight behaviours, from near-motionless hovering to acute turns at high speeds. Here, we review the optical, neuronal, and behavioural adaptations that underlie the dragonflies' ability to achieve such remarkable predatory success.

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Benjamin H. Lancer
Contribution to the Paper	Figure generation, wrote the manuscript
Overall percentage (%)	75
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 11/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Manuscript Feedback
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Please cut and paste additional co-author panels here as required.

Thesis Prelude: *The Visual Neuroecology of Anisoptera* began as an invited review extended to my Primary Supervisor, Dr. Steven Wiederman, from Basil el Jundi on behalf of Current Opinion in Insect Science. Upon hearing Bernard Evans and I discussing how to decompose the behavioural strategies of flying pursuit predators into meaningful categories, Steve invited us onto the paper and I expressed interest in taking the brunt of it, as I had already been working on a similar kind of review article on my own, which became the nucleus of the Introductory chapter to this Thesis. The instructions were to focus on the concept of 'Neuroecology', or the interaction between the development and function of the brain and the environment and ecological interactions the organism resides in. Although I was unfamiliar with the term at first, Neuroecology is a subject I am very interested in. After all, if 'nothing makes sense in biology except in the light of evolution' (Dobzhansky, 1973), then nothing in biology makes sense except in the light of the environment and behaviour that shapes evolution.

I have elected to include this review as an Appendix rather than in the main text as the majority of the content is presented in greater detail in the introduction, and it would be repetitive (and narrative breaking) to reproduce it as a main text chapter chronologically (It would reside between chapters 3 & 4). Instead, I have left it here as-published in *Current Opinion in Insect Science*, with acknowledgment that much of the relevant sections in the introduction were expanded from this text (or the other way around).

Introduction

Dragonflies *(Odonata: Anisoptera)* are highly successful predators both in ambush as aquatic larvae and as adult aerial pursuit specialists. The earliest known flying animals, *Odonatoidea* originated in the carboniferous period approximately 300 Ma (Jarzembowski and Nel, 2002; Nel et al., 2009; Petrulevicius and Gutierrez, 2016) in a terrestrial environment dominated by vast swampy forests and moorlands. The modern form of true dragonflies (*Anisoptera*) arose in the Jurassic approximately 150 Ma later (Huang et al., 2017; Nel et al., 2017). Early Odonata show wing, eye, and leg specialisations suggestive of highly effective aerial predation (Nel et al., 2018). Odonata are predominantly visual creatures, lacking a tympanic membrane and carrying morphologically abridged antennae (Corbet, 1999). This behavioural reliance on vision is reflected in the complexity of their visual system (Evans et al., 2019; Lancer et al., 2019).

Across the lifespan, Odonata inhabit environments of diverse aquatic and terrestrial light conditions. As adults, dragonflies exhibit behaviours ranging from near-motionless hovering, high-speed pursuits, and complex aerobatics that far exceed the flight performance of typical prey (Combes et al., 2012; Bomphrey et al., 2016; Lohmann et al., 2019; Nakata et al., 2020; Rüppell and Hilfert-

Rüppell, 2020). During predation, potential targets rarely span more than 1^{o} of visual space (Lin and Leonardo, 2017), stimulating only two or three ommatidia of the compound eye (Horridge, 1978). Targets are often observed against clutter and pursued in variable luminance conditions, with swarms of distractors and conspecifics nearby. Yet despite these sensory and aerobatic challenges, dragonflies are highly successful predators boasting capture rates reaching up to 97% (Olberg et al., 2000). Here we review adaptations in the dragonfly visual system from the optical architecture of the compound eye to behavioural pursuit strategies that underlie their role as the top insect predator within complex ecologies.

Differentiation in optical architecture and photoreceptor physiology is driven by differing task demands.

The dragonfly compound eye is of an apposition type (Sherk, 1978), an architecture where individual ommatidial units are optically isolated, receiving the photons from a limited region of space. Each ommatidium presents a crystalline lens that focusses incoming light onto a single rhabdom, a cylindrical structure containing several photoreceptors, which is surrounded by the pigmented ommatidial wall. Compound eyes typically have lower spatial resolution than vertebrate camera-lens equivalents. As each ommatidial unit functions as an individual sampling point, spatial resolution is limited by both the overall number of ommatidia (more sampling points leads to higher visual acuity), and the angle between neighbouring ommatidia (interommatidial angle, where a higher angle implies greater distance between the sampling points and reduced resolution). Dragonflies have some of the smallest interommatidial angles measured, as low as 0.24° in *Aeshnidae* (Land, 1997), and the largest eyes among insects, with as many as 30,000 ommatidia (Sherk, 1978) sampling across a large visual field (Fig. 1A).

Most insects exhibit non-uniform interommatidial angles and ommatidial sizes across the eye surface, supporting specialised regions for different visual tasks. Like many predatory insects, dragonflies possess an acute zone analogous to the vertebrate fovea (Horridge, 1978; Sherk, 1978), with increased visual acuity due to increased facet diameter, increased ommatidia count, and decreased interommatidial angles. This results in a region of improved capacity for resolving small moving targets. Due to improved photoreceptor sensitivity in this region, the dragonfly can detect targets well below the sampling resolution of a single ommatidium (Rigosi et al., 2017). The major acute zone in dragonflies is located fronto-dorsally as a laterally-extending 'strip' from the midline to the periphery (Dorsal Acute Zone, Fig. 1B). This area is well situated to detect moving targets at maximum contrast, against the clear sky (Labhart and Nilsson, 1995). Many dragonfly species fly low, such that a target will be interposed between the dorsal eye and the sky (Olberg et al., 2007; Mischiati et al., 2015; Bomphrey et al., 2016; Supple et al., 2020). The *Odonata* compound eye has an additional,

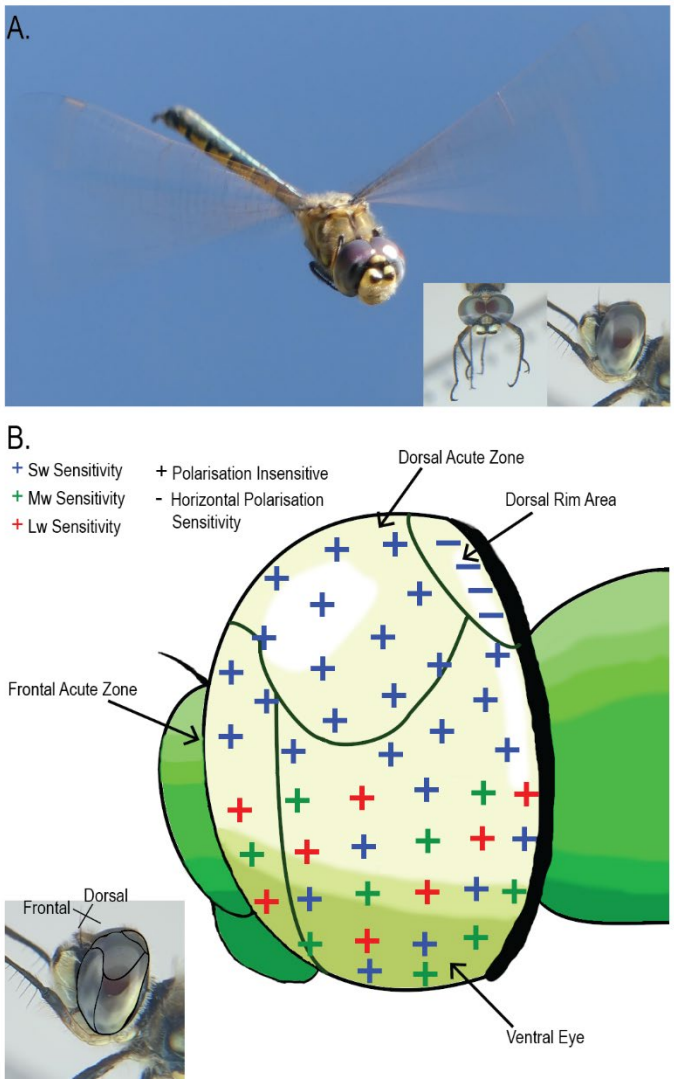

***Figure 45**: **The Compound Eye of the Dragonfly.** A) The hawking dragonfly Hemicordulia tau in flight, showing large eyes relative to head size. Insert: the same dragonfly under a magnifying glass. B) Illustration of the dragonfly eye, illustrating prominent zones, spectral sensitivity (Sw = short wavelength, Mw = Medium wavelength, Lw = Long wavelength), and polarisation sensitivity. Note the ventral eye also contains horizontal polarisation sensitive photoreceptors, but their distribution is currently unknown. Insert: Boundaries of the major zones superimposed on an image of the dragonfly eye. Compass indicates direction. From front in clockwise: the Frontal Acute Zone, the Dorsal Acute Zone, the Dorsal Rim Area and the Ventral Eye.*

forward-facing frontal acute zone (Horridge, 1978), in which the closely related damselflies *(Odonata: Zygoptera)* fixate prey during pursuit (Supple et al., 2020).

In addition to differences in optical architecture, ommatidia also exhibit a diverse distribution of colour-sensitive opsins across regions of the compound eye. This distribution varies between the aquatic and terrestrial stages of development. Transcriptomic analysis of multiple families revealed expression of up to 33 distinct opsin genes (Futahashi et al., 2015), a large variety compared to other insects studied. Spectral sensitivity distributions show a pattern across multiple species (Fig. 1B). Dorsal regions exhibit narrow shortwave sensitivity (Labhart and Nilsson, 1995; Futahashi et al., 2015) matching optical and behavioural specializations for the rapid detection of objects against the sky. Many dragonflies feed during crepuscular flights (Corbet, 1999), when sunlight becomes dim and shortwave skewed. Prey or conspecifics positioned in the dorsal acute zone dominated by shortwave opsins are thus perceived as silhouettes contrasting against the sky, even in this dimmer environment.

Comparatively, ventral regions display a wide variety of sensitivities from UV to red (Bybee et al., 2012; Futahashi et al., 2015), congruent with habitat selection, as well as territorial and reproductive behaviours. Among both damselflies and dragonflies, colour represents an important cue for the identification of conspecifics which are often first observed flying on the same horizontal plane through lateral and frontal ommatidia. Colour can trigger sexual selection and male-male

competitive interactions (Schultz and Switzer, 2001; Schultz and Fincke, 2009; Brydegaard et al., 2018; Schröder et al., 2018), allowing territorial males to pursue potential mates and evict intruders. Although in Dragonflies predatory selection and pursuit behaviour is primarily driven by target size (Olberg et al., 2005; Duong et al., 2017; Lin and Leonardo, 2017) and velocity (Lin and Leonardo, 2017), In damselflies colour may also serve as an important cue for predatory pursuit initiation (Schröder et al., 2018). Ventrally-directed colour sensitivity and form recognition may also play an important role in predatory gleaning, the practice of catching still prey from a surface or spider web. Gleaned prey are often camouflaged and stationary, making it unlikely narrowly-tuned shortwave motion pathways play a significant role. Although gleaning is mostly associated with damselflies, dragonflies can opportunistically hunt this way (Waltz, 1998; Corbet, 1999).

In the ventral region of the adult compound eye, polarised, ultraviolet photoreceptors play a critical role in habitat selection (Bernáth et al., 2002; Kriska et al., 2009). Highly sought-after freshwater territories are required for oviposition and are sensed via polarised light reflected off the water surface (Laughlin and McGinness, 1978; Kriska et al., 2009). The distribution of polarisation sensitive photoreceptors in the dragonfly ventral eye is currently unknown (review (Heinloth et al., 2018)). Three main patterns of polarization sensitivity have emerged. Two of these patterns belong to water-surface dwelling insects and are characterized by ventrally directed zones of polarization sensitive ommatidia for observing the subsurface. However, dragonflies utilize polarization cues from a range of visual angles, making such a limited subregion of polarization sensitivity insufficient. The third pattern has been observed in another riparian predator, the long-legged fly (*Dolichopodidae*), which exhibits alternating rows of ommatidia that show either vertical or horizontal sensitivity (Trujillo-Cenóz and Bernard, 1972). This architecture allows the horizontally sensitive columns to detect water bodies, while vertically sensitive columns support predation by filtering horizontal glare. While dragonflies are not known to habitually hunt prey at the water surface, conspecific interactions can be elicited by ventrally-observed targets (i.e., an ovipositing female or close-by female-guarding male). Polarization sensitivity in the ventral eye of the dragonfly may be similar to the columns observed in long-legged flies due to their similar behaviours. Alternatively, polarization-sensitive ommatidial units may be stochastically distributed across the ventral eye, as are colour-channel subtypes in *Drosophila* (Wernet et al., 2006).

Dorsally, dragonflies possess a polarisation-sensitive Dorsal Rim Area similar to many other insects (Meyer and Labhart, 1993), which in those species is important for celestial navigation. Dragonflies may rely on a similar mechanism for long-distance migration (Merlin et al., 2012; Troast et al., 2016; Hallworth et al., 2018), though they are thought to rely on landmarks for short flights (Bernáth et al., 2002; Eason, Perri K., 2006). Comparatively, dragonfly nymphs exploit polarisation

sensitivity in their aquatic habitat for predation. Here, polarisation sensitivity aids object-background segmentation, enhancing prey contrast (Sharkey et al., 2015), which is otherwise obscured by short-wave spectral attenuation and photon scattering in underwater environments. *Anisoptera* thus exploit polarisation in multiple environments to meet varied behavioural demands across the lifespan, indicating significant developmental plasticity in the polarisation system.

The dragonfly target detection system is specialised for hunting amongst swarms of prey

Visually based pursuit predation requires a system capable of tracking moving targets at high speeds, often amidst distractions. Dragonflies possess neurons likely to underlie such behaviour, which respond robustly to 1-3° targets moving within a limited region (i.e. a receptive field). These 'Small Target Motion Detector' (STMD) neurons (O'Carroll, 1993) in the third neuropil of the optic lobe, are sensitive to target contrast (O'Carroll and Wiederman, 2014) and tuned for both size and velocity (Geurten et al., 2007), making them well-matched to ecological demands (Labhart and Nilsson, 1995; Olberg et al., 2005). Downstream, between the brain and thoracic ganglia, a series of 'Target Selective Descending Neurons' (TSDNs) (Gonzalez-Bellido et al., 2013), are also well-matched to behavioural demands. TSDN input dendrites are thought to collocate with the outputs of some STMD neurons and together, TSDNs form a population code of target position that can modulate wing musculature in the thorax (Gonzalez-Bellido et al., 2013) to direct wing steering. A recent comparison between dragonflies and damselflies found TSDN receptive field properties were matched to the behavioural strategy of the hunter (Supple et al., 2020); whereas dragonflies fixate prey in the dorsal acute zone (Olberg et al., 2007), damselflies fixate prey frontally (Supple et al., 2020).

Dragonflies forage on a variety of prey, often hunting amongst swarms. In one *Libellula*, increased *Drosophila* density actually increased prey-capture success (Combes et al., 2012). Although this can be attributed to reduced flight-space under swarm conditions inducing less erratic, more predictable flight-patterns by the prey. It is notable that these dragonflies did not suffer from the *confusion effect*, a reduced attack-to-capture ratio experienced by predators across taxa (Jeschke and Tollrian, 2007). This confusion results from an inability to distinguish individuals from high-density groups due to an overwhelming of the predator's nervous system. Behavioural evidence on the effects of confusion in *Odonata* is limited, but suggests that some families of dragonfly, such as the *Libellulidae* may be immune or highly resistant (Jeschke and Tollrian, 2007; Combes et al., 2012), while others, such as the *Aeshnidae,* are not (Jeschke and Tollrian, 2007). Alternately, as *Aeshnidae* have only been studied in their larval form, resistance to the confusion effect may be a property of the adult visual system.

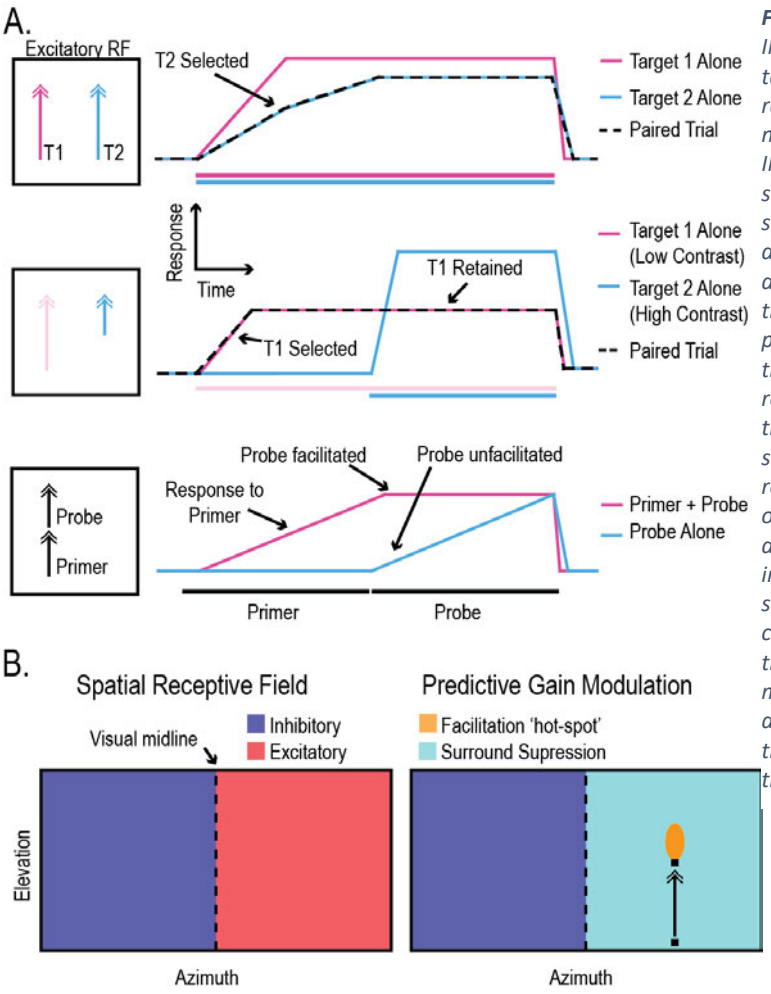

Figure 46: Complex Properties of CSTMD1. A) *Illustration of CSTMD1's response to moving targets. Left: Pictograms illustrating the spatial relationship of targets presented within the neuron's excitatory receptive field (RF). Right: Illustrative responses. Lower lines indicate stimulus timing. Top: Selective attention to a single target amongst a pair. Middle: Selection of a low contrast target can be maintained despite an abruptly-appearing high-contrast distractor. In this example, selection of T1 is retained in the presence of a distractor that appears half-way through the trial. Bottom: Spatial facilitation results in a build-up of neuronal response over time (pink line), compared to the response of a stimulus (blue line) recently appeared in the same region of the receptive field.* ***B)*** *Spatial properties of CSTMD1. Left: CSTMD1's receptive field covers a large portion of the visual field and is divided into excitatory (contralateral relative to recording site) and inhibitory (ipsilateral) hemifields. The contralateral optic lobe CSTMD1 would display the opposite pattern. Right: Predictive gain modulation involves both enhancement (known as 'facilitation') of a spotlight ahead of a target's trajectory, and response suppression elsewhere in the receptive field.*

The visual neuron 'Centrifugal Small Target Motion Detector 1' (CSTMD1) (Geurten et al., 2007) described in *Hemicordulia tau* (Family: *Corduliidae*, sister to *Libellulidae* (Carle et al., 2015)), exhibits a kind of winner-takes-all selective attention that may play a role in overcoming confusion (Wiederman and O'Carroll, 2013a; Lancer et al., 2019) (Fig. 2A: top). This selective attention may underlie a predators' ability to isolate individual targets, thus reducing the confusion effect (Landeau and Terborgh, 1986). CSTMD1 is a binocular, efferent neuron that straddles the optic lobe and midbrain (Geurten et al., 2007), and is thought to be an output integrator of the broader pre-synaptic STMD network. CSTMD1 is tuned to detect the motion of small targets against a bright background (Geurten et al., 2007; O'Carroll and Wiederman, 2014), with a receptive field that spans a large region of the visual field. However, it exhibits a sharp distinction between excitatory and inhibitory hemispheres on either side of the visual midline (Geurten et al., 2007) (Fig. 2B: left). When presented with two targets CSTMD1 selects and responds to only one, encoding the *absolute* strength of the selected target as if it were presented alone (Wiederman and O'Carroll, 2013a). This system can select a lower contrast target (Lancer et al., 2019), without interference from distractors, as is characteristic of vertebrate attentional systems (Ghose and Maunsell, 2008). Additionally, CSTMD1 is able to flexibly 'lock on' to

a selected target and retain this selection in the face of an abruptly-appearing, high-contrast distractor (Lancer et al., 2019) (Fig 2A; middle).

During the presentation of a moving, single target, there is a gradual enhancement of CSTMD1's response, known as facilitation (Nordström et al., 2011; Dunbier et al., 2012) (Fig 2A: Bottom). This predictive 'spotlight' of gain enhancement spreads ahead of the target such that if a target disappears (e.g. prey becomes temporarily occluded) the facilitation enhances responses ahead of the predicted trajectory (Wiederman et al., 2017). In concert with this forward enhancement, distal areas are supressed (Wiederman et al., 2017) and together, this neuronal enhancement and suppression is referred to as predictive gain modulation (Fig. 2B; right). This modulation occurs for the presentation of a single target (Nordström et al., 2011; Dunbier et al., 2012; Wiederman et al., 2017; Fabian et al., 2019), and is not purely an attentional-enhancement effect as observed in vertebrates (Martínez-Trujillo and Treue, 2002).

What role could the predictive gain modulation play in target pursuit? Fabian *et al* (2019) have suggested that facilitation drives CSTMD1 towards saturation, minimising neuronal variability and maximising target detectability despite noisy visual input (Fabian et al., 2019). This is a critical computational function for target-tracking in highly cluttered environments, where the angular size, velocity, and contrast of a target may change drastically throughout pursuit. For effective tracking under such conditions, representation should be robust to a wide range of dynamic stimulus properties. Evidence suggests that CSTMD1 is robust to this scenario (Wiederman and O'Carroll, 2011; Lancer et al., 2019). The antagonistic surround may function as a gating mechanism to suppress inconsistent, transient, highly-salient distractions (Lancer et al., 2019). It is likely that prediction and selection have evolved to allow dragonflies to successfully hunt amidst swarms in highly cluttered conditions. These 'higher-order' properties beyond target detection, help to avoid confusion and pattern noise (clutter) by selectively locking-on to a moving target.

Predictive interception and complex wing control allow dragonflies to outfly competition

Odonata are extremely agile, capable of hovering, acute turns, high-speed pursuits, and even backwards flight (Combes et al., 2012; Bomphrey et al., 2016; Lohmann et al., 2019; Nakata et al., 2020; Rüppell and Hilfert-Rüppell, 2020). This is enabled by intricate wing innervation, allowing independent control of all four wings (Nakata et al., 2020; Rüppell and Hilfert-Rüppell, 2020). After detecting prey, *Anisoptera* can launch with an acceleration of 15 ms^{-2}, reaching a maximum speed of 7.2 ms^{-1} after only 0.2 seconds (Rüppell and Hilfert-Rüppell, 2020), and perform turns with a radius of curvature as small as 4.1 ± 2.4 cm (Combes et al., 2012) at up to 1000°/s (Bomphrey et al., 2016).

In contrast to species that exhibit a narrower range of flight behaviour, such as hovering hawkmoths (O'Carroll et al., 1996; Theobald et al., 2010; Stöckl et al., 2019) or fast-flying butterflies and bees (Ibbotson, 1991; O'Carroll et al., 1996), dragonflies require strategies to encode self-motion over a broad range of speeds. Dragonflies must determine their motion visually, unlike other species which augment their vision with mechanosensory organs, such as halteres derived from wing-pairs (Fraenkel and Pringle, 1938; Pix et al., 1993), or complex antennae in the four-winged *Lepidoptera* (Sane et al., 2007; Dahake et al., 2018). To achieve this, the dragonfly optic-flow system has developed a striking variation in spatiotemporal tuning properties. Here, a subset of neurons rapidly adapt over time, providing additional velocity-tuned channels (Evans et al., 2019). Together, these neurons of the dragonfly visual system cover a broader velocity range than analogous neurons in other studied insects (O'Carroll et al., 1996; Theobald et al., 2010), subserving the dragonflies' broad extent of behavioural demands.

There are two broad behavioural strategies a pursuer can apply to the problem of catching a target (Fig. 3). The first, classical pursuit[2], is where the pursuer actively follows the target by steering to minimise the angle between its own flight path and that of the target (Land and Collett, 1974). These following chases ensure a successful capture if the pursuer is faster than the target. Alternatively, proportional navigation[3], seeks to maintain a constant acute relative-angle to the target, allowing the pursuer to ignore many target uncertainties (size, speed etc). Target trajectory changes can be countered by steering to minimize the target's relative angular movement. Proportional navigation can be implemented through various computational mechanisms, by maintaining constant either; the angle between the target and pursuer trajectories (Olberg et al., 2000) (Fig. 3); the target's direction (Ghose et al., 2006) or bearing (Olberg et al., 2000) relative to the pursuer.

Dragonflies often exhibit proportional navigation in both predatory (Olberg et al., 2000, 2007; Mischiati et al., 2015) and territorial (Bomphrey et al., 2016; Lohmann et al., 2019) engagements, but also display classical pursuit in some conspecific engagements (Bomphrey et al., 2016). What factors lead dragonflies to choose one pursuit strategy over another is currently unknown. Dragonfly pursuit behaviour is driven by an internal model (Mischiati et al., 2015) that predicts prey image drift, ego-motion, and body position in order to drive interception steering with minimal time-lag (Olberg et al., 2007), in stark contrast to a purely reactive 'neuronal autopilot' (Collett and Land, 1978; Gonzalez-Bellido et al., 2013), where sensory information passed to motor actuators without significant

[2] CLASSICAL PURSUIT IS ALSO REFERRED TO VARIOUSLY AS TRACKING, SMOOTH PURSUIT, PURE PURSUIT, AND SIMPLE PURSUIT.

[3] PROPORTIONAL NAVIGATION ('PROPNAV') IS ALSO REFERRED TO AS PARALLEL NAVIGATION, AS THE RANGE VECTORS REMAIN PARALLEL THROUGHOUT PURSUIT (FIG. 3).

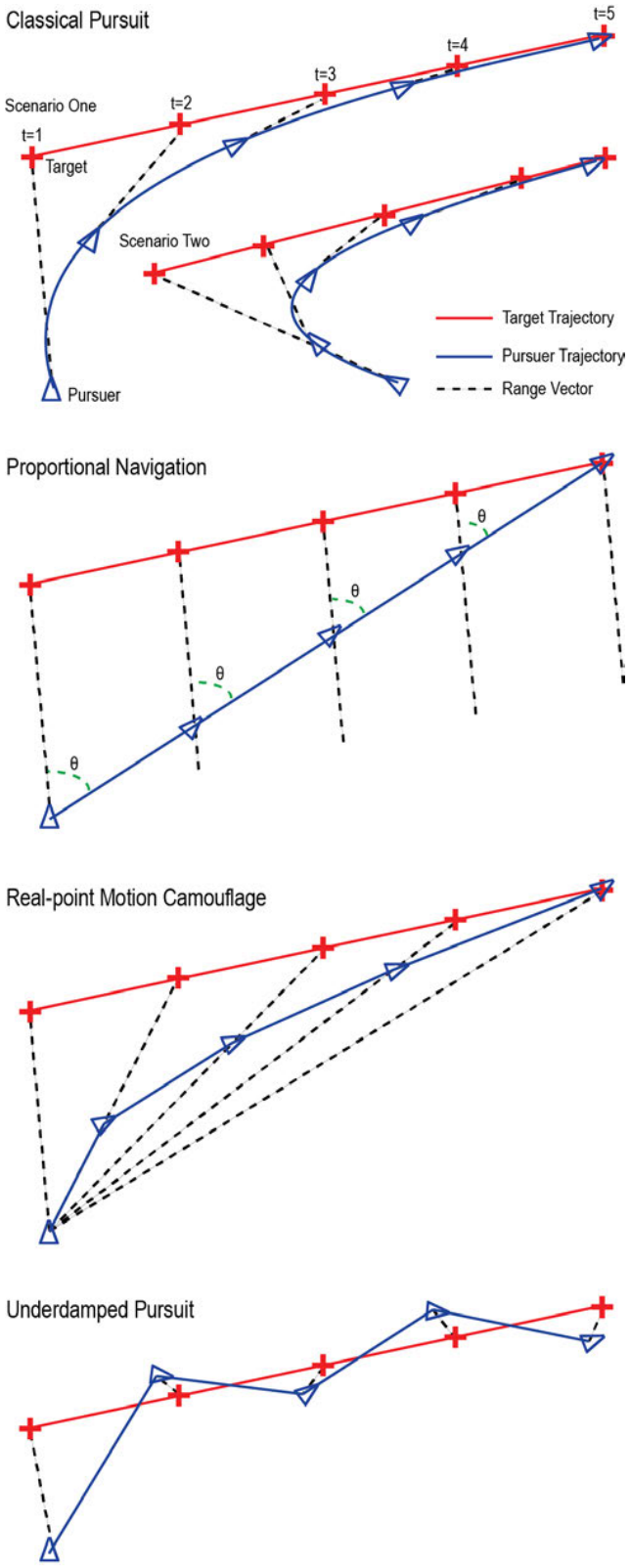

Figure 47 Pursuit Strategies Observed in Dragonflies. *Classical (also known as tracking, smooth pursuit, pure pursuit, and simple pursuit) and Proportional navigation (also known as Interception or Parallel navigation) are two strategies used to catch a target. In Classical pursuit, the pursuer moves towards the target along the current range vector, resulting in in a 'chase' (Scenario one). If the pursuer notices the target before it has crossed ahead of the pursuer, the pursuer's trajectory will turn in one direction and then turn as the target crosses ahead (Scenario Two). In Proportional navigation the target position is held constant relative to the pursuer while the length of the range vector is minimised. There are a number of steering laws that can be used to implement proportional navigation. Here, we have illustrated the Constant Error Model (CEM) where the error between the target and pursuers' trajectory is maintained stable. Real-point Motion Camouflage is a special case of proportional navigation in which a real-point landmark, rather than the target, is held constant relative to the pursuer. Here, the pursuers approach is disguised in background information, by remaining on a path where it is interposed between the target and a constant real point behind the pursuer. Underdamped pursuit is an aggressive strategy involving repetitively overshooting a target that is employed in conspecific engagements where the goal is to evict a territorial intruder, rather than make physical contact.*

transformation. It is feasible that the predictive gain modulation observed in STMD neurons underlies such predictive computations (Nordström et al., 2011; Dunbier et al., 2012; Wiederman et al., 2017), by conveying predicted target location to the wing motor system to drive predictive steering.

Dragonfly flight is not limited to predator-prey interactions. Dragonflies regularly engage both conspecifics and heterospecifics (Moore, 2000) in territorial defence and for reproduction. These interactions involve complex aerial dogfights, with rapid and acute manoeuvres for territorial defence (Beckemeyer, 2009; Bomphrey et al., 2016; Lohmann et al., 2019; Nakata et al., 2020), tandem flight in mating, and mate refusal. When engaging a conspecific, dragonflies make use of distinct behavioural strategies (Fig. 3). For example, they can 'motion camouflage' where the pursuer embarks on a trajectory that keeps its

image stationary relative to a fixed point on the target's retina (known as 'Real Point Motion Camouflage). This disguises the pursuers own motion cues and therefore they appear stationary (though looming) from the target's perspective (Srinivasan and Davey, 1995; Mizutani et al., 2003). In addition to camouflaging the pursuers approach, this strategy is more efficient than classical pursuit (Glendinning, 2004). Dragonflies can also use an aggressive strategy that involves overshooting a target from alternating directions (Lohmann et al., 2019) (Fig. 3). This may evoke evasive responses from the target, effectively 'herding' it out of the territory. As this aggressive behaviour involves high translational and angular velocities, it may also function as a display of flight performance and an honest signal of fitness.

Conclusion

Dragonflies are highly successful predators that specialise in hunting amongst swarms, in conditions that confuse and disadvantage many predatory species. They are capable of extreme aerial manoeuvres and flight performance, which has allowed them to dominate their niche for millions of years. This has been enabled by acute optimisation of the visual system towards the challenging task of target identification, tracking, prediction, and pursuit in a highly complex 3-dimensional environment. These adaptations begin in the optical architecture of the dragonflies' compound eye and continue through specialisations in retinal and neuronal processing, culminating in behavioural performance.

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