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Investigation of insect selective attention during visual object tracking using neurophysiology, neuroanatomy, computational modelling

Bekkouche, Bo

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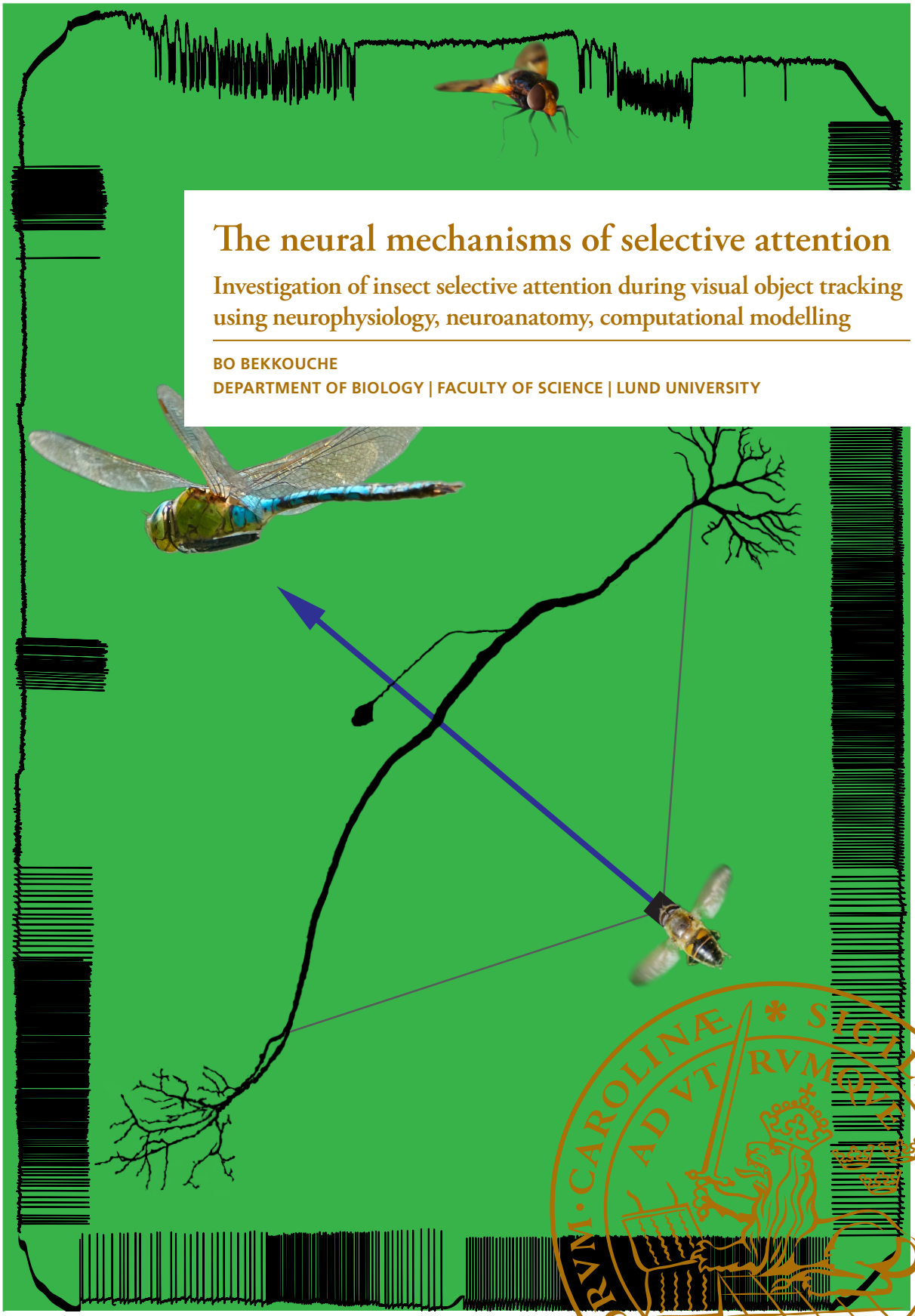

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Bo Bekkouche

LUND UNIVERSITY

DOCTORAL DISSERTATION

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Abstract
The brain simulates the world around us using sensory information and provides an estimation of reality in which actions can be executed. This estimation of reality is controlled by attention which decides which information is accepted, further processed and ignored. The process of attending a certain part of the sensory information while ignoring other parts is called selective attention. I have studied visual selective attention on a neuronal level in hoverflies and dragonflies. These insects are highly skilled at object tracking in behaviors related to defending territories, mating or hunting prey. They have very small brains with few neurons compared to mammals and yet execute object tracking tasks with impressive accuracy. In Paper 1 we compare insect brain tissue preparation techniques for optimizing the amount of neuronal morphology details that can be captured during microscopy imaging. We then use these techniques to acquire a highly detailed neuron morphology and further apply the techniques in the other papers. In Paper 2 we captured the morphology of a hoverfly target-tracking neuron using techniques from Paper 1. I measured a type of short-term memory called response facilitation in a population of these hoverfly target-tracking neurons. This was measured by comparing the response of long (primed) versus short (unprimed) target traveling paths. In the next experiment I measured the neuronal response while distracting the neuron with another target moving outside the part of the visual field in which that neuron responds. Both primed and unprimed distractors reduced the response, indicating that the attention was sometimes moved to the distractor. This phenomenon could potentially be implemented using long range inhibition as part of an attention mechanism. Paper 3 & 4 involved computational modeling of target tracking neurons using a neuronal morphology from the dragonfly. We show that a receptor (N-methyl-D-aspartate receptor), known for its involvement in short term memory processing, have some of the properties required to generate facilitation. Altogether, the results of this thesis have improved our knowledge and understanding of the neural mechanisms of selective attention in hoverflies and dragonflies. It has also paved the way for future studies to further expand on this knowledge and understanding.

Key words selective attention, object tracking, small target motion detector, STMD, lobula, hoverfly, dragonfly, insect brain

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To my family.

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2. **Bekkouche B.**, Rigosi E., O'Carroll D. Response facilitation and selective attention in hoverfly target tracking neurons. *(Manuscript)*
3. **Bekkouche, B.**, Shoemaker, P. A., Fabian, J., Rigosi, E., Wiederman, S. D., and O'Carroll, D. C. (2017). Multicompartment simulations of NMDA receptor based facilitation in an insect target tracking neuron. in *Artificial Neural Networks and Machine Learning – ICANN 2017. Lecture Notes in Computer Science, eds. A. Lintas, S. Rovetta, P. Verschure, and A. Villa (Springer, 609 Cham), 397–404.* doi:10.1007/978-3-319-68600-4_46
4. **Bekkouche B.**, Shoemaker P., Fabian J., Rigosi E., Wiederman S. O'Carroll D. Modelling nonlinear dendritic processing of facilitation in a dragonfly target-tracking neuron. *(Submitted. Preprint doi:10.1101/2021.03.24.436732)*

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1. DO'C, BB, and HF: conceptualization. BB and HF: clearing experiments and measurements. BB: tracer injection. BB and DO'C: confocal imaging and manuscript writing. DO'C: DLS imaging. ER, DO'C, and BB: bead experiments. All authors: comments on figures and writing.
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Papers not contained in this thesis

5. **Bekkouche B.***, Iannella N.*, Wiederman S., Shoemaker P., O'Carroll D. Selective target tracking using a biophysically inspired spiking network model of dragonfly (*Manuscript*. *Equal contribution)
6. Singh P., **Bekkouche B.**, O'Carroll D., Shoemaker P. Non-spiking models of facilitation and prediction (*In preparation*)
7. **Bekkouche B.**, Rigosi E., O'Carroll D. Investigation of selective attention in hoverfly target tracking neurons using frequency tagging (*In preparation*)
8. **Bekkouche B.**, O'Carroll D. Computational model of a predictive focus of gain using graded synapses in a spiking neural network (*In preparation*)
9. **Bekkouche B.**, O'Carroll D. Morphological comparison of motion detector neurons: the morphological characteristics of target tracking neurons (*In preparation*)

Popular summary

The brain simulates the world around us using sensory information and provides an estimation of reality in which actions can be executed. This estimation of reality is controlled by attention which decides which information is accepted, further processed and ignored. The process of attending a certain part of the sensory information while ignoring other parts is called selective attention. I have studied visual selective attention on a neuronal level in hoverflies and dragonflies. These insects are highly skilled at object tracking in behaviors related to defending territories, mating or hunting prey. They have very small brains with few neurons compared to mammals and yet execute object tracking tasks with impressive accuracy. In **Paper 1** we compare insect brain tissue preparation techniques for optimizing the amount of neuronal morphology details that can be captured during microscopy imaging. We then use these techniques to acquire a highly detailed neuron morphology and further apply the techniques in the other papers. In **Paper 2** we captured the morphology of a hoverfly target-tracking neuron using techniques from Paper 1. I measured a type of short-term memory called response facilitation in a population of these hoverfly target-tracking neurons. This was measured by comparing the response of long (primed) versus short (unprimed) target traveling paths. In the next experiment I measured the neuronal response while distracting the neuron with another target moving outside the part of the visual field in which that neuron responds. Both primed and unprimed distractors reduced the response, indicating that the attention was sometimes moved to the distractor. This phenomenon could potentially be implemented using long range inhibition as part of an attention mechanism. **Paper 3 & 4** involved computational modeling of target tracking neurons using a neuronal morphology from the dragonfly. We show that a receptor (N-methyl-D-aspartate receptor), known for its involvement in short term memory processing, have some of the properties required to generate facilitation.

Altogether, the results of this thesis have improved our knowledge and understanding of the neural mechanisms of selective attention in hoverflies and dragonflies. It has also paved the way for future studies to further expand on this knowledge and understanding.

Sammanfattning

Hjärnan simulerar världen omkring oss med hjälp av sensorisk information och ger oss en estimering av verkligheten där handlingar sen kan utföras. Den här verklighets-estimeringen kontrolleras av vår uppmärksamhet som bestämmer vilken information som accepteras, vidarebehandlas och ignoreras. Att hålla uppmärksamheten på en viss del av den sensoriska informationen medan andra delar ignoreras kallas selektiv uppmärksamhet. Jag studerade visuell selektiv uppmärksamhet på nervcells-nivå hos blomflugor och trollsländor. De här insekterna är väldigt skickliga på objektspårning i beteenden relaterade till att försvara territorier, parning eller att jaga byten. De har en väldigt liten hjärna och få nervceller jämfört med däggdjur och trotts det utför de objektspårning med hög träffsäkerhet. I **Artikel 1** jämför jag förberedelse-tekniker för insekt-hjärnvävnad med syftet att optimera mängden detaljer i de neurala morfologierna som går att utvinna med mikroskopi. Jag använde teknikerna för ta fram en exempel-morfologi med hög detaljrikhet. Teknikerna användes även i **Artikel 2** där jag avbildade mofologin till en objektspårande nervcell från Blomflugan. I Artikel 2 mätte jag även en typ av korttidsminne som kallas responsfacilitering från en population av objektspårnings-nervceller. Det mättes genom att jämföra responsen till små objekt som färdas på en lång (primad) eller kort (oprimad) sträcka. I nästa experiment mätte jag igen den neuronala responsen men visade samtidigt ett distraktions-objekt som färdades utanför den delen av det visuella fältet som nervcellen mottaglig inom. Både primade och oprimade distraktionsobjekt ledde till minskad respons för objektspårnings-neuronet. Det här skulle kunna implementeras med långdistans-inhibering some en del av en uppmärksamhetsmekanism. **Arikel 3 & 4** involverade beräkningsmodellering av de objektspårande neuronen. Jag visade att en receptor (N-metyl-D-aspartat receptor), känd för sin inblanding i korttidsminnet, har några av egenskaperna som krävs för att generera facilitation. Sammantaget har resultaten från avhandlingen förbättrat vår kunskap och förståelse för de neurala mekanismerna för selektiv uppmärksamhet hos blomflugor och trollsländor. Den har också banat väg för framtida studier att expandera den kunskapen och förståelsen.

1 Introduction

1.1 What is selective attention?

The brain can be considered the organ in which our minds exist (Penfield, 1972). The perceived reality of the mind can be controlled by the saliency of external stimuli as well as by the brain itself using memories for example. These two control processes are the basis for the ability to allocate information processing power to a certain part of the neural representation of the world, and is called attention. A famous metaphor used in the attention research field is the "attentional spotlight" (Müller et al., 2003). The "attentional spotlight" is movable and facilitates processing within the beam of the spotlight. Fig 1.1 illustrates clay pigeon shooting and how it relates to attention. In the single target case the shooter must pay attention to a clay pigeon target being shot out from the machine in the right lower corner. The target which takes the path indicated in red must be attended, its movement predicted and then shot. In the more advanced case with multiple targets, a target must first be selected. Then, the target must be attended while ignoring the distractor targets. This ability is called selective attention. Finally, the shooter can predict the target movement and shoot the target.

The neural mechanisms of attention are not completely understood (Knudsen, 2007; Petersen and Posner, 2012), but it is generally hypothesized to be controlled through two processes. Firstly, if the decision to allocate processing power comes from the brain itself, it is called top-down attention (TD). Secondly, if attention is controlled by the saliency of a stimulus it is called bottom-up attention (BU) (Moore and Zirnsak, 2017). The ability to maintain the "attentional spotlight" on a certain part of the sensory input, while ignoring distractor stimuli, is called selective attention (Moran and Desimone, 1985). Humans use selective attention all the time with or without thinking about it consciously. Selective attention is not exclusive to humans and has been found in many animals such as non-human primates (Moran and Desimone, 1985), birds (Sridharan et al., 2014), dragonflies (Wiederman and O'Carroll, 2013; Lancer et al., 2019) and potentially other insects (De Bivort and Van Swinderen, 2016; Nityananda, 2016). The ability to attend a certain part of the sensory input and ignore other distracting sensory information is fundamental for the survival of all animals. For example, dragonflies can chase swarms of flies and still manage to catch one of them with very high probability (Olberg et al., 2000). Another insect with impressive aerobatic skills and ability to track objects is the

hoverfly (Collett and Land, 1975; Wijngaard, 2010). In this thesis I investigate visual selective attention using hoverflies and dragonflies.

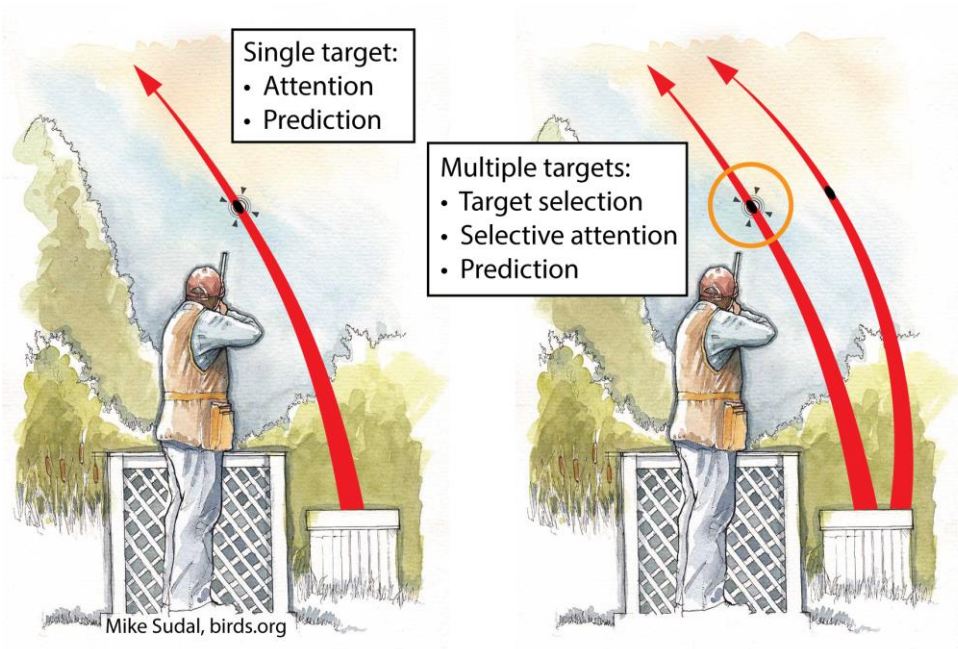

Fig 1.1 Illustration of attention using clay pigeon shooting.
(A) A single clay pigeon target is shot out from a machine in the lower right corner taking the path indicated in red. The target is attended, the path is predicted and the target is shot. **(B)** A more advanced case of shooting involving multiple targets. One of them is selected and attended while ignoring the other (distractor) targets. The path of the selectively attended target can be predicted and the target shot. Figure modified with permission from Mike Sudal.

1.2 Why study attention in insects?

One of the great challenges in neuroscience is that the brain has so many neurons and synapses, which makes it extremely hard to understand (Lisman, 2015). The insect brain is much smaller and has less neurons and synapses (e.g. 135 000 neurons in the fruit fly) (Alivisatos et al., 2012) compared to vertebrate brains (e.g. 71 000 000 neurons in the house mouse) (Herculano-Houzel et al., 2006) which in theory should make them easier to understand. Despite this relative theoretical simplification, there are still plenty of complex and interesting behaviors in insects, such as target tracking (Collett and Land, 1978; Olberg et al., 2000).

Dragonflies and hoverflies have neurons that are specialized for tracking small targets which are called “small target motion detectors” (STMDs) (O’Carroll, 1993; Nordström et al., 2006). The dragonfly STMDs have turned out to be a highly useful

tool for studying selective attention (Wiederman and O'Carroll, 2013; Lancer et al., 2019). Selective attention has been measured in a dragonfly STMD neuron by drifting two small squares (targets) across a screen (chapter 3.9 and 3.10). The neuron has the ability to switch between targets and even select a target that has lower contrast compared to the other target (Lancer et al., 2019). While evidence for selective attention in the dragonfly is starting to accumulate (Wiederman and O'Carroll, 2013; Wiederman et al., 2017; Lancer et al., 2019), the same is not the case for the hoverfly. Given the relatively small brain of the hoverfly compared to dragonfly and vertebrate brains we hypothesize that its STMDs form a highly efficient and effective target tracking system. This thesis presents the first evidence of attention-related processing in the hoverfly STMD neurons (Paper 2). Understanding this system could inspire the development of object-tracking systems enabling improved accuracy and predictability, or computationally miniaturized tracking systems.

1.3 Advantages of studying a dipteran brain

While the performance of the dragonfly as an object tracker is likely superior to that of the hoverfly, the dragonfly brain research suffers from three disadvantages compared to the hoverfly. The first disadvantage is also seen in many vertebrate brains: the brain is bigger, containing more neurons and synapses, making it more complex to understand. The second disadvantage that dragonfly researchers suffer from is that the species are not easily reared in a lab. Finally, the dragonfly is not dipteran and has known and unknown differences from dipteran flies regarding neurophysiology/neuroanatomy (e.g. olfaction) (Rebora et al., 2012). The extensive research on dipteran flies, such as the fruit fly and blowfly, is much more applicable to hoverflies than it is to dragonflies, leaving the dragonflies with a relatively less extensive research context. These reasons encourage research on dipteran flies with known skill in object tracking, such as the hoverflies (Collett and Land, 1975).

1.4 The lack of an explicit understanding of selective attention

Although great progress has been made on the neurophysiological understanding of visual selective attention in dragonflies, the computationally based mechanistic understanding (biophysically plausible) remains limited (Paper 3 & 4), or on an abstract (bioinspired) level (Wiederman et al., 2008; Bagheri et al., 2017). Computational models of visual selective attention, not specific to dragonflies, are also limited (Chik et al., 2009; Avery et al., 2012; Farah et al., 2017) or on an

abstract level (Tsotsos et al., 1995, 2015). One way to better understand the mechanisms is to build data driven computational models that mimic the underlying system as closely as possible. This thesis presents the first biologically plausible computational model that includes the dendritic morphology of a dragonfly small target tracking neuron (Paper 3 & 4).

1.5 General aims of the thesis

This thesis aims to investigate the underlying neural mechanisms of visual selective attention in hoverflies and dragonflies using neuroanatomy, neurophysiology and computational modelling.

To the best of my knowledge, there are no neuronal morphologies of hoverfly STMDs that have been reconstructed in detail and publicly shared and thus the neuroanatomical objective is to identify, characterize and describe STMD neuron morphologies using intracellular tracer injection. I addressed this aim in **Paper 1** by trying to find the best tissue clearing method for optimizing the amount morphological detail visible to confocal and light sheet microscopy. I then utilized these methods in **Paper 2** where I injected, recorded and imaged a hoverfly small field (SF) STMD.

SF-STMDs are presumed be to be upstream to to large field STMDs, and together with other neuron types, they form a target tracking system. With the pieces of evidence of selective attention in the dragonfly large field STMD neurons in mind (Wiederman and O'Carroll, 2013; Lancer et al., 2019), one can ask if similar evidence could be found in the hoverfly neurons. The SF-STMDs in dragonflies have only recently been shown to display response facilitation (Wiederman et al., 2017) which is a form of short term memory, but have not yet shown an involvement in selective attention processing. Can selective attention processing be found already in the SF-STMD layer that are upstream to LF-STMDs? And do hoverfly SF-STMD neurons show response facilitation?

A second aim of this thesis was to investigate these questions by recording the neural activity from the hoverfly STMD neurons while showing visual stimulus related to attention, such as moving targets. In **Paper 2**, I thus performed experiments focused on measuring response facilitation. I also displayed a target together with a distant distractor target to investigate how the putative facilitation influences selective attention. We found evidence for SF-STMD response facilitation and show that distractors do indeed modulate attention.

To further investigate and test the putative neural mechanisms involved in visual selective attention, as well as potentially generate new hypotheses, I developed a computational model (**Paper 3 & 4**) using the neuronal morphology of a dragonfly

STMD, and tried to replicate *in vivo* experimental results in computer simulations. We found that a receptor called N-methyl-D-aspartate (NDMA) receptor, famous for its involvement in short-term memory (Xia and Chiang, 2009; Purves, 2012) can generate some of the facilitation properties seen in dragonfly STMDs (Wiederman et al., 2017). We also found stronger facilitation when a dragonfly neuron was used compared to a control neuron (blowfly wide-field motion neuron).

In summary, the work in this thesis have resulted in an increased knowledge and improved understanding of the neural mechanisms of selective attention in hoverflies and dragonflies. Furthermore, it has as opened many opportunities for future projects to expand on using suggested experimental protocols or the provided computational modeling framework.

1.6 The structure and aim of the chapters ahead

The following sections are designed to ease you into the neural mechanisms of selective attention in insects, starting (chapter 2) by taking a step back from the very specific and mechanistic to gain a more general understanding of attention from a conceptual and cognitive perspective. After this, the reader is introduced to the puzzle pieces needed to understand the neuroscience of selective attention (chapter 3), namely the relevant neuroanatomical compartments, neuron subtypes and their neurophysiology. With the puzzle pieces in place I then introduce another type of puzzle pieces, the putative neural mechanisms of selective attention (chapter 4), that are connected to the previously introduced neuroanatomy, and also show how previous research has studied it on a single neuron level. I then take all of the puzzle pieces and introduce how researchers have been utilizing computational modelling (chapter 5) to test if the puzzle pieces fit together or not. Next, I explain my method for how to make sense of the puzzle pieces (chapter 6) by explicitly discussing the research projects of this thesis. The following three chapters (7, 8 and 9) describe the papers of the thesis. In the final chapter (10) I discuss and conclude based on the literature study and results.

2 Conceptual attention models

2.1 Attention models

Selective attention is a sub-task/phenomena of attention in general. In order to better understand selective attention, I here describe more general research on attention. It should be said that there is no strong general consensus on how attention works and which brain areas are involved (Knudsen, 2007; Petersen and Posner, 2012), but the following are two popular theories rooted in neuroscience experiments on monkeys, humans, birds and other animal species.

2.1.1 Attention model 1: a competition for working memory

One way of thinking of attention is in terms of sensory input information competing for access to working memory for further processing and control (Knudsen, 2007). The idea is that attention has four fundamental components:

- Working memory: stores selected information for detailed analysis over periods of seconds.
- Competitive selection: determines which information gets access to the working memory.
- Top-down sensitivity control: regulation of relative signal strengths of different information channels that compete for working memory.
- Salience filters: filtering for stimuli that are likely to be behaviorally important (salience filters). For example, stimuli that are infrequent or of instinctive/learned biological importance.

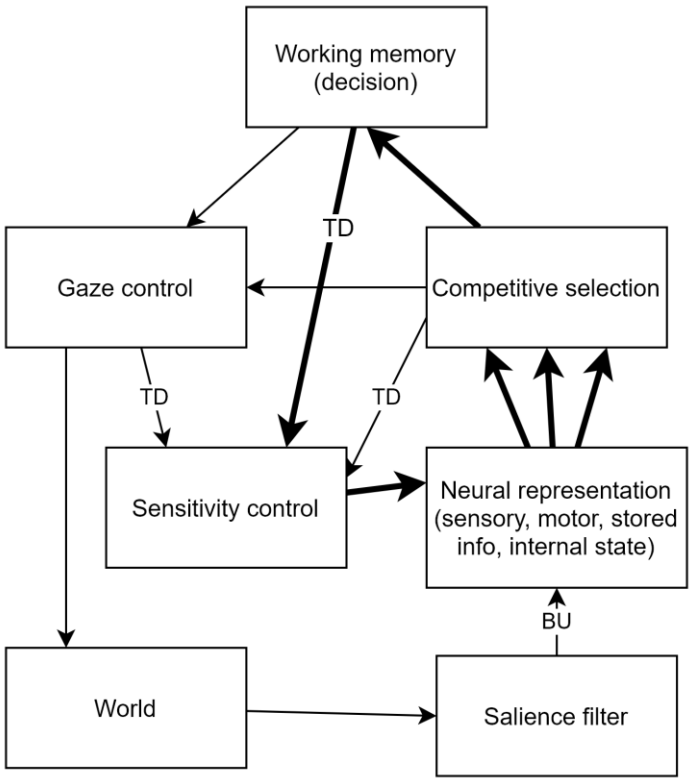

Fig 2.1. Functional components of attention. Includes indication of bottom-up (BU) and top-down (TD) attention processing. The thick arrows illustrate a recurrent loop underlying voluntary attention. Figure remade from figure 1 in Knudsen 2007.

Different parts of the sensory input information compete for access to working memory and their strengths are affected by signal quality, top-down bias and bottom-up salience filters. The winner gains access to the working memory and thereby access and control of the top-down bias signal underlying voluntary attention.

In Knudsen's 2007 review, there is a translational gap between the functional (Fig 2.1) and anatomical connectome models (Fig 3.4) of attention which is discussed in the next chapter. This translational gap means that finding the corresponding functional part in the anatomical model is challenging or not possible. Considering the large number of brain areas, neurons and synapses in the mammal brains it is not so strange that this is not known or hard to extract from data. Despite the translational gap, Knudsen's models are important since they improve and are fundamental for a systematic understanding of attention.

2.1.2 Attention model 2: a set of independent network systems

A second way of thinking of attention stems all the way back to the 1990s but was recently updated (Petersen and Posner, 2012). Posner and Petersen describe attention as consisting of three major systems that operate largely independent from each other. That is the alerting, orienting and executive network.

- Alerting network: This network involves arousal systems and systems related to sustained vigilance. In other words, becoming and staying attentive towards the surroundings. The network is modulated by norepinephrine (a neurotransmitter) and is supported by experiments showing that norepinephrine release influences alertness.
- Orienting network: This network directs attention towards a specific stimulus. Acetylcholine is a major neurotransmitter that is involved in the network.
- Executive network: Functions as top-down control which is used when there are multiple conflicting attention cues. They speculate that there might be two relatively independent parallel executive systems within this network.

2.1.3 Comparison of attention model 1 and 2

Without mentioning the specific brain area names here, the two attention models involve roughly the same areas brain areas. Although both models use experimental neuroscience and cognitive psychology to back up their models, and have their own fair share of speculation, Knudsen's reasoning and explanations are more explicit with the functional diagrams used for explaining system connectivity and mechanistic explanations, sometimes on a neuron level, as opposed to a brain area level. These two ways of modeling attention on a functional level may not be incompatible, but they are two ways of understanding the same system. There are some more apparent overlaps and differences when comparing on a neuro-anatomical level (section 3.2). The heterogeneity and variances in the explanations and the fact that these models have accumulated over many years of research indicates to me that it is a highly complex problem rooted in the extremely complex vertebrate brain in terms of number and types of neurons, synapses, and the brain areas that they form. This neural complexity is reflected in complex behavior and limits how deep our understanding of the brain is today. Research on animals with smaller brains (such as insects) with less and/or different neural and behavioral complexity could aid in understanding brains in general. Some evidence indicating that this is true will be presented in chapter 5.6 discussing neuron level connectomics. It should be noted that what has been discussed so far has been related to general attention and not selective attention, which have its own repertoire of (more conceptual) models.

2.2 Selective attention models

In cognitive psychology there are a number of conceptual models for selective attention which are potentially useful as reference models when investigating the phenomena in insect brains, despite the fact that the models mainly arose from studying selective attention in humans or other mammals. There are four major models that have arisen during the past 60 years. These models differ from the models described in the previous chapter in that they are less based on neuroscience and instead more general and based on psychology.

- Early selection model (Broadbent, 1958): unattended information is filtered out completely, early in processing.
- Attenuator model (Treisman, 1964): unattended information is attenuated early in processing.
- Late-selection model (Deutsch and Deutsch, 1963): all information is processed, and unattended information is filtered out only late in processing.
- Theory of perceptual load (Lavie, 1995): selection is early in difficult tasks, and late in easy tasks.

These conceptual models can be used to explain complex observations in which results may or may not show absolute/modulated selective attention. For example, many of the visual stimulus experiments in the work of this thesis could be considered as simple since they are one or two small black squares moving across a computer screen, with slight variations. According to the Lavie model, simple stimuli may thus promote late selection. Indeed, attentional switching in the dragonfly centrifulgal STMD1 (CSTMD1) neuron are not uncommon (Wiederman and O'Carroll, 2013; Lancer et al., 2019) (chapter 3.9). If the stimulus was more challenging, perhaps several targets moving in a complex pattern, then maybe the STMDs would have higher probability to lock on attention to a target early on and not switch.

3 The neuroanatomy and neurophysiology of selective attention

This chapter aims to describe the most important basic neuroanatomical and neurophysiological components of visual selective attention (including prediction). A lot of important attention research comes from studies using vertebrate brains. Thus, the first section describes and compares insect and vertebrate brain anatomy and function mainly related to the visual pathway, and the following three sections focus on vertebrate neuroscience attention research. The final five sections focus on insect neuroscience attention research.

3.1 Analogous visual neuroanatomy between the vertebrate and insect brain

This section describes basic insect and vertebrate visual neuroanatomy, the function of the brain areas and insect-vertebrate analogies. The insect brain can seem very different and odd to someone that is used to looking at vertebrate brains. The fly brain (Fig 3.2A) consists of the following compartments with respective function:

- Insect retina: the photoreceptors in the insect retina are the first cells that process the visual information and sets the limits for the visual acuity for feature detection (Rigosi et al., 2017). Many insects, including dragonflies and flies, have compound eyes (Fig 3.1) which is different in many ways from the vertebrate camera eye. The functional unit of the compound eye is called the ommatidia, which essentially contain a lens and subsequent photoreceptors which receive and transduce the visual information to the lamina (Fig 3.3B) (Land and Nilsson, 2012).
- Lamina (LA): consists of 12 neuron subtypes. The neurons are involved in contrast detection and contribute to motion detection but are not motion selective (Tuthill et al., 2013).

- Medulla (ME): selective involvement in motion detection including direction selectivity and small receptive fields (Tuthill et al., 2013). Has around 59 neuron subtypes (Takemura et al., 2008).
- Lobula complex (LO): consists of two subcompartments called Lobula plate that mainly processes wide-field motion vision, and Lobula which has neurons that selectively detects small target motion (Nordström et al., 2006; Keles and Frye, 2017), and has been implicated in prediction and attention in the dragonfly (Wiederman and O'Carroll, 2013; Wiederman et al., 2017).
- Mushroom bodies (MB): are associated with learning behavior including visual and olfactory modalities (Troy Zars, 2000), and has been suggested to be involved in attention-like behavior (De Bivort and Van Swinderen, 2016). The main neuron type is called Kenyon cells which consists of seven subtypes (Christiansen et al., 2011; Shih et al., 2019).
- Noduli (NO) and Central body upper/lower (CBU/CBL): the NO, CBU (also called fan-shaped body) and CBL (also called ellipsoid body) are part of a system called central complex which is involved in navigation (Green et al., 2017; Honkanen et al., 2019), but has also been suggested to be involved in attention-like behavior (De Bivort and Van Swinderen, 2016; Grabowska et al., 2020).
- Protocerebrum (P): is a large and complicated region that connects other regions, has premotor function and is a common output area for sensory information from optic lobes, central complex and MB.
- Antennal lobe (AL): is the main input center for olfactory sensory information from the antennae (Hansson and Anton, 2000).

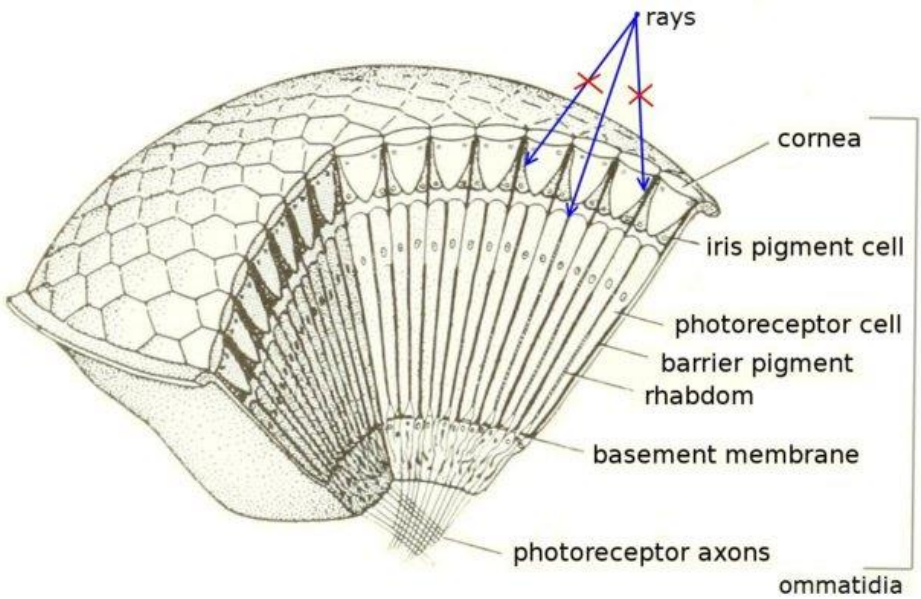

Fig 3.1. Illustration of a typical insect compound eye with illustration of the photoreceptors in the ommatidia. The blue light rays illustrate the directionality of the light that can enter the ommatidia and reach the photoreceptors. Figure taken from Khamukhin (2017) with original source Duke-Elder (1958).

The human brain roughly consists of the cortex and a large set of subcortical nuclei. The cortex wraps around the subcortical nuclei and this anatomy inherently makes it challenging to visualize functional pathways anatomically. This section is limited to include the brain areas involved in the initial and simplified part of the visual pathway, illustrated in Fig 3.2B. The following is a short functional description each of the brain areas:

- Retina: apart from photoreceptor cells the retina also contain interneurons and retinal ganglion cells which consist of many subtypes (Sanes and Masland, 2015) and perform computations, such as predictions to compensate for lag in the salamander & rabbit (Berry et al., 1999). The visual information is further fed to the optic nerve.
- Optic nerve is a bundle of axons that signals the visual information from the retina to the hypothalamus, pretectum and lateral geniculate nucleus.
- Hypothalamus: involved in regulation of circadian rhythm, hunger (Purves, 2004) and thirst (McKinley and Johnson, 2004).
- Pretectum: is important for reflex control of pupil and lens (Frost, 2010).

- Superior colliculus: is responsible for orienting the movements of the head and eyes. The owl homologue brain area (optic tectum) has been associated with attention (Knudsen, 2007).
- Lateral geniculate nucleus: is mainly a relay station and the activity is similar to that of the retina, and has for example been shown to relay attentional information across sensory modalities (auditory to vision) (McAlonan, 2006), but has shown involvement in receptive field refinement through connections with visual cortex (Tailby et al., 2012).
- Striate cortex: is the first visual cortical processing area (also called Primary visual cortex) and has neurons that respond to light-dark bar edges, with certain orientation, within the receptive filed. This population of neurons includes a cell type that respond selectively to small target motion called hypercomplex cells. The subsequent brain area in the continuation of the visual pathway is called the extra striate cortex and has been shown to process selective attention filtered information from the striate cortex (Moran and Desimone, 1985).

The processing pathways of each of the brain areas are further illustrated and described in Fig 3.3 and in relation to attention in section 3.2, 3.6.

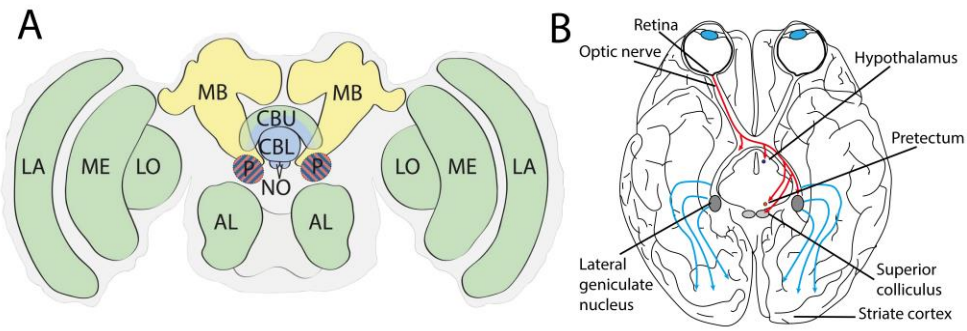

Fig 3.2. Illustration of insect and human brain. (A) General insect brain structure. Lamina (LA), medulla (ME), lobula complex (LO), mushroom bodies (MB), central body upper/lower (CBU/CBL), antennal lobes (AL), noduli (NO), protocerebrum (P). Optic lobe is a group involving visual processing including LA, ME, LO. Image taken from Klein and Barron (2016). **(B)** Human brain showing simplification of the initial part of the visual pathway that starts from the retina through lateral geniculate nucleus (LGN) and ends in striate cortex (Primary visual cortex) and branches to other visual processing areas. Redrawn with inspiraton from Purves (2004).

The optic lobe is a group of neuropils involving visual processing including LA, ME, LO. Although there is no strong consensus in the scientific literature, the protocerebrum and optic lobe putatively corresponds to the vertebrate retina (partially), lateral geniculate nucleus and visual cortex, illustrated in Fig 3.3. The authors of the article from which Fig 3.3 was taken (Sanes and Zipursky, 2010) has the opinion that the insect protocerebrum is homologous to vertebrate visual cortex. An alternative hypothesis (Nordström and O'Carroll, 2009) is that the lobula is

partially analogous with the visual cortex. The alternative hypothesis is based on the small target motion detector neurons (STMDs) and an analogous neuron subtype in the vertebrate, the hypercomplex cells of the visual cortex (Rose, 1977; Grieve and Sillito, 1991). The basic neurophysiology of these neuron subtypes are further discussed in chapter 3.7 and 3.8.

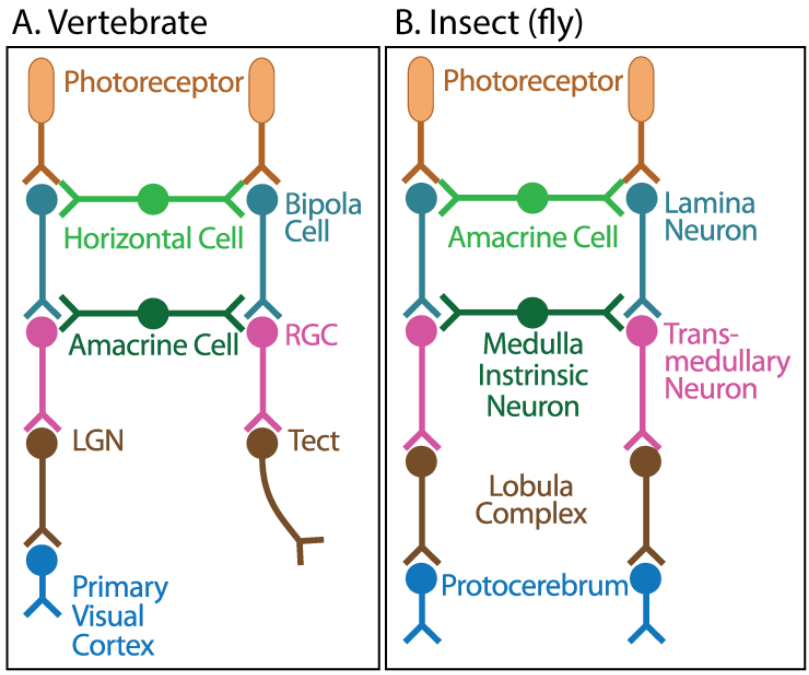

Fig 3.3. Analogy between vertebrate and insect (fly) visual processing pathway. (A) The main neuronal subtypes/brain areas involved in the visual pathway of vertebrate brains. From the retina there are the photoreceptor, horizontal, amacrine and retinal ganglion cells (RGC). These retina cells are connected to the lateral geniculate nucleus (LGN) and tectum. LGN is then projecting to primary visual cortex. (B) In the insect brain there are Photoreceptors and amacrine cells as well as lamina, medulla, transmedullary neurons, performing similar computations as the vertebrate retina. The lobula complex and protocerebrum then contains many of the neurons that respond similarly to those of LGN and visual cortex. Image taken from Sanes and Zipursky (2010) with modification.

Although the visual areas are analogous as described in Fig 3.3, in humans and some vertebrates, the source of top-down attention is often attributed to the prefrontal or parietal cortex (more discussed in next section). It is not known if a corresponding part in the insect brain exists. It may be built into the lobula or somewhere in the midbrain, such as the protocerebrum, central complex or mushroom bodies (De Bivort and Van Swinderen, 2016; Grabowska et al., 2020).

3.2 Vertebrate brain areas involved in attention

In this section we return to attention model 1 from Knudsen (2007) (chapter 2.3), and present the brain areas involved. According to Knudsen the fundamental components of attention are: working memory, competitive selection, top-down sensitivity control and salience filters. Fig 3.4 illustrates the different brain areas involved in top-down sensitivity control. Information from the world (already salience filtered) enters visual cortex and superior colliculus and from there travels to the complex path of the attention network. A brain area that is often associated with working memory is the prefrontal cortex. Although it was not specifically indicated in the original figure from Knudsen (2007), it was added here as the area responsible for working memory. This figure serves to list the brain areas involved in attention in mammal brains and convey the complexity of understanding attention on a neural level. For further understanding I refer to Knudsen's (2007) review.

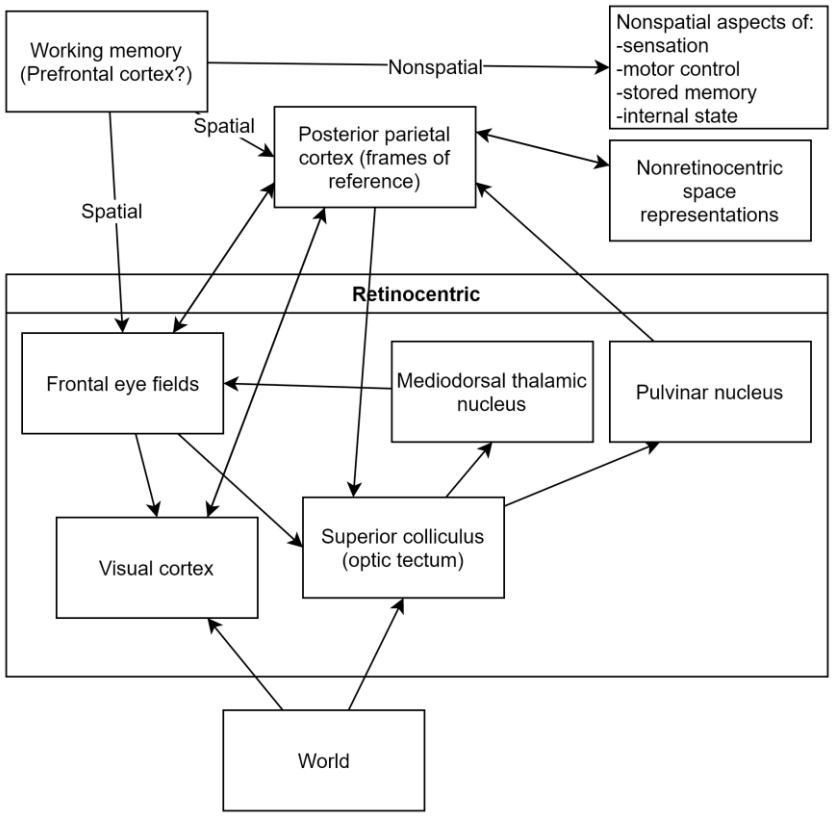

Fig 3.4. A network diagram explaining top-down sensitivity control. Information from the world (already salience filtered) enters visual cortex and superior colliculus. Working memory was assumed to be associated with prefrontal cortex. The translation of head vs retina centered position is believed to be taking place in the posterior parietal cortex, hence the text “frames of reference” in the parenthesis. Figure remade from Knudsen (2007).

Petersen and Posner's model for attention also involve many of the mentioned brain areas. Their model consists of three major systems for attention. The alerting, orienting and executive network:

- The alerting network (becoming and staying attentive towards the surrounding) is associated with locus coeruleus, the source of the neurotransmitter called norepinephrine.
- The orienting network (directing of attention towards a specific stimulus) is associated with parietal cortex. More specifically they see two attention networks that exist in that area. The dorsal attention system (frontal eye fields and intraparietal sulcus/superior parietal lobe) and the ventral attention system (temporoparietal junction and ventral frontal cortex). This system is associated with acetylcholine.
- The executive network (top down control for multiple conflicting attention cues) has been suggest to consist of two relatively independent parallel executive systems: the frontoparietal network (precuneus, medial cingulate cortex, dorsal frontal cortex, dorsolateral prefrontal cortex, intraparietal sulcus&lobe), and the cingulo opercular network (anterior prefrontal cortex, frontal operculum).

The mentioned brain areas for the Knudsen and Petersen-Posner model do not overlap entirely but there is a common focus on the frontal and parietal cortex. After reading this list of brain areas the reader is likely convinced that a brain with fewer brain areas, neurons and synapses (such as the insect brain) should be easier to fully understand.

3.3 Neurophysiological studies of vertebrate selective attention

In order to describe a more complete picture of the frontier of attention research, this and the following two chapters focus on attention research related vertebrate brain studies. There are many experimental studies of selective attention using the vertebrate brains involving mice (Zhang et al., 2014; Wang and Krauzlis, 2018), birds (Asadollahi et al., 2010; Sridharan et al., 2014), monkeys (Moran and Desimone, 1985; Rinne et al., 2017) and humans (Driver and Frackowiak, 2001). In this section two neurophysiological studies are described, presenting single neuron recordings during attention stimulus protocols, from the barn owl and the macaque. The barn owl study was selected since Attention model 1 (chapter 2.1, 3.2) was created by the same lab (Kundsen) and it has an interesting way of simulating top down attention. The macaque paper was selected since it is focusing on the visual cortex which is largely ignored by Attention model 1 and 2 (chapter 2.1, 3.2) and

records from visual cortex, a brain area with neurons that have similar response properties as those in the hoverfly and dragonfly lobula complex (Nordström and O'Carroll, 2009).

3.3.1 Bottom-up and top-down selective attention in the barn owl

In barn owl brains, the researchers (Mysore and Knudsen, 2013) tested bottom-up and top-down selective attention in the optic tectum (OTid, superior colliculus mammalian homologue) by blocking nucleus isthmi pars magnocellularis (Imc, called 'lateral tegmental nucleus' in mammals) activity using kynurenic acid (kyn, inhibits ionotropic glutamate receptors). Their hypothesis was that Imc mediates competitive inhibition to the OTid. They measure single neuron activity extracellularly in the OTid while showing visual stimulus on a screen (bottom-up stimulus) or by applying electric stimulus to the brain (top-down stimulus). In the case of both bottom-up (visual) and top-down (electric stimulation of brain) distractors, they show that blockage of Imc removes competitive inhibition in OTid recordings. This indicates that Imc is a key brain area involved in bottom-up and top-down selective attention.

The study is inspiring in the way that electrical stimulation is used to control attention. Perhaps the same could be done in the type of intracellular recordings of STMDs performed in this thesis (chapter 8.1). For example, attention could be modulated in a neuron by injecting positive or negative current to it before two moving targets are shown as visual stimulus. The hypothesis would then be that the neuron responds more likely to one of two the targets depending on the electrical charge sign.

3.3.2 Discovering a selective attention gate in monkey visual cortex

In this study (Moran and Desimone, 1985), the researchers show that one can attenuate the effectiveness of a visual stimulus using an ineffective distractor. An ineffective distractor is a visual stimulus that does not elicit a response in the recorded neuron. The visual stimulus response was attenuated when the stimulus and ineffective distractor were within the receptive field of the neuron. They found this effect in visual cortex area 4 (V4) and inferior temporal cortex, but not in striate cortex (V1) indicating a selective attention gate in the visual cortex.

The study is inspiring in the way that ineffective distractors are used to control attention. Such a stimulus could perhaps also be used in the type of intracellular recordings of STMDs performed in this thesis (chapter 8.1). For example, could a moving bar instead of a target (Wiederman et al., 2017) be used to enhance attention in a small target motion detector neuron? Or could a bar be used as a distractor instead of another target (Wiederman and O'Carroll, 2013; Lancer et al., 2019)?

This could reveal information about which neuron subtype is responsible for attentional modulation.

3.4 Studies of vertebrate prediction

From the introduction (Fig 1.1) we recall that target prediction is a type of brain computation that becomes possible when attention or selective attention (if distractors) is directed at the selected target. Object motion (small target motion) is a visual stimulus and experimental protocol that is used in this thesis for studying visual selective attention. Research on vertebrate selective attention rarely use small moving objects as stimuli. The fact that the selective attention stimulus used in this thesis is different from much of the other attention research makes it challenging to compare the results. Research involving prediction on the other hand, often use visual object motion stimulus. Predictive stimuli are also interesting since they are related to response facilitation which is hypothesized in this thesis to be a mechanism for selective attention. Furthermore, prediction requires attention as illustrated in Fig 1.1. I here present two studies that have measured object motion prediction in salamanders, rabbits and humans:

3.4.1 Prediction in the salamander and rabbit

Cells in the retina of salamanders and rabbits have been shown to predict object motion (Berry et al., 1999). In this study, the retina was extracted and mounted on a multi-electrode array and then shown a moving dark bar. The moving bar elicits a wave of response that does not lag behind as one might have expected due to cell physiology processes. The wave travels near the leading edge of the bar and it is believed to compensate for visual latency. The fact that prediction of object motion computation begins so early in the visual processing is quite interesting. That said, due to the recording method, they cannot specify any cell subtype responsible for the computation apart from "retinal ganglion cells" which is a very general cell type (Sanes and Masland, 2015).

This study tells us that predictive processing may be a type of computation that is spread out in the brain acting through multiple networks of neurons. Thus, in the insect brain, it can be worth studying the predictive properties not only in target motion detecting neurons, but also more general motion sensitive neurons such as many of the lobula plate tangential cells.

3.4.2 Hidden predictive information in human brain recording

In this study (Hogendoorn and Burkitt, 2018) the researchers used electroencephalography (EEG) with 64 electrodes on the scalp of humans. In the following experiments the subjects were asked to fix their gaze on a fix point but attend a square around the point at the same time. The square could take 8 different positions on a circle around the fix point. A machine learning classifier was trained to identify which EEG activity pattern that corresponded to each individual step position of the square on the circular path (trained to 55-60% correct identification).

In the first test protocol the subjects were shown the square, moving (with steps) on the circular predictable path. In the second protocol the subjects were shown the target at the same step positions but in scrambled (pseudo random) order. For each of the two protocols they used the classifier to estimate the chosen position using EEG data recorded continuously over the time course of the experiment. The first (predictable) protocol was identified around 16ms faster than the second (scrambled) one, indicating that the square position was predicted. The classifier analyzed EEG patterns from electrodes placed on the whole scalp and therefore the researchers could not conclude what brain area was responsible for the prediction. For this to be possible, the researchers would need to retrain the classifier using a subset of electrodes corresponding to specific brain areas. Although the researchers did not perform this extended analysis in this study, they speculate based on other research literature, that the predictive information mainly comes from the visual cortex.

This study reminds us that predictive and attention processing information is likely a general brain phenomena based on processing of many brain areas (De Bivort and Van Swinderen, 2016) and one target could be represented in different or the same way by multiple neurons.

3.5 Insect selective attention behavior

This and the following sections now turn the focus back to attention research based on insect brains. It is important to have behavioral studies in mind when designing, executing and analyzing neurophysiological, neuroanatomical and computational modelling experiments and data. Behavior is perhaps the most basic and important first type of observation one can collect when attempting to capture a cognitive phenomenon.

The relatively small size of the insect brains and low number of neurons compared to mammal brain gives the insect brain theoretically less complexity and should therefore be easier to understand. Despite the lower complexity there are plenty of complex behavior that insects perform. One such behavior is target tracking.

Dragonflies and hoverflies have been shown to have impressive target tracking and aerobatic abilities related to catching prey, mating, and guarding territory. The dragonflies have been shown to have up to a 97% chance of success when trying to catch a prey (Olberg et al., 2000).

In non-predatory insects such as the hoverfly the target detection is mainly used for courtship, mating and defending territory (Collett and Land, 1975, 1978; Wehrhahn et al., 1982; Boeddeker et al., 2003).

While the males are the primary pursuers, the females, while feeding on flowers, identify and keep track of males and other bees which are hovering above them or approaching (Thyselius et al., 2018). These behavioral experiments can help understand neural recordings and put them into a context. For example the female STMDs often have more lateral receptive fields compared to the males (Nordström and O'Carroll, 2006). This makes sense in light of the behavior data showing that they keep track of who is approaching or hovering around them (Thyselius et al., 2018).

Despite the dragonfly being a more superior target tracker compared to hoverflies, the main experimental animal used in this thesis is the hoverfly. Apart from the fact that the hoverfly is more abundant in the specific location in which the thesis work was being executed (Lund, Sweden), the behavior of the hoverflies makes them easier to catch compared to the dragonflies. Hoverflies are pollinators and often sit on flowers or defend territories in wooded areas/paths protected from wind on both sides. The dragonflies on the other hand often fly high in trees or over lakes to hunt swarms, mate and defend territories. This makes the dragonflies relatively more challenging to catch. Also, due to the hoverfly being a pollinator, companies such as Spanish Polyfly (Polyfly SL, Almeria, Spain) are rearing and selling flies and shipping them to various countries, including Sweden. Finally, the pollinating behavior enables future studies to utilize stimulus properties inspired by flowers (colors, shape) to bias attention or sugar (nectar) as rewards to control behavior in an attention related task. The same is not impossible in the dragonfly but would be a bit different and perhaps less straight forward. For example, a stimulus shaped like a small fly or with colors of conspecifics (Lancer et al., 2020) could be used to bias attention toward that target compared to a square target. To train the animal for attentional control one could use chemical drugs as reward or a laser for punishment.

The behavior controlled by the wings have, in dragonflies, been shown to be encoded in eight pairs of target-detecting descending neurons (TSDNs) (Gonzalez-Bellido et al., 2013). These types of neurons also exist in hoverflies (Nicholas et al., 2018) and receive putative input directly or indirectly from STMD neurons. Further description can be found in section 3.8.1.

3.5.1 Behavioral tracking strategies

Much like primates hoverflies perform saccades to optimize the extraction of spatial information from the retinal image displacements (Collett and Land, 1978; Geurten et al., 2010).

Some dragonflies use a "sit and wait"-strategy (perching) to catch their targets (Olberg et al., 2000). They sit and wait until they have spotted a prey that they predict is suitable based on target size and speed (Lin and Leonardo, 2017), and then quickly fly off on a target interception course. During the perching, they perform saccade-like movements. Other dragonflies begin the hunt while flying, for example while guarding territory or approaching a swarm (Lancer et al., 2020). One study claimed to have evidence that dragonflies also perform in-flight saccade-like movements (Olberg et al., 2005).

Dragonflies and hoverflies track and fly in an interception course rather than steering directly towards the target as other flies and insects do (e.g. houseflies) (Land and Collett, 1974) and honeybees (Zhang et al., 1990).

It is important to be aware of these tracking behaviors since the experiments of this thesis use experiments that locks the position of the body and head of the insect using wax. For example, the temporal frequency of the saccade-like movements may be able to explain some of the temporally related attention measurements like the time course of response facilitation in dragonflies and hoverflies.

3.6 Insect brain areas involved in attention

The STMD neurons of the dragonfly lobula have been strongly associated with attention (see chapter 3.9) (Wiederman and O'Carroll, 2013; Lancer et al., 2019). Other studies using the fruit fly have also mentioned the lobula, but also other areas such as the lateral protocerebrum (including the anterior optic tubercle), central complex (fan-shaped and ellipsoid body) and mushroom bodies (De Bivort and Van Swinderen, 2016; Grabowska et al., 2020). To the best of my knowledge however, no one seem to have created a brain area based wiring diagram for attention in insects, of the type shown in Fig 3.4. For this reason, I compiled the following (Fig 3.5) speculative brain area level wiring diagram of the insect brain with primarily visual, motor, attention and working memory related brain areas. The diagram is based on information from studies: Shih et al. (2015), De Bivort and Van Swinderen (2016), which are mainly based fruit fly brain research. Some connections are not included though to keep it simple and avoid speculation. The diagram can bee seen as a set of puzzle pieces with some relevant connections. Future studies could use this diagram to further connect the pieces.

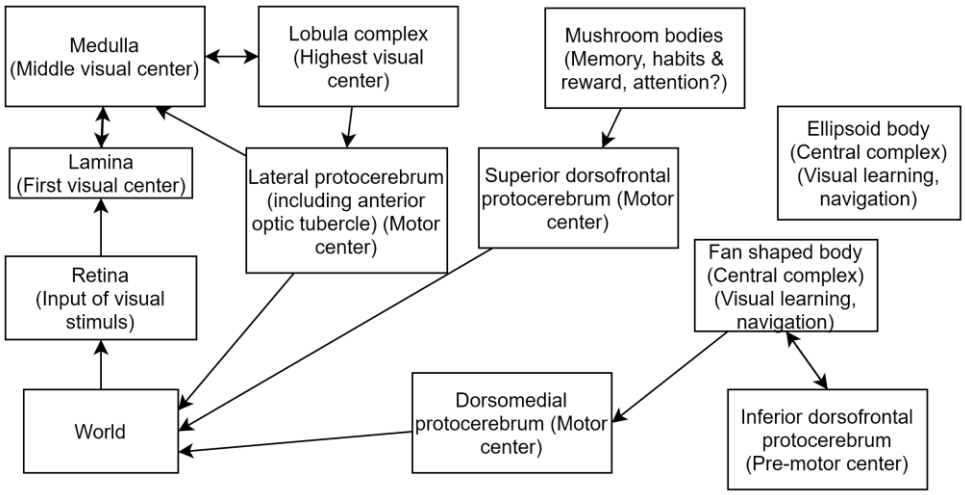

Fig 3.5. Wiring diagram of the insect brain with primarily visual, motor, attention and working memory related brain areas. Based on information from studies: Shih et al. (2015), De Bivort and Van Swinderen (2016). Plenty of arrows were left out to not make the diagram too cluttered and speculative. For example central complex is connected to the medulla/lobula complex.

3.7 Introduction to small target motion detector neurons (STMDs)

The small target motion detector neurons (STMDs) of the insect brain are a group of neuron subtypes in the lobula that responds selectively to small moving targets and ignores larger features such as bars and gratings. This neuron subtype is the main component in the method used to study selective attention and prediction in this thesis. A method to record activity from the STMDs is illustrated in Fig 3.6A, showing an animal in front of a computer screen with electrode that has penetrated a neuron of interest. Using the electrode, the membrane potential of the neuron is then amplified, recorded and stored for further analysis. This method was used in this thesis and to obtain the insect data in Fig 3.6B & C. Fig 3.6B shows the response of a hoverfly STMD neuron to a moving small target, bar and grating (Nordström and O'Carroll, 2009). A clear response is elicited in the case of the small target, and the bar merely generates some fluctuations from excitatory and inhibitory inputs and one spike. The grating does not generate much response at all. Fig 3.6C (top) shows a similar type of neuron in the mammal brain (primary visual cortex) called the hypercomplex cells and the dragonfly STMD (bottom).

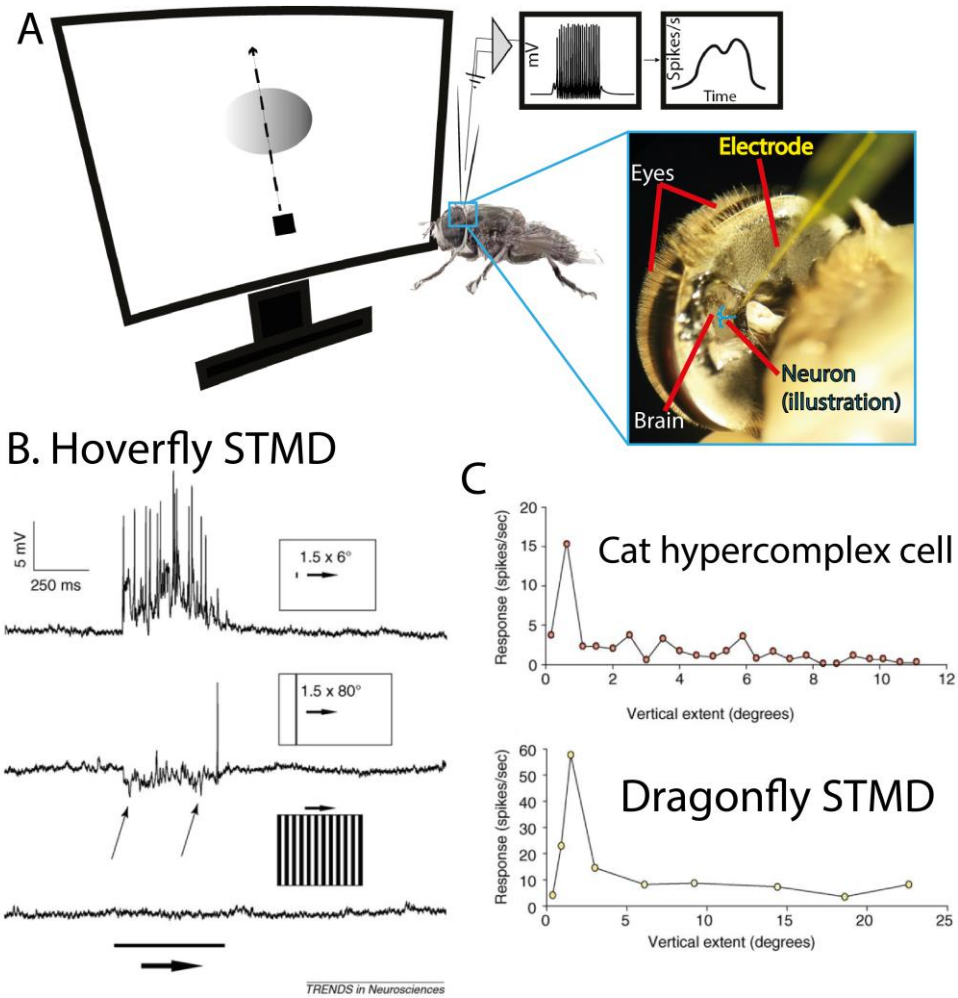

Fig 3.6. Illustration of the recording method and response characteristics of STMDs and a homologue in the mammal primary visual cortex. (A) Illustration of the general recording method used to record the response activity shown in the rest of the sub-figures. The gray shaded area indicate the neurons receptive field (visual area in which the neuron responds). **(B)** The response of a hoverfly STMD neuron to moving targets of varying size. **(C)** The top graph shows an STMD homologue from the mammals called the hypercomplex cells. The bottom graph shows the response characteristics of a dragonfly STMD. B and C was taken from Nordström and O'Carroll, (2009).

3.8 Neuronal subtypes of STMDs

To figure out how visual selective attention to small targets is achieved in insect brains it is important to know the neuronal subtypes that are likely to be involved in this neural network. In addition to looking at different insect species, it can be worth to look at cells with similar response properties in vertebrates.

3.8.1 STMD cell types of the dragonfly and hoverfly

Several main STMD subtypes have been characterized so far, including groups of small field STMDs (SF-STMD) and large field STMDs (LF-STMD), as well as identified neurons such as the centrifugal STMD 1 (CSTMD1) and binocular STMD 1 (BSTMD1). The main thing in common with these neurons is that they respond selectively to small targets and almost nothing to other types of stimuli. In addition, there are highly relevant subtypes such as the lobula giant motion detector (LGMD), target-selective descending neuron (TSDN) and the descending contralateral motion detector (DCMD) (Nordström and O'Carroll, 2009). For all of these subtypes there are many sub-subtypes of based on variations in excitability, direction sensitivity and size of the receptive field (part of visual field in which the neuron is responsive). The following is a brief description of the STMD neurons:

- CSTMD1 is an identified STMD neuron in the dragonfly, characterized by a receptive field on the contra lateral side compared to where the output and electrophysiological recording is made. It adapts quickly to repetitive stimulus and has been shown to have a predictive gain in front of the small targets that are being tracked (Wiederman et al., 2017) and it seems to be able to switch on and off between two targets in its receptive field (Wiederman and O'Carroll, 2013). While a CSTMD1-like neuron has been described in the hoverfly, they have not been analyzed as extensively as in the dragonfly (Nordström et al., 2006).
- BSTMD1 is an identified dragonfly STMD neuron that responds to visual stimulus from both sides of the visual field, but the graded potential on which the spikes ride is depolarizing when the visual stimulus is on the ipsilateral side and hyperpolarizing when on the contralateral side (Dunbier et al., 2012). In Paper 3 & 4 I used the morphology of this neuron to test if NMDA synapses could be the basis for its facilitation mechanism.
- The SF-STMDs have been found in both dragonflies and hoverflies and are often hypothesized to be the input to large field STMDs such as BSTMD1, but have been shown to have its own facilitation mechanism (Wiederman et al., 2017) in dragonflies and hoverflies (Paper 2). The receptive field is relatively small but there is a continuum of receptive field sizes in between SF-STMD and LF-STMDs. The receptive field is located at the ipsilateral side and as far as I

know there are no contralateral SF-STMDs. That said, there are SF-STMDs that have a receptive field location near the edge of the visual field and more so in the females compared to the males (Barnett et al., 2007). This can be explained by behavioral differences (chapter 3.5). In the fruit fly, recently, a study showed the selective response of L11 neurons to small target motion with receptive field sizes similar to that of SF-STMDs (Keles and Frye, 2017).

- The LF-STMDs have also been found in both dragonflies and hoverflies (Nordström and O'Carroll, 2006, 2009). They have been well characterized in the female hoverfly. Two out of three of the subtypes also respond to bars to some extent but they do not respond to gratings which is a critical characteristic. One of the subtypes does not respond to bars at all (maximally at tiny 0.8° drifting targets).

Apart from connections from STMDs to the same or different STMD types, there are likely direct or indirect output connections to the TSDNs (Gonzalez-Bellido et al., 2013). The TSDNs thus likely act as a link that process and transfer target tracking information from the optic lobe to the wing motor center. When the TSDNs are electrically stimulated the wings muscles are activated. The TSDNs exist both in hoverflies and dragonflies (Nicholas et al., 2018). A study in hoverflies show that background motion moving in the same direction as the targets, attenuates the response (Nicholas et al., 2018). They also show, and recently with larger evidence (preprint), that background motion in the opposite direction enhances the response (Nicholas and Nordström, 2021). Many STMDs ignores the background to a large extent and some are attenuated, but to date no study has shown an STMD with enhanced response from background motion in the opposite direction (Nordström et al., 2006).

The inputs and outputs of STMDs are not entirely known but there is some hypotheses and evidence based on overlapping arborization areas, electrophysiology, calcium imaging and pharmachological blocking. In the next section I describe some evidence which I combine with speculations to construct a putative connection diagram.

3.8.2 STMD-related cell types of the blowfly, housefly and fruit fly

There are many neurons that do not respond selectively to small target motion but could be involved in the computation of it. One large group of neurons are the lobula plate tangential cells (LPTCs) which generally respond to wide field motion (Nordström and O'Carroll, 2009). Wide field motion could potentially be used for subtracting away background movement when tracking small targets. A subtype of the lobula plate tangential cells are the figure detecting cells (Egelhaaf, 1985) that respond to gratings of circular size 6-40° (angular width) with less tuning for smaller features. They are clearly different from STMDs since they have a preference for

larger sized features and also have some response to wide field gratings (Nordström and O'Carroll, 2009). The LPTCs are illustrated in the connection diagram illustration in Fig 3.7. They are known to receive inputs from T4 and T5 cells which have their own set of inputs which will not be further discussed here (Haag et al., 2016). T4 and T5 are essentially feature detecting cells with preference to small targets compared to large bars (Keleş et al., 2020).

In the fruit fly these neurons have been is a genetically, morphologically and functionally defined (Keleş et al., 2020). There is another neuron that responds similar to SF-STMDs and is called Lobula Columnar cell 11 (LC11) (Keles and Frye, 2017). The inputs to LC11 are not well known but recently believed to come from a pair of "on-off" neurons with selectivity towards small objects, called T2/T3 (Keleş et al., 2020). The neuronal outputs overlap with the inputs of LC11 and when they blocked T3 the LC11 response was strongly reduced. They respond to both brightening and dimming events with preference toward small objects. T3 responds more strongly to dimming events (black targets). The fact that they found likely "on-off" unit (T2/T3) inputs to a STMD-like cell (LC11) supports the hypothesis that the dragonfly/hoverfly STMDs receive inputs from a "on-off" unit, also called rectifying transient cell stated in computational modelling studies (Wiederman et al., 2008, 2013) called elementary STMD model (ESTMD). The rectifying transient cell in the modelling studies never showed any evidence for "on-off" units with selectivity for small objects. Thus, the discovery of the likely connections from T2/T3 to LC11 adds support to the underlying motivation for the ESTMD computations. From the evidence presented in this and the previous section I constructed a hypothetical STMD pathway connection diagram (Fig 3.7). Due to the speculative nature of this diagram, it should be considered a set of unfinished puzzle pieces rather than evidence based illustration. I put a T5 as well as T3/T2 connections as input to the SF-STMD neuron to indicate that the other insect species may have subtypes of the SF-STMDs, such as direction selective SF-STMDs, that utilize the T5, which is a direction selective neuron.

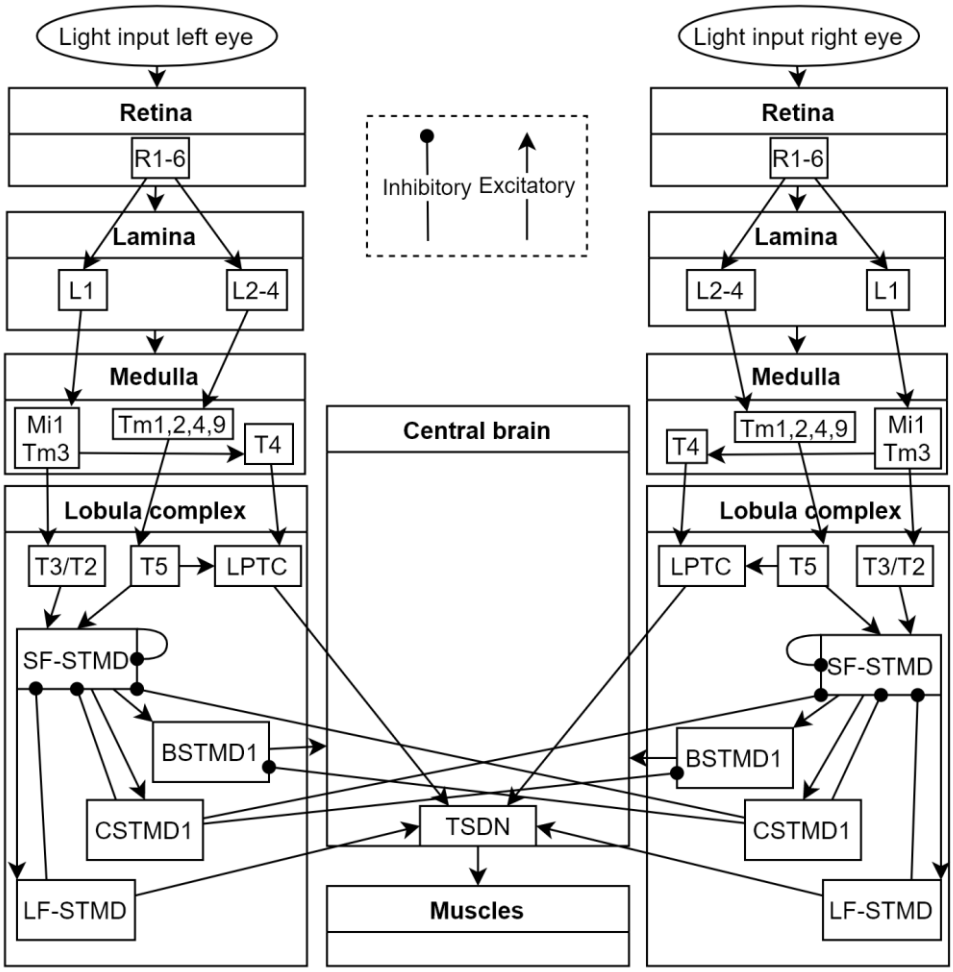

Fig 3.7. Illustration of neuron connections in the fly and dragonfly brain based on evidence (Haag et al., 2016) and speculations. Shows how light is input to the photoreceptors of the retina (R) connecting to the Lamina (L), transmedullary (TM) neurons, medulla interneurons (Mi), T2, T3, T4, T5 neurons and lobula plate tangential cells (LPTCs). The connection from Mi1 and Tm3 to T2 is not evidence based. Connection from the retina to the LPTCs are evidence based (Haag et al., 2016). Some of the SF-STMD to CSTMD1/BSTMD1 connections were inspired by studies which also speculated regarding some connections (Bolzon et al., 2009). There is some evidence for the existance of interaction between BSTMD1 and CSTMD1 (Dunbier et al., 2012). The additional neurons and connection are speculative. It is not known how they connect and to what extent they exist in flies. The BSTMD1 and CSTMD1 information (intracellular recordings and tracer fills) are mainly from dragonflies (Dunbier et al., 2012).

3.8.3 STMD-like cells in vertebrates

There are indeed many STMD subtypes in the insect brains, some of which appear to be found in the simpler (fruit fly) as well as the more advanced species (regarding target tracking, dragonfly). As mentioned before, in mammals the hypercomplex neurons have very similar response to STMDs of insects (Nordström and O'Carroll,

2009) and is located in the primary visual cortex (V1) of for example cats (Kato et al., 1978), monkeys (Hubel and Wiesel, 1968), mice and rats (Metin et al., 1988). It should be said that many of these studies used light stimulus as opposed to the commonly used dark stimulus for STMDs and often state "mix" or state it unclearly in relation to the specific hypercomplex cells, likely due to many cell types being studied simultaneously. The hypercomplex cells appear to be less well studied compared to the insect STMDs, possibly because there are more brain areas and neuron subtypes in the mammalian brain and that the mammals have stricter ethical rules (or that insects lack ethical rules). One example of an attention-related experimental paradigm that does not seem to have been tested a lot in the primary visual cortex is selective attention to object motion.

Another brain area that contain neurons which respond to small target motion and has been associated with attention is the superior colliculus (optic tectum in birds) (Gale and Murphy, 2016; Zahar et al., 2018). The neurons in this brain area consist of various subtypes depending on how they respond to target properties. Some of the neurons seemed to have small target selectivity, but most neurons also responded to very large sizes (e.g. 30°). The size response tuning curves of Gale and Murphy (2016) is somewhat similar to that of the blowfly feature detecting neurons rather than the highly selective STMDs of dragonflies and hoverflies.

3.9 Studies of insect selective attention and prediction

This section discusses studies of selective attention and prediction. The studies were selected based how related/similar they were to the experimental protocols of thesis papers. Thus, it was preferred if the stimulus resembled a visual moving target, if frequency tagging was used, if the insect had flying abilities or even better, if it was a hoverfly/dragonfly. The similarity between these studies and the thesis papers simplifies the comparison of the results. The most convincing evidence for selective attention in insects was reviewed in De Bivort and Van Swinderen (2016). Two studies were selected from the review, and are presented in the next sections. The studies both involve a method called frequency tagging, in fruit flies (van Swinderen, 2012) and bees (Paulk et al., 2014) respectively. The last three studies describe studies involving the small target motion detector neurons (STMDs), one of which also uses the frequency tagging method.

3.9.1 Attentional fixation on frequency tagged stationary objects

In the first study (van Swinderen, 2012), van Swinderen put tethered fruit flies in front of a screen while recording the local field potential (LFP) from the brain (mainly from optic lobe neurons). The flies could control the horizontal position of

objects on the screen using their wing beat. A behavioral indication of attention is fixation. That is when an animal locks its position toward a certain direction. The visual stimulus consisted of objects in shapes of "+" or "x" of size 30°.

In the first experiment, one object was shown that was either a "+" or "x", and it was flickering at 7 or 9 Hz (frequency tagging). This frequency could be measured in the LFP recording indicating which of the objects the animal was paying attention to.

In the second experiment, two objects were shown ("+" or "x", on top of each other) with different frequency (7 or 9Hz) for each. A spectrogram (frequency power over time) of the LFP response during fixation behavior showed that the response power to the two frequencies were sometimes uncorrelated. The uncorrelated activity was interpreted as the result of attentional processing.

In the two final experiments, van Swinderen tests how novelty affects the response by setting the object to alternate between "+" and "x" with random time in between 5-50s, without changing frequency. Dots in the visual surround were used as distractors. The flies increase response power in all behaviors, even when "Flying but not fixating" and "not flying". These results indicate that increased attention due to novelty may be independent from behavioral indications of attention, such as fixation. For example, the fly could attend to something even though it is not directing its body towards that target.

Finally, it is concluded that LFP responses to visual flicker is dependent on behavioral state, stimulus history and salience. This suggests that endogenous mechanisms modulate synchronized neuronal response to visual flicker. One major conclusion is that changes in the direction of attention does not have to be associated with active behavior. This last observation is interesting since a major experiment of this thesis involves locking the position of the insect body and head with wax while showing visual stimulus on a computer screen in front of it.

3.9.2 Attentional fixation on frequency tagged moving bars

In the second study (Paulk et al., 2014), bees were put in front of a screen which they could control by walking on an air-supported ball. The researchers showed bright green bars on the screen, flickering with certain frequencies. They could measure the individual tagging frequency in the neural response (local field potential).

In a first experiment, the bar moved 90° during 300ms to the left or right and the bee re-fixated the bar back in front of itself.

In later experiment, when showing two bars, the bee either fixated between the bars (bars close together 30/60°) or alternated fixation on either bar (90-180°). Further testing using 90° between bars revealed that in 33% of cases the bee refocused the

"original" fixated bar after displacement (even if the distractor bar was in front of the animal).

The movement of the two bars were recorded and replayed to the fly. The researchers found that when the stimulus was replayed stimulus the neural response was weaker compared to when the fly was in control. This means that the behavior itself was inducing some of the response activity indicating top-down attentional involvement.

The response to the two competing frequencies (90° separated bars) was anti-correlated. This was also the case when the bee was not in control of the screen (open-loop). However, when the bee controlled the screen (by walking), they found medulla and lobula frequency specific activity (selectivity) BEFORE the behavioral switch (BEFORE=selected object not yet in front of animal). When the animal was not in control of the screen (but same stimulus was shown) the optic lobe activity only increased AFTER the object switch. They interpret this as an association between fixative behavioral and top-down attention, whereas if the animal lacks behavioral control to fixate, it is bottom-up attentional activity.

One interpretation of both of these two studies is that while attention may be involved in experiments that do not enable active behavior, that "active behavior lacking"- attention could be either bottom-up (study two indicates this) OR potentially top-down attention (first but not second study indicate this). Another observation is that the first study hint that there may be independent attentional systems, which relates to Posner and Petersen's attentional model (Attention model 2, chapter 2.1.2).

3.9.3 The dragonfly STMD neurons as a tool to investigate selective attention

The following three studies focus on selective attention and prediction in the dragonfly using STMD recordings. In the first study (Wiederman and O'Carroll, 2013) the researchers recorded from the CSTMD1 neuron while drifting small targets into its receptive field. As illustrated in Fig 3.7, two target paths were used and the response pattern for each path was first established by showing the moving targets one at a time. The researchers then displayed the two targets simultaneously and observed that the response followed the response pattern of either one of the targets or the other but not a combination of both (such as sum or average), except for occasional switch between them as illustrated in the Fig 3.7 (Response to both). This indicated that one target was attended while the other was ignored which is the definition of selective attention.

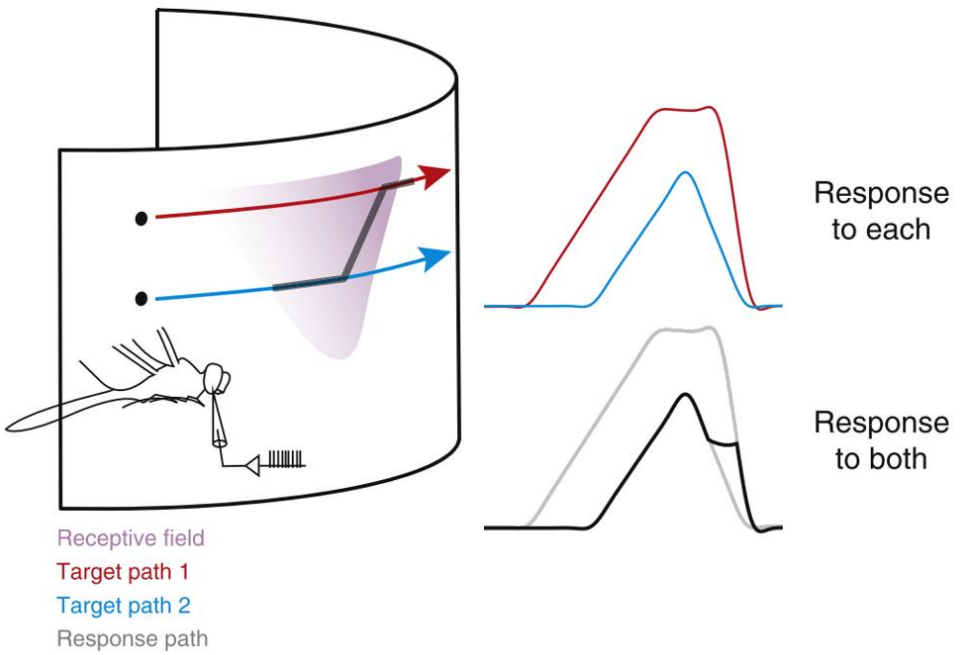

Fig 3.8. Illustration of a selective attention experiment using dragonfly CSTMD1. A dragonfly is set in front of a computer screen while an electrode is recording from a CSTMD1 neuron. Small moving targets are shown on the screen and drifted into the receptive field (purple shaded area) of the neuron. A response pattern (red and blue, "Response to each"), showing spike histogram over time, for each of the two indicated paths (red and blue) was established by showing the targets one at a time. Then the two targets were shown at the same time and "Response to both" indicates a response. The figure was adapted from: Frye (2013), which is based on research from Wiederman and O'Carroll (2013).

3.9.4 Using frequency tagging on dragonfly STMDs to further study selective attention

In the second study (Lancer et al., 2019) the dragonfly CSTMD1 was again used. The novelty of the study was firstly the application of a stimulus technique called "frequency tagging" on intracellular neuron recordings. Secondly, the researchers show even more convincing evidence that the CSTMD1 selectively attends one target and ignores the other as in the previous research (Wiederman and O'Carroll, 2013). Finally, they show (Fig 3.9) that if one of the targets had been previously shown moving (priming) and then continues on the same path with a distracter target that pops up on the side, then the dragonfly is more likely to keep its attention on the primed target. This was also the case if the primed target had lower contrast than the unprimed distractor. This concept of priming is described and discussed in the next section in relation to object motion prediction.

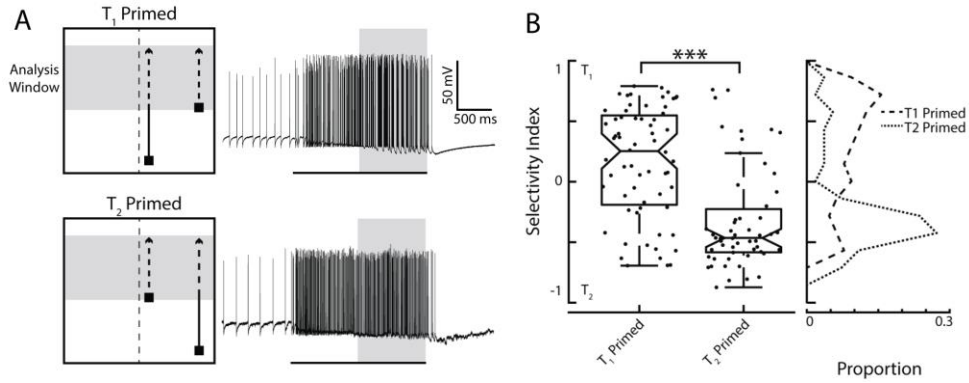

Fig 3.9. Illustration and response of frequency tagged paired targets with one of the targets primed. (A) Shows the experiment and the analysis window in gray from which the spike rates in (B) were averaged. **(B)** Boxplot and distribution of responses of paired targets with either T1 or T2 target primed. Each of the two targets are flickering with different frequencies. The selectivity index is a measurement of which target (T1=1, T2=0) that was attended based on how much frequency that was encoded in the spike rate. Figure was taken from (Lancer et al., 2019).

3.9.5 STMDs as a tool for studying prediction

The dragonfly STMDs have for many years been hypothesized to be involved in object motion response amplification, with facilitation as the neural mechanism (Nordström et al., 2011). Facilitation is a type of short term memory mechanism (Zucker and Regehr, 2002).

Recently it has been shown that the facilitated neural activity of dragonfly STMDs contain predictive information (Wiederman et al., 2017). Given the high performance of the dragonfly as a target tracker it is highly likely that this predictive information is used for object motion prediction (Wiederman et al., 2017). To demonstrate this, the researchers used the so called primer & probe experiment illustrated in Fig 3.10A. In the first trial type (Probe) the target drifts inside of the receptive field of the neuron generating a spiking response. In the next trial type (Primer & probe) the probe is preceded by a target starting further back on the same path, called primer. The idea is that the preceding target (primer) lets the neuron build up neural activity (facilitation) that enhances the activity during the probe path compared to when the probe was shown alone without the primer. This facilitation phenomenon had already been shown in the dragonfly CSTMD1 in 2011 (Nordström et al., 2011), but in the 2017 study the researchers pushed the protocol further by calculating the so called "facilitation field". The probe path was shortened and its position varied systematically over the receptive field of the neuron (Fig 3.10B). The primer position was locked to a certain location and the probe response was measured with and without the primer (Fig 3.10B right). The difference between these two values is the so called facilitation measurement (ΔResponse). The facilitation field is indicated in Fig 3.10B (right), with the red being facilitation and blue being negative facilitation. The facilitation hotspot, indicated in red, is in

front of the primer, indicating that is carries predictive information. The authors observed that the facilitation field spread over time by comparing a pause between the primer and the probe of 0 and 300ms (Fig 3.10B top vs. bottom right).

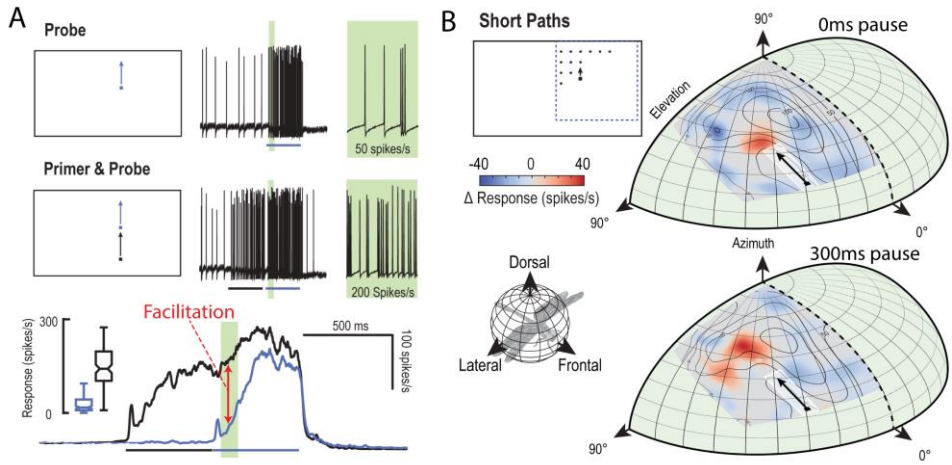

Fig 3.10. Illustration of the Primer & Probe experiment and its adaptation to measure the facilitation field. (A) The Primer & Probe experiment (top and mid) with an indication of the facilitation measurement (bottom). **(B)** The short paths (top left) illustrates the shortening and variation of the Probe position used to calculate the facilitation field. The facilitation fields (right) indicating the fixed primer position. The red indicates facilitation and blue indicates negative facilitation. The facilitation field at the top has a 0ms pause between the primer and the probe whereas the facilitation field at the bottom has a 300ms pause, demonstrating the spread of facilitation over time. The figure was adapted from Wiederman et al. (2017).

4 The neural mechanisms of selective attention

This chapter lists and discusses evidence for potential neural mechanisms underlying attention in insects and mammals.

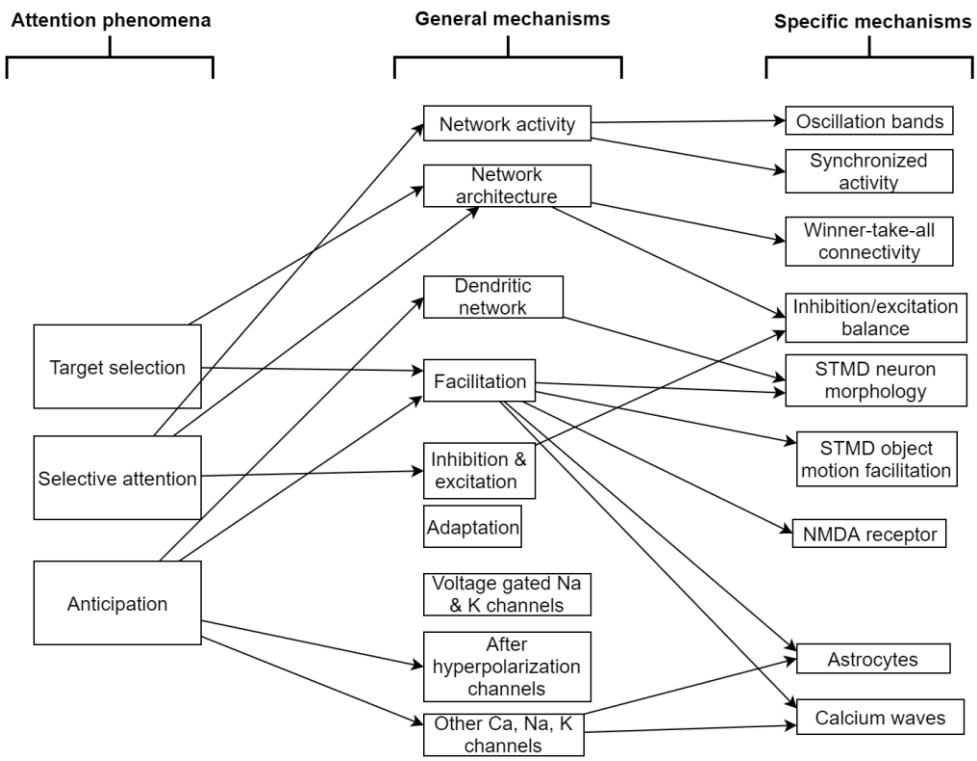

Fig 4.1. A connection diagram between attention phenomena/functions and the putative general and more specific neural mechanisms. The references for the diagram is presented in the following sections. The arrows between "Attention phenomena" and "General mechanism" indicate an example mechanism that could be responsible for the attention phenomena. Similarly, the arrows between "General mechanism" and "Specific mechanism" is an example of a more specific neural mechanism. The specific mechanisms also relate to the specific PhD sub-projects, which are described in chapter 6.

4.1 General synaptic function: Excitation and inhibition

The brain contains many excitatory synapses, and one large group is the glutamate receptors which includes both ionotropic (receptor binding leads to cell membrane channel opening) and metabotropic (receptor binding leads to signal cascade and later channel opening) receptors. The ionotropic receptors are faster than the metabotropic receptors but the metabotropic often have more sustained signaling effect. For example, for the neurotransmitter glutamate, ionotropic receptors include the subtypes kainate, AMPA and NMDA receptors (Purves, 2012).

Local inhibition likely serves as a filter for small target selectivity (Wiederman et al., 2008) and is likely involved in inhibition between targets that are close in space (Bolzon et al., 2009). It is not known however if this inhibition is related to attention or only small target selectivity. Long range inhibition is likely to have a role in attention. For example, in dragonflies a neuron called CSTMD1 responds strongly to small target motion in the contralateral visual field to the recording site and is inhibited by motion in the ipsilateral side (Bolzon et al., 2009). This function would be needed for attention to work in an interocular rivalry tasks. Furthermore, a study utilizing the mouse retina connectome and electrophysiology on goldfish, using 2.4° moving dark bars, concluded that motion prediction arises from excess of inhibitory over excitatory inputs to retinal ganglion cells, and that these inputs need to be randomly distributed (Johnston and Lagnado, 2015). They show evidence that excess inhibition to excitation input on to dendritic trees of retinal ganglion cells, rather than lateral inhibition or feedback inhibition, is the mechanism for prediction at this early stage of processing.

Excitation and inhibition enable neurons to enhance or silence the activity of other neurons. However, there are more advanced synaptic mechanisms that silence or enhance neuronal activity over time, such as facilitation, which is a form of short-term plasticity. Synaptic inputs lead to enhancement of the response to subsequent excitatory input. Another mechanism is adaptation, with which synaptic inputs lead to reduction of the response to subsequent excitatory input.

4.2 More advanced synaptic function: Facilitation and adaptation

Facilitation and adaptation are both synaptic functions which serve to remember recent spiking activity (short-term plasticity). Facilitation implies that previous synaptic input makes the neuron spike more easily (increased excitability) for a certain time period whereas adaptation means that the neuron becomes less

excitable. This section focus on facilitation, but adaptation could also be involved in attention.

To achieve attention, it is reasonable to assume that an amplifying mechanism is involved (Shoemaker et al., 2013). A candidate receptor with this ability is the N-methyl-D-aspartate receptor (NMDA) receptor. When an NMDA receptor binds to a neurotransmitter (glutamate) and opens the ion channel it lets through sodium and calcium ions. NMDA receptors respond nonlinearly to input and more specifically: the higher membrane potential the more positive response (facilitation) up to a certain turning point after which it responds more linearly. Assuming that there is winner-take-all inhibition between a network of cells with NMDA input synapses, the first neuron or group of neurons to gain a slightly higher membrane potential should become a winner and silence its neighbors. The winner could thus be thought of as the neuron which acquired the attention.

Typically, when a NMDA receptor is activated by receptor binding, it drives the membrane potential from the resting membrane potential (e.g. around -70mV) towards 0mV. A characteristic of the NMDA receptor is that it is blocked by a magnesium ion, which enables it to respond less to receptor binding when the membrane potential is low (e.g. -70mV) but more when the potential is a bit higher (e.g. -50mV) as the magnesium ion is removed and not blocking any more. This response characteristic is not typical of excitatory receptors (it is non-linear). Usually a receptor would respond less the closer it gets to the membrane potential level (linear) it is driving towards (0mV in our example) (Groc et al., 2002; Shoemaker, 2011; Amakhin et al., 2018).

NMDA receptor homologues have been found to be widely expressed in many insect brains (including optic lobe), such as fruit flies and bees which could be considered close relatives of hoverflies and dragonflies (compared to mammals) (Xia and Chiang, 2009; Davis et al., 2020).

In chapter 3.9.5 I describe that facilitation measurements from the dragonfly has been shown to be predictive several hundreds of milliseconds after the target disappeared. The evidence of hoverfly response facilitation presented in Paper 2 only demonstrates weak predictability due to experiment design limitations. However, even without predictability stretching far in to the future, facilitation could be involved in saliency based target selection and sustained attention since it enables a history based sensory input representation and not just some potentially transient fluctuation in saliency. Sustained attention is the ability to keep attention on one thing for an extended (relative to behavior and species) period of time. Although dragonflies are capable of switching attention between targets, the tendency is to keep attention on one target at a time when shown two small moving targets (Wiederman and O'Carroll, 2013). This observation supports a short-term memory based attentional focus. Another interesting observation (Wiederman et al., 2017) is that the predictive facilitation in dragonflies seems to be spreading like a

wave-like spotlight, and it is not entirely clear what type of synaptic mechanism is required to do this, leading to speculations of other types of signaling, such as calcium waves.

4.3 Calcium signalling and waves

Astrocytes are a type of glial (neuron support) cell and are usually associated with mechanical support, ion channel control and neurotransmitter uptake in the brain (Purves, 2012), but in this section we will look at an alternative role that they may have. The insect brains contain astrocyte-like glia cells that have similar functions. There are generally less glia cells in insect brains (10-25%) compared to mammalian brains (around 50% in humans) (Edwards and Meinertzhagen, 2010; von Bartheld et al., 2016).

During recent years astrocytes have become a hot topic of discussion regarding alternative roles, potentially involving more direct neural processing (Bazargani and Attwell, 2016). The primary ideas are that they are regulators of neuronal spiking, synaptic plasticity and brain blood flow. Another question is whether or not this hypothetical form of processing is involved in visual attention. Indeed, there seems to be an interesting link between astrocytic calcium, NMDA activation and attention in the mouse somatosensory cortex (Takata et al., 2011). The researchers found that astrocytic calcium signaling links cholinergic activation with attention and vigilance states, and somatosensory plasticity. Another study found that astrocytic glutamate release, through NMDA receptors, synchronized the spiking of neurons in a hippocampal rat brain slice, and concluded that calcium waves were not the mechanism in this scenario. Although these are very different animals and brain areas, they provide clues that can be used for hypothesis generation.

One purpose for the astrocytes could be to prepare neurons for intense activation by increasing blood flow to that area and perhaps in that way even control which neurons that will be able to handle the intense firing required for sustained attention (Bazargani and Attwell, 2016).

2-photon calcium imaging has been frequently used in recent years. However, the specific role of calcium is often not clear, and instead, calcium is used as an indicator or correlator of sodium and potassium based spiking activity (Ramirez and Stell, 2016). The LC11 neuron in the fruit fly has been characterized as an object detecting neuron (Keles and Frye, 2017) and respond similar to STMD neurons regarding small target selectivity. In the study, all measurements were made using 2-photon calcium imaging. But it is hard to know the functional purpose of that calcium. A connection between calcium waves, astrocytes and NMDA receptors and insect attention is thus still very hypothetical.

Excitation/inhibition, facilitation/adaptation and calcium waves tell us about the type of neuronal connections, what is enhanced/silenced and how activity can persist and spread, but it does not say so much about the action potentials (spikes) which is the main information transfer method for neurons.

4.4 Voltage-gated ion channels

The voltage gated sodium and potassium channels in a neuron enables it to generate action potentials (Purves, 2012). There are various ways a neuron can generate action potentials, especially if one considers the theories of dynamical systems neuroscience (Izhikevich, 2007). A simplified interpretation of the theories is to think of the neuron membrane potential as something that orbits on a 2D plane where one axis is the potassium conductance and the other axis is the sodium conductance. As input to the neuron arrives and initiates the journey, the neurons membrane potential then wanders on this orbit as it performs the action potential and can gravitate towards or repel from certain points in the plane (action potential turning point, or resting membrane potential point). This enables interesting neuronal subtypes to be defined which could of course matter for the cognitive function in which the neuron is involved. Therefore, it is tempting to ask what type of spiking the STMD neurons are executing.

Although the subtypes that the theories of dynamical systems neuroscience enables are diverse, they require significant simplification of the conductances. There are various subtypes of ion channels and one diverse group are the afterhyperpolarization channels that can generate fast, medium and slow effect afterhyperpolarizing currents (Shah and Haylett, 2017).

Excitation/inhibition, facilitation/adaptation, calcium waves and voltage-gated ion channels tell us about the type of neuronal connections, what is enhanced/silenced and how activity can persist and spread, and how neurons can spike, but it does not say so much about the timing of the collective spiking activity between neurons and how they are connected as a whole.

4.5 Network activity and architecture

It has been proposed that synchrony between neurons is a mechanism for selective attention and there is some correlation between the two phenomena that has be measured in vertebrate brains (Womelsdorf and Fries, 2007). Not only network activity is likely to be important though, but also the way in which the network is connected.

A common network architecture that is associated with attention is the winner-take all architecture (Itti et al., 1998; Shoemaker et al., 2013). In this architecture, typically, each neuron inhibits all or a subset of the other neurons in the network by direct inhibitory connections or by activating a separate inhibitory network that inhibits all neurons in the network (including itself). In this way, the neuron with highest activity stays active. In the case of multiple input of varying strength, the strongest (saliency) would be active in the neural network.

Another type of network architecture is the one within the neurons defined by the neuronal morphology. Neurons are built up of various combinations of input and output arborizations (dendrites and axons) and a cell body (Purves, 2012). The axons and/or dendrites often form tree like structures which have been speculated to reflect or enable function. For example, in one study (Stiefel and Sejnowski, 2007) the authors selected neuron morphologies that were either optimized to linearly sum or to distinguish the temporal order of the excitatory input potentials.

With these kind of studies in mind it is reasonable to ask if the dendritic structure of STMD neurons are optimized for target selection, prediction or selective attention.

4.6 Imaging methods: a key to understanding the neural mechanisms of attention

The above mentioned putative neural mechanisms of selective attention all have a common set of *in vivo/vitro* methods with which they have been measured and analyzed. One is electrophysiology which is already described with an example in chapter 3.7. The other is imaging methods, which are discussed here. Innovation and development of imaging methods is key to improving our understanding of biological systems. The following sub-sections discuss two recent important developments for insect brains.

4.6.1 Serial block-face scanning electron microscopy on fly brain

A cutting-edge imaging method called serial block-face scanning electron microscopy (SBFSEM) was recently applied on an entire fruit fly brain (Zheng et al., 2018). The imaging resolution enabled neuron morphologies and synapses to be mapped. The dataset is also called ‘hemibrain’ because they have reconstructed neurons from around half of the brain. The dataset has a web-tool interface (neuprint.janelia.org) enabling visualization and download of reconstructed neurons (Xu et al., 2020). First of all, this level of resolution enables connectomics at a level that has so far only been known in animals with even smaller brain, such as the nematode (Szigeti et al., 2014). Second, this number of individual neuronal

morphologies from the same brain have never been available before. Researchers could for example begin to systematically compare if there are morphological differences that enable specific neuronal functions like object motion detection (Paper 9). While synaptic weights and proteins will still be unknown, the project is a huge leap in figuring out how fly brains work. Since the fruit fly and hoverflies are both dipteran flies it is of high interest to find the similarities and differences. Also, a hoverfly brain SBFSEM dataset is hopefully not too far away given the rate of the imaging size developments. To date, researchers have started to map out the potential connectomes between neurons in the fruit fly brain. Fig 3.7 illustrates the fruit fly optic lobe neuron pathway and some speculations regarding how dragonfly/hoverfly STMD neurons may be involved.

4.6.2 Rapid and flexible tissue clearing of insect brains

A commonly used method to visualize neurons in a brain is to inject them with a fluorescent dye such as Lucifer yellow. After the injection the brain must then be put in to fixative and made transparent (cleared) so that the neuron can be visualized through the brain by looking at it in a fluorescence microscope. To achieve high resolution, confocal microscopy is commonly used. The resolution is limited by how well matched the refractive indexes of the materials/solutions that are between the microscope objective and the neuron. One of these solutions is the mounting medium. Traditionally, toxic solutions have been commonly used and some of these require specific pre-treatment conditions to work. In recent years, researchers have been developing new clearing and mounting solutions that are less toxic and more flexible. One example of these recently developed clearing solutions is Rapiclear (Sunjin lab). In addition to its time efficient protocol and high transparency it has a wide range of available refractive indexes enabling optimization of refractive index match and thus improved resolution. It also works well on insect brain tissue which is especially challenging to work with due to air filled tracheal tubes that needs to be successfully infiltrated by the clearing medium in order for them to become transparent.

Studies that have used Rapiclear include Frank et al. (2017) that looked at humidity neurons in *Drosophila melanogaster*, Govindan et al. (2020) that developed a work flor for looking at neuronal morphologies in in vitro organoid brain model, or finally our own study (Paper 1) that compared a set of clearing media showing an example of a neuron in a hoverfly brain cleared with Rapiclear.

These recently developed clearing solutions enable important developmental steps for extending resolution to the tiny neurites of neurons. These details may be important for understanding neuronal function. As previously mentioned, part of the selective attention functions could potentially be executed through dendritic computations. One way to investigate such dendritic computations is to perform computational modelling.

5 Computational modelling of object motion attention

In this chapter I group (for simplicity) computational models in to four general types. Firstly, if the design of the model is inspired by neurobiological observations, then the model is called bioinspired. These models are often capable of performing tasks in (or near) real time. Secondly, if the model is not inspired by neurobiological observations and is (often) capable of performing tasks in (or near) real time, the model is non-bioinspired. Thirdly, if the design of the model is mimicking neurobiological observations (such as ion channels, synapses or neuronal morphology) but is not (usually) capable of performing tasks in (or near) real time, then the model is called biologically plausible. Finally, if the model is a combination of one of the previously mentioned types then it is a hybrid model. In reality, the different types are on a scale rather than discrete types and how a model is classified can vary depending on how model properties are interpreted as bioinspired/biologically plausible.

This section has been limited to only include models related to object motion attention. I first briefly discuss non-bioinspired, bioinspired and biologically plausible models of object motion attention, and then move on to discuss important research projects and platforms related to biologically plausible models.

5.1 Non-bioinspired models for object motion attention

Generally speaking, this type of object trackers can be divided into two types. Correlation filter trackers and non-correlation filter trackers (but there are some that mixes both). In this sub-chapter I start by discussing the context and basics of correlation filter algorithms, and then discuss two commonly used methods for predicting future object position.

5.1.1 Object tracking by detection: correlation filter methods

The non-bioinspired object tracking algorithms are often used in real life applications such as in surveillance cameras (Eom et al., 2009), humanoid robots

(Mohamed et al., 2016), unmanned aerial vehicles (Nundal and Skjong, 2014), anti-collision systems and autonomously driving cars (Wang et al., 2018b). The algorithms often involve object detection of for example a face or a car. The initial object is selected based on some general object detector or manual selection of an area in the first video frame (image) of a video sequence that contains the object. The object thus gets an initial position. The tracking challenge is to update the position of the object over the progression of the video (Bolme et al., 2010).

Some tracking algorithms work by re-running the object detector over the whole image (computationally expensive) and some limit the search to a certain search window based on the previous object position. Furthermore, some algorithms have a pre-trained classifier that has often been trained offline on translated and scaled version of the object to maximize the chance of recognition as the object moves in the video sequence. Other algorithms have an adaptive classifier (online training) that adapts the definition of what constitutes the object as the video progresses (Bolme et al., 2013) (Fig 5.1). Thus, many modern trackers are essentially computationally cheap object detectors, able to keep track of the identity of the object(s), and often keep tracking (estimating) the position in case of object occlusion.

A popular and successful way to estimate the position of the a previously defined object in a video frame (without re-running the object detector) is to use correlation filters. The correlation filter method is essentially a quick object detector. First a filter template is created using the initially selected object. This can simply be a small image of the object but is in practice some method involving average or sum of a few images. The template and the video frame are then translated into the frequency domain (fast fourier transform), which enables quick correlation to be computed between them. The resulting correlation image then shows a peak at the location of the target.

For these types of correlation filter algorithms, a linear correlation filter and not a non-linear is usually used because of the speed. Let us think of the object as a group of dots in a 2D plot where the dots constitute the same object but in different angles, translations and scale sizes. A linear filter would then be a straight line that separates the object dots from all other types of backgrounds and other object types. A non-linear correlation filter would be a more complex line that could for example be a circle around the objects dots in the 2D plot (also called Kernelized correlation filter [KCF]). A method for computing a KCF was recently achieved using ordered translated object images in a circular manner, giving the algorithm computation times similar to that of linear correlation filters (circulant matrises diagonalized with FFT gives fast comp. times) (Henriques et al., 2015).

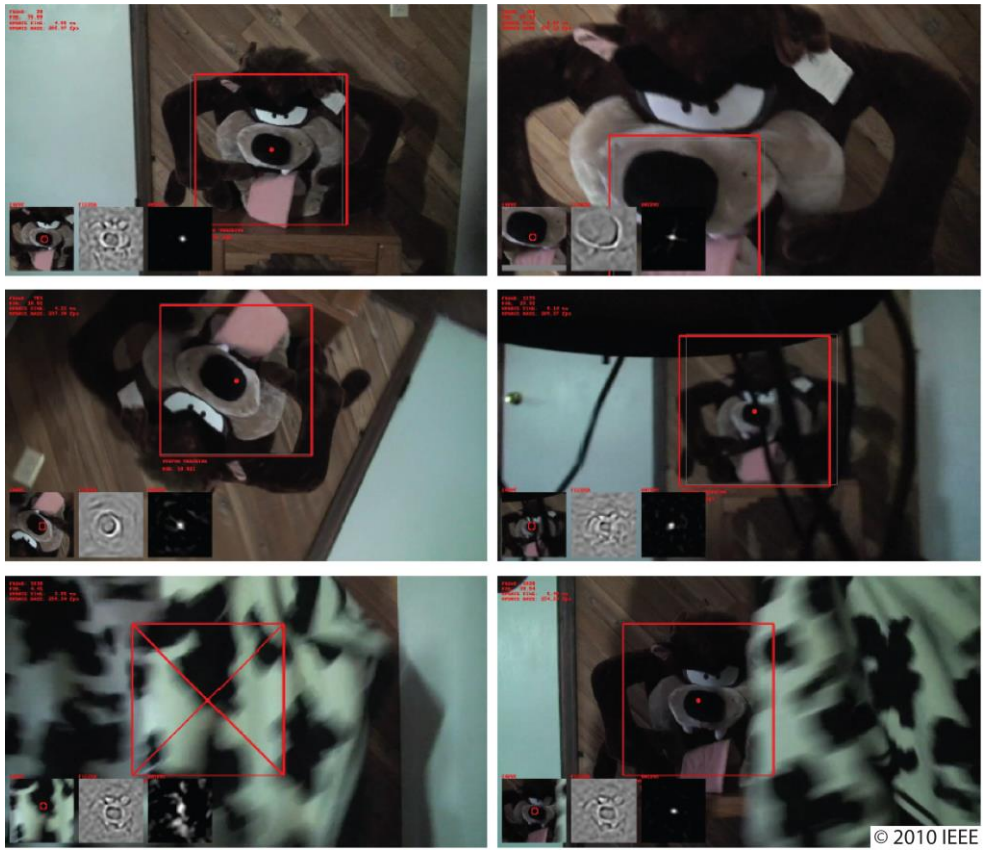

Fig 5.1. Example of a correlation filter based adaptive tracker (MOSSE) that is able to keep track of the target despite zooming in and re-locate the target after an occlusion. The miniature pictures respectively display the input from the estimated target position, correlation filter, and the result of convolving the correlation filter over the image (estimated new position). Figure taken from Bolme et al. (2013).

5.1.2 Estimate future position based on previous data

Previous algorithms have focused on quick object detection as a way to track objects and ignored a more probability based approach of tracking and predicting object position. Two fundamental techniques that are almost always mentioned in general literature on object tracking is the Kalman and Particle filters (Marsland, 2011). They are both general methods for estimating the future value of any signal, and I will briefly explain them from the object tracking perspective on a simplified level. The Particle filter aim is to build up a probability map in the spatial input space. So if you have a regular 2D image, the algorithm would place out points (particles) on the image, and more points would end up where the algorithm thinks that the object will be in the next time step. The predictions are based on the current and previous

target positions. The previous observations form a probability function which is updated at each time step based on the latest position. The noise distribution can take any form, which is one of the unique things about this method since a main alternative method, the Kalman filter, is bound to use the Gaussian distribution (Normal distribution) to model the noise.

The Kalman filter take previous information about the object position and builds up a Gaussian distribution. One useful ability of the Kalman and Particle filters is that they can rely on signals that hint about the object position rather than the position measurement itself. For example, if I see a moving object, like a running person, that then moves from left to right and goes behind a wall (occlusion) I can guess that the runner will come out to the right from the other side of the wall, which may be wrong since the runner could suddenly start to run the other way when behind the wall. However, if I use sound in combination with the object position I could better estimate the runner position. We thus have two probability functions (auditory and visual) that we can join in order to better estimate the object position. This is useful when we have some computationally/time intensive measurement of object position that we do not want to use very often, as mentioned in the previous section with the object detectors.

The algorithms are also both based on a prediction model, so in theory, these algorithms could keep predicting object position even though all measurements (auditory and visual) are occluded. Because the methods are quite general, many modern tracking algorithms use Kalman or Particle filtering frameworks without stating it in the algorithm name because some other property of the algorithm is what makes it unique (Fiaz et al., 2018). It can thus be challenging to systematically find algorithms using these methods.

An example of a tracking method that uses particle filters is the incremental visual tracker algorithm (IVT). The algorithm learns a low-dimensional representation (principal components) of the object. This representation is updated by including the latest representation of the object in the mean and at the same time forgetting a certain degree of the oldest representations (Ross et al., 2008).

5.1.3 Infrared-based small target detection methods

In military contexts, such as in a passive defence system, it is of high importance to quickly detect small targets in sometimes cluttered environments. It is common to use infrared camera for generating the input to the detection system, and the object of interest is often very small. Thus, object classification is not necessarily performed in the same system but instead, similar to the STMDs, it simply detects small targets. Many such models have been developed (Gao et al., 2013; Wei et al., 2016; Bai and Bi, 2018), some with minor bioinspired-properties. However, it is not clear that they perform well in highly cluttered environments since the examples

shown are relatively uncluttered compared to those of the ESTMD papers. For example, a sky with clouds or sea with waves (Wei et al., 2016) compared to forest with bushes, trees and leaves (Wiederman et al., 2008; Wang et al., 2019). A recent study show that an ESTMD-based method outperforms state of the art infrared-based small target detection methods (Uzair et al., 2021).

5.2 Bioinspired models for object motion attention

The bioinspired models/algorithms are developed by deliberate observation of what is known/hypothesized about animal brains or some other biological system such as evolution, while most often achieving or aiming at high computational efficiency. Here, I describe two motion trackers which are inspired by the insect and mammal brain respectively.

5.2.1 An object motion tracker inspired by dragonfly brains

The elementary STMD (ESTMD) model (Wiederman et al., 2008; Bagheri et al., 2017) is an example of an object tracking model that was developed with the aim to both mimic insect neurophysiology and be computationally efficient. The model consists of two stages where the first performs early visual processing and the second subsequently performs target matched filtering. In the early visual processing stage the green channel is extracted, blurred and subsampled followed by temporal and spatial band-pass filters to mimic the photoreceptors and lamina of the insect optic lobe and reject redundancy from the image. The next brain region is the medulla in which ESTMDs are putatively located. At this target matched filtering stage, the response to brightening events ('ON') and dimming events ('OFF') are separated into two different pathways by half-wave rectification of the input signal. Movement of a target is assumed to consist of a moving target that triggers either an ON or OFF detector (the leading edge of the target) followed by an opposite sign stimulus with a short delay at the same location as the trailing edge passes. A low-pass temporal filter delays the signals from each detector so that a correlator within the ESTMD compares each delayed signal with the undelayed signal of opposite sign. Combination of this "target template" with fast adaptation (to reject background texture) and center-surround antagonism provides a sharp selectivity for small, moving targets within the input images. This ESTMD model was used as the first processing stage of the hybrid model which is further described in the attached manuscript and briefly in chapter 8.

Several extensions of this model have been developed in recent years. One extension implemented a form of bioinspired facilitation that improved the success rate for simulated pursuits (Bagheri et al., 2015). The facilitation was applied by multiplying

the ESTMDs with a Gaussian map placed ahead of the current target position, with the target velocity determining how far ahead. Another study (preprint) used a delayed feedback from the ESTMD output onto the final (Lobula) stage of algorithm where signals from the ON and OFF channels are being multiplied (Wang et al., 2018a). Although the authors do not mention facilitation, this feedback could be interpreted as a type of facilitation acting more as a short term memory than predictive estimation. Nevertheless, the feedback enabled improved accuracy when tracking small targets in cluttered environments.

5.2.2 An object motion tracker inspired by mammal brains

There are a lot of algorithms that utilize artificial neural networks (ANN) (Wang et al., 2015; Fiaz et al., 2018) and one could argue whether the usage of a ANN makes the model "bioinspired". I consider them bioinspired here due to the fact that they to some extent mimic the way that neurons learn, for example weights between units can be considered equivalent to the neurons synapses.

One interesting development is a type of model that utilizes principles from neuroscientific attention research to guide the development of the object tracking algorithms. When utilizing artificial neural networks for target tracking, a bottle neck is that large regions in the input image must be analyzed. Large regions are selected because it is challenging to know how far away the target might have traveled in the next input image, and one does not want to select a too small window leading to the algorithm having to search again using a larger window.

Based on selective attention research on superior colliculus, a study suggested a region proposal network based on a saliency map (Yohanandan et al., 2018). The saliency map is generated using low resolution versions of the original images. This proposal system removes the parts of the image which do not contain high saliency and thus likely do not contain a target. The model consists of a convolutional neural network that has been trained to choose which areas it should attend or not attend using loads of object motion examples. That said the saliency is generated from one image at a time using low resolution object detection and thus does not base the saliency map on previous object motion. Nevertheless, this selective attention function allowed the object tracker to perform at much higher rates (500frames/s) than ANN object trackers usually do.

5.3 Biologically plausible models for object motion attention

The biologically plausible models go further than the bioinspired models and try to mimic specific biological molecules (receptors, neurotransmittors, ion channels etc). This most often lead to that the models not being computationally efficient enough to run in real time.

Here, I present a selective attention model based on the sensory cortex (Lee et al., 2013). The authors wanted to investigate if top-down oscillations (synchronized at 20Hz) would generate spiking activity similar to that of experimental recordings (mostly local field potential recordings). They used a network model based on single-compartment Hodgkin Huxley type neuron models with 9 cortical neuronal subtypes included. From these they designed two cortical columns that could be thought of as representing two pixels or two sensory areas. The model received unsynchronized input spikes (100Hz poisson excitatory postsynaptic potentials) to both columns. The activity that was generated from the noise was their control. They then biased one of the columns by stimulating one of the columns with input spikes synchronized at 20Hz. The resulting network activity was similar to that of experimental recordings (successful entrainment of 20Hz oscillation).

5.4 Hybrid models for object motion attention

Hybrid models, as defined in this report, contains both biologically plausible and bioinspired/non-inspired algorithms.

In a conference paper (Farah et al., 2017), the researchers describe a model of the dragonfly brain that has been set up to operate in a robotic simulation environment. Much similar to Paper 3, the model starts with an ESTMD model to capture small target movement, followed by mapping of this to a neuron morphology (they do not have STMD morphology) with Hodgkin Huxley type spiking mechanisms. The model further processes the signal using artificial neural networks and machine learning methods for pattern recognition and action selection and finally closes the loop by motor output enabling the agent to move in the simulation environment. Although some parts of the results seem unclear or questionable due to the publication limitations (conference paper), the model is an important proof of principle for hybrid models.

5.5 Important platforms for biologically plausible models

When modelling insect visual selective attention one has many potential types of previously published models that can be used for inspiration or directly adapted and used. Since computational principles and mechanisms are often the focus of the models and that one is often forced to make a lot of assumptions and simplifications, models from other animals or brain areas can be of high value when designing new models. The following is a list of a few selected important modeling projects/platforms:

- Neurokernel: is an open source modelling project for simulating the fly brain on graphics card cluster/cloud environment (Givon and Lazar, 2016).
- Biospaun: is an open source way of simulating model (potentially robotics in virtual environment) made of spiking neurons (including detailed Hodgkin Huxley model type neurons) (Eliasmith et al., 2016).
- Spinnaker: is an open source modelling project for simulating brain on graphics card cluster/cloud environment (Mundy, 2016; Sen-Bhattacharya et al., 2017). This project could potentially be combined with any of the other mentioned platforms since it is more a way of computing platform than a modelling platform itself.
- Neurorobotics platform: is one of the subprojects of the human brain project which aim to provide a virtual 3D environment for developing and simulating spiking neural networks and/or other algorithms (Falotico et al., 2017).

It is important to be aware of these projects/platforms when considering future research projects since they are relatively large scale compared to most other modeling studies.

6 Aim and research questions

This PhD thesis presents 4 papers (Paper 1-4) and excludes results from 5 other papers (Paper 5-9) related to the thesis but still at earlier stages of preparation. All papers are based on combinations of methods from neurophysiology, anatomy and computational modelling. Fig 6.1 is a diagram of the papers and their connection to attention and putative neural mechanisms. The papers are summarized as following:

- **Optimizing the tissue preparation method to maximize morphological details of insect neurons (Paper 1).**

  One of the hypotheses of this thesis is that the key to some neural mechanisms of selective attention lies in the morphological network properties of target detector neurons. In order to visualize such networks to the finest detail, high resolution imaging techniques such as confocal microscopy can be used. Transparency and shrinkage are important determinants for imaging resolution and in this paper we compare the performance of recently developed clearing methods with more traditional ones in order to find the more optimal methods.

- **Investigating facilitation and selective attention in the hoverfly using intracellular STMD recordings (Paper 2).**

  In this project I recorded intracellularly from STMD neurons of hoverflies using an experiment technique called priming (See primer & probe experiment in Fig 3.10). The project focuses on measuring facilitation in hoverfly STMD responses to small target motion. Evidence for facilitation in small field STMDs is shown. An experiment is performed to test if the facilitation can be used to control selective attention. Results show that unprimed distractors can be at least as distracting as primed ones and priming could activate selective attention.

- **Computational simulations of NMDAR based facilitation in a hybrid STMD model (Paper 3 & 4).**

  In these papers I developed a hybrid computational model, combining abstract processing and detailed neuronal morphology, to test hypotheses relating to NMDARs, facilitation and attention. The response from continuously traveling small targets are compared with randomly appearing targets and targets traveling a short distance. The model response is compared with *in vivo* intracellular BSTMD1 recordings (Primer & probe experiment). We show that NMDA receptors enable response facilitation in a BSTMD1 and a control

neuron morphology. We discuss future experiments to test traveling wave for showing extended predictive facilitation.

In addition to these papers, I am currently still working on 5 additional projects that were not completed in time for being included in this thesis. These papers represent important follow-up or related studies, in some cases done with external collaborators, as follows:

- **Selective target tracking using a biophysically inspired spiking network model of dragonfly (Paper 5).**

  In this computational modelling project, the focus is not on neuronal morphology as in Paper 3 & 4. Instead, single compartment neurons are simulated in a network to investigate the effect of lateral inhibition and the winner-take-all principle in relation to selective attention. We demonstrate that a spiking winner-take-all network can be used to display selective attention when two moving targets are presented. A substantial amount of work still remains to finish the paper.

- **Non-spiking models of facilitation and prediction (Paper 6).**

  In this project I assisted in creating three types of facilitation wave models. One is based calcium waves in astrocytes, neurons and one on NMDA receptors. All synaptic communication is graded and no action potentials are involved. We investigate velocity and shape of the facilitation wave as well as the various constant value ranges that generate waves that mimic those that have been observed in other studies *in vivo/vitro*.

- **Investigating selective attention in the hoverfly using intracellular large field STMD recordings (Paper 7).**

  This paper aims to investigate selective attention using the frequency tagging technique or the receptive field response pattern comparison techniques similar to the ones explained in chapter 3.9.

- **Computational modelling of predictive facilitation in a spiking neural network with graded synapses (Paper 8).**

  In this project I supervised a bachelor student (Kuzmin, 2020) in designing a spiking neural network model with graded NMDA synapses. A substantial amount of work still remains to finish the paper.

- **Morphological comparison of target tracking neurons (Paper 9).**

  This project aims to compare target tracking neurons from various species to investigate if there is any network property in the dendritic/axonal arborizations that is related to target tracking or related computations. We utilize traditional tracer injections and recently available serial block-face scanning electron

microscopy dataset of a *Drosophila melanogaster* brain. I supervised a bachelor student in this topic (Federley Ottosson, 2020). A substantial amount of work still remains to finish the paper.

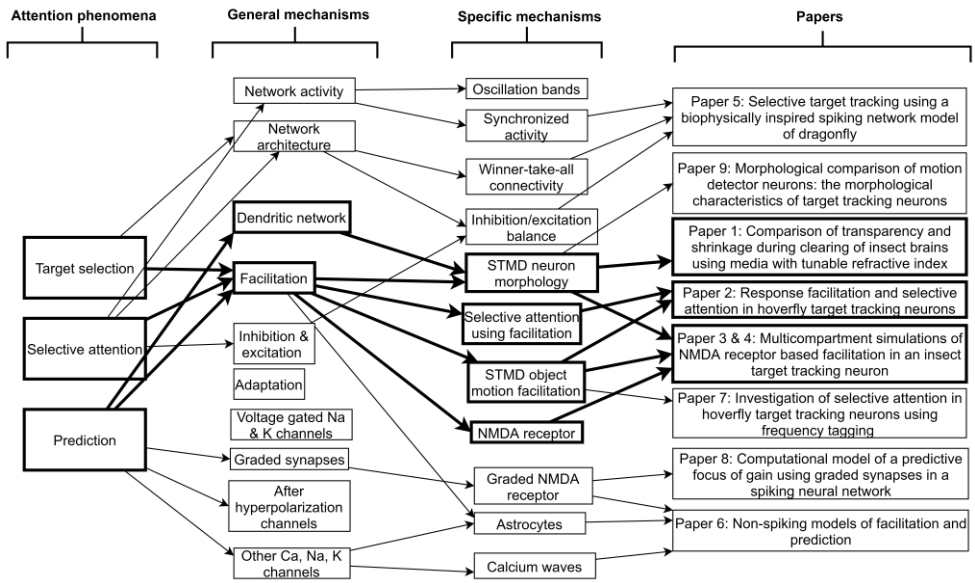

Fig 6.1. A connection diagram between attention phenomena/functions and the putative general or more specific neural mechanisms and what PhD projects in which the mechanisms are being investigated. The bold arrows and borders indicate the phenomena/ mechanisms/papers which have been selected for presentation in this thesis.

In this thesis I present **Paper 1-4**.

A general overarching question that the PhD thesis aims to answer is:

What are the underlying neural mechanisms responsible for visual selective attention in dipteran flies and dragonflies?

7 Optimizing neuronal imaging by comparison of Clearing methods (Paper 1)

This paper was motivated by the hypotheses that a key to some neural mechanisms of selective attention lies in the morphological network properties of target detector neurons. To visualize such networks down to the finest level, high resolution imaging techniques such as confocal microscopy and, or as we show: digital light sheet imaging, can be used.

This paper compares insect tissue clearing techniques in order to find out how one can optimize microscopy imaging resolution of insect brains and neuronal morphologies. Four clearing techniques were tested:

- Methyl salicylate and permount (MS/P). A commonly used and "traditional" technique that requires ethanol dehydration before being transferred in to methyl salicylate.
- Thiodiethanol (TDE). Another commonly used clearing media, reported to have good point spread function (Ke et al., 2016) which is an indicator for how high imaging resolution that can be achieved.
- SeeDB2 (SDB2). A recently developed method based on iohexol, which is commonly known to be used as contrasting agent in X-ray imaging.
- Rapiclear (RC). Another recently developed clearing media which works very quickly and effectively but the creators have not released all information about its constituents.

These methods were then combined with or without ethanol treatment, vacuum treatment and for some also various refractive index values. For each clearing method the tissue transparency and shrinkage were measured. Shrinkage was measured using the length brains before and after clearing (Fig 7.1). Transparency was measured by calculating the relative contrast over the brain versus the background (Fig 7.1C) Ethanol dehydration and rehydration was a sub-protocol which was tested and increased performance of the clearing medias.

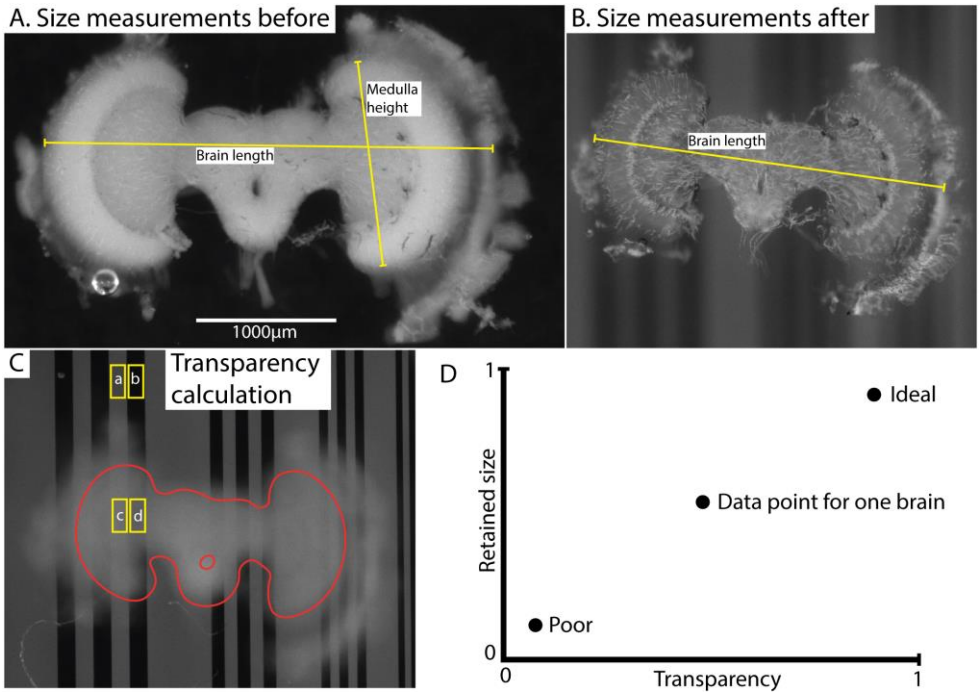

Fig 7.1 Illustration of measurement method (using hoverfly brain) and results. (**A,B)** illustrates size measurements for brain length and medulla height before **(A)** and after **(B)** clearing. "Brain length" indicates the typical length used for comparison of shrinkage. Characteristic landmarks rather than actual anatomical structure length were used to enable measurements on highly transparent brains. "Medulla height" indicates the length used for normalization of transparency values. This measurement was based on the anatomical height of the medulla and was measured only in uncleared brains. **(C)** shows selected areas used to calculate contrast; $C_{brain} = \frac{c-d}{c+d}$ and $C_{ctrl} = \frac{a-b}{a+b}$, and transparency $t = \frac{C_{brain}}{C_{ctrl}}$. The labels (a, b, c, d) indicate the average grayscale value of the area. The brain structure (excluding lamina) is outlined in red. (D) illustrates the interpretation of the comparative data plot (which is used in the next figure). Transparency and retained size are the two dimensions, and ideal versus poor performance are indicated with two illustrative data points. Figure taken from (Bekkouche et al., 2020).

The results of the paper are shown in Fig 7.2A as a scatter plot with transparency versus retained brain size, and in Fig 7.2B as a box plot. As explained in Fig 7.1D, the ideal cleared brain would have retained its original size while becoming as much transparent as possible. The scatter plot clearly shows that the Rapiclear and SeeDB2G protocols dominate the ideal part of the plot whereas MS/P and TDE are located in the middle/bottom or to the left, further away from the ideal. One exception is when Rapiclear and SeeDB2 did not receive ethanol treatment (hollow symbols) in which case they perform much worse in terms of transparency. The boxplot in Fig 7.2B confirms that Rapiclear and SeeDB2 give higher transparency than TDE and MS/P as seen by the non-overlapping notches. Regarding retained size there is more mixed performance. In summary all methods had a retained size median near 80% except MS/P which was below 70% and SeeDB2 had the highest retained size.

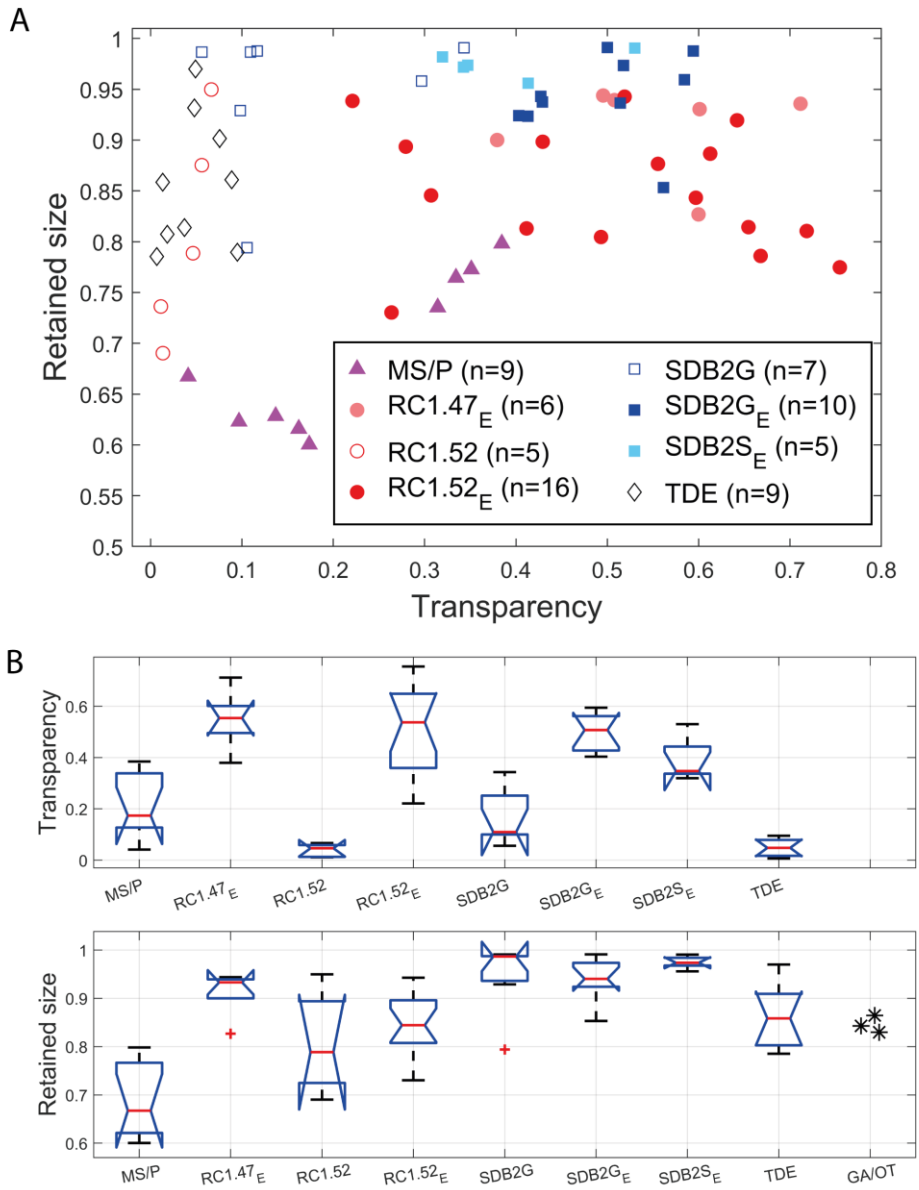

Fig 7.2 Clearing performance measured using transparency and retained size. (A) A scatter plot of the transparency vs. retained brain size. "E" stands for ethanol treatment. The numbers in the acronyms refer to refractive index, for example RC1.52 uses rapiclear with refractive index 1.52. The parenthesis after the clearing method name (n = …) indicates the number of samples (brains). **(B)** Box plot (Matlab R2016b built-in boxplot function) showing median (red line), notches, 25th and 75th percentiles (edge of boxes), whiskers (extreme data points). MS/P always includes ethanol dehydration but no rehydration. The three glutaraldehyde and osmium tetroxide (GA/OT) samples were used in a side experiment (more info in paper 1). Each transparency measurement was normalized using the medulla height to compensate for differences in brain thickness. Figure taken from (Bekkouche et al., 2020).

Fig 7.3 shows an example of neuronal branches from a neuron that was injected with a fluorescent dye and then cleared with the Rapiclear1.49 (Refractive index=1.49). Fig 7.3B highlights the very thin neurites that connect two neurite areas with blebs, that is areas filled with mitochondria and is considered as a hallmark of a synaptic output area (Hausen, 1976; O'Carroll et al., 1992). The figure illustrates the type of high resolution detail that could easily be missed with suboptimal imaging methods. With the results showing optimal usage of clearing methods researchers (including myself) are better informed and ready to perform intracellular dye injections to interesting and challenging neuron subtypes such as the small field STMD in Paper 2.

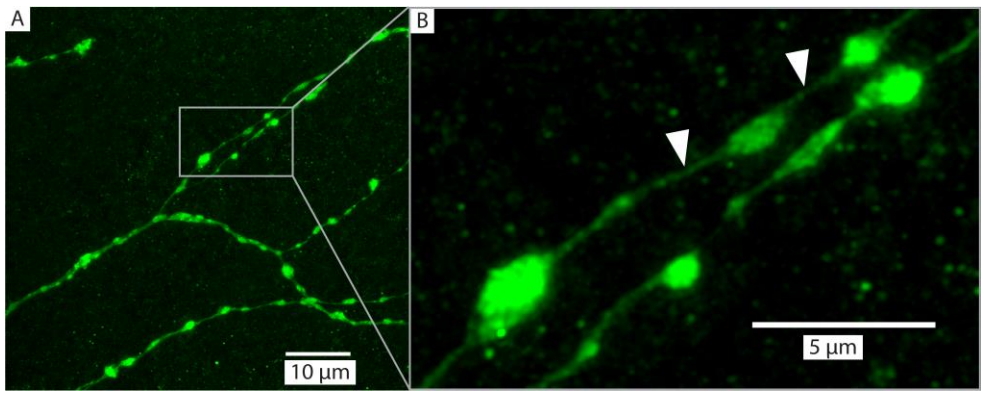

Fig 7.3 Example of detailed branches from a tracer injected wide-field motion neuron. The brain was cleared with Rapiclear1.49E and imaged with a glycerol objective (63x, NA: 1.3). The images were captured from a sample following 9 months of storage at room temperature. The imaging depth was around 50µm bellow brain surface. (A) shows an overview of a group of branches and (B) zooms in on a subset to illustrate the details of a few "blebs," which are considered to be an indication that the branches have output synapses (Hausen, 1976; O'Carroll et al., 1992). The white arrows indicate very fine neurites that have a diameter of between 136 and 271 nm. Figure taken from (Bekkouche et al., 2020).

8 Investigating prediction and attention in hoverfly STMDs (Paper 2)

In this project I recorded intracellularly from STMD neurons of hoverflies using a stimulus technique called priming. The project aim is to find evidence of object motion facilitation in hoverfly (*Volucella pellucens* is used here) STMDs and investigate if this facilitation can be used to control attention. Using the primer & probe experiment, I present evidence that STMDs display facilitated response for long (primed) target paths when compared to short (unprimed) paths. Furthermore, I show that a distractor can be used to take attention away from one target toward the distractor. First I performed a facilitation experiment, then I performed a selective attention experiment. Before describing these I briefly illustrate the general electrophysiology recording method that was used.

8.1 General neurophysiology method

This section describes the general recording method used in Paper 2. I recorded intracellularly (Fig 3.6A) from small target motion detector neurons of the lobula while showing visual stimulus of small targets moving across a computer screen. Intracellular recordings from neurons can vary a lot in difficulty depending on the type of recording setup, neuron and species. The recordings were made using the bridge mode current clamp technique with a silver wire in an aluminosilicate glass capillary. The electrophysiology setup is illustrated in Fig 8.1 showing apparatus view and Fig 8.2 showing the electronics view. These kinds of illustrations are rarely shown at such detail in electrophysiology studies due to the focus of the study being something else. However, for researchers interested in repeating the study, this is of high importance and small shifts in method that is not mentioned could lead to unreproducible results. Fig 8.2 is useful for understandings why and how the steps of the recording method called bridge balance adjustment and capacitance compensation is used.

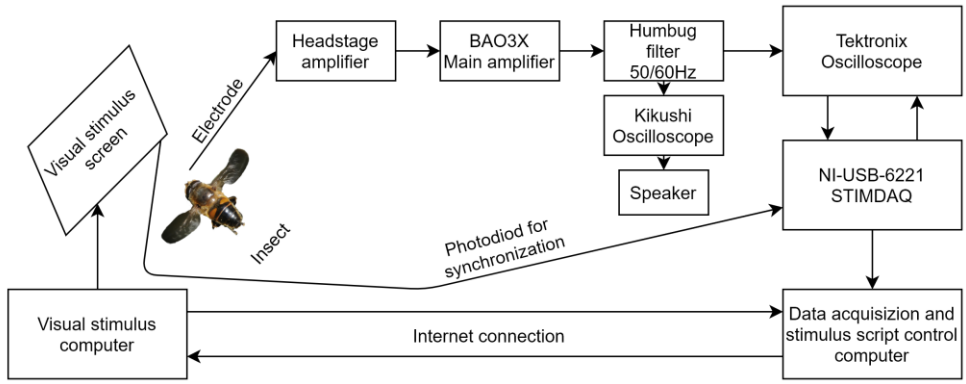

Fig 8.1. Electrophysiology setup and instruments used in intracellular recordings and tracer injection.

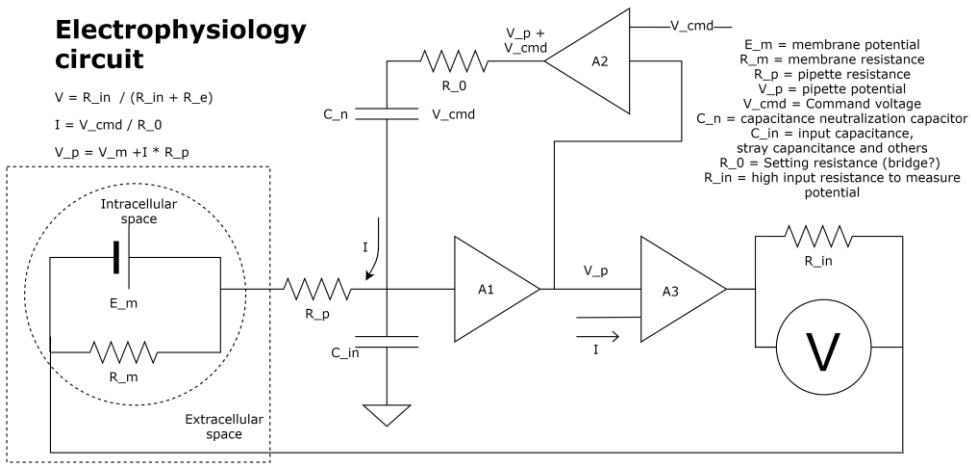

Fig 8.2. Simplified electrophysiology electronics circuit.

8.2 Method for measuring facilitation in STMDs

I recorded intracellularly from small and large field STMDs as well as some lobula plate tangential cells that were used as a control. The neuronal morphology shown in Fig 8.3A is a small field-STMD (SF-STMD) neuron that was injected with a fluorescent dye. It looks and responds like a typical SF-STMD (Barnett et al., 2007). The response from a moving small target and bar is shown in the Fig 8.3A and shows the typical low spontaneous activity, lack of response to moving bars and strong response to small moving targets.

The facilitation experiment involves a short probe path (small square moving across screen) acting as a test stimulus and a priming stimulus preceding the primer. Fig 8.3 is an illustration of the visual stimulus and the concept of priming including STMD characterization. First of all, a moving bar is shown to identify if the neuron is an STMD or a wide field motion detector neuron (or something else). The priming is used to induce facilitation that can be captured in the response of the subsequent part of the stimulus. One trial type includes the primer (primed) and one shows the probe alone (unprimed). The stimulus does not use a pause between the primer and the probe which has often been the case in previous studies using the dragonfly (Wiederman et al., 2017). Thus, the stimulus including both primer and probe is just a continuously drifting target.

The selective attention experiment consists of two targets moving in the same direction with one path outside of the receptive field acting as a distractor. The distractor was either be primed or unprimed while the target in the receptive field was always unprimed (except for a few control trials).

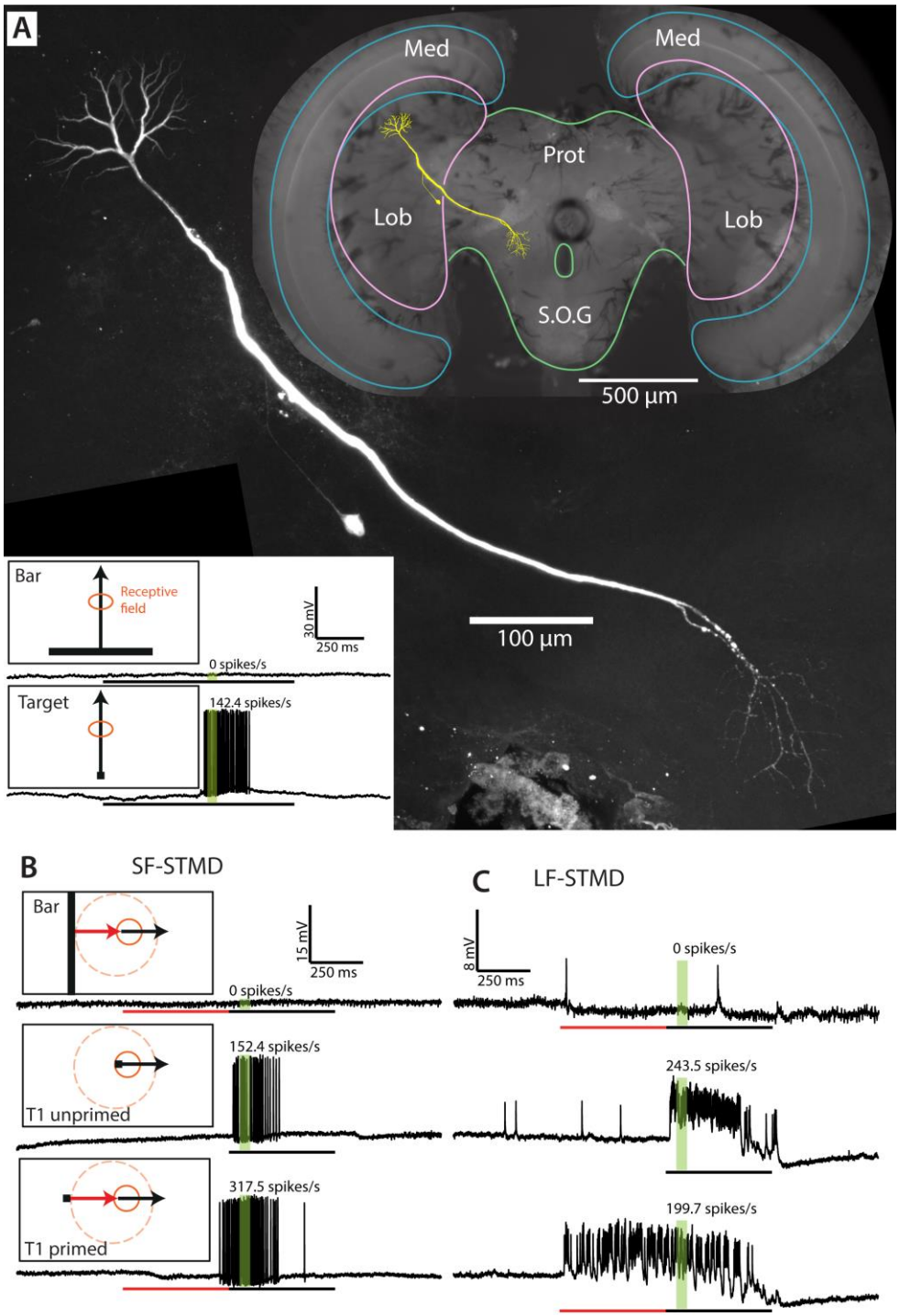
A
Med
Med
Prot
Lob
Lob
S.O.G
500 µm
100 µm
Bar
Receptive field
30 mV
250 ms
0 spikes/s
Target
142.4 spikes/s
B
SF-STMD
Bar
15 mV
250 ms
0 spikes/s
152.4 spikes/s
T1 unprimed
317.5 spikes/s
T1 primed
C
LF-STMD
8 mV
250 ms
0 spikes/s
243.5 spikes/s
199.7 spikes/s

Fig 8.3. Illustration and example anatomy, electrophysiology and stimulus. (A) A fluorescent-dye injected small field STMD (SF-STMD) neuron from *Vollucella Pellucens*. Top right inset illustrates where in the brain the neuron is located. The labelled brain areas include medulla (Med), lobula (Lob), protocerebrum (Prot) and subesophageal ganglion (S.O.G). The lower left inset is an illustration of a moving bar and target stimulus and the raw intracellular membrane potential response from the same SF-STMD. The orange receptive field indicates the part of the visual field, in front of the animal, in which the neuron responded. **(B and C)** Illustrates the visual stimulus which was performed in all neurons to identify subtype (Bar vs Target response) and measure facilitation (Unprimed vs primed response). Shows example responses from an SF-STMD **(B)** and a large field STMD **(C)**.

8.3 Evidence of facilitation in STMDs

The results can be seen in Fig 8.4 showing boxplots of the average responses. Each dot in Fig 8.4 is an average of 3-8 repeated trials from one cell. First, we classified the cells using background activity (No stimulus), the response to a moving bar and a moving target (unprimed and primed). We assumed that STMDs have low background activity, low response to bars and strong response to small moving targets. We grouped all neurons with response to moving bars and equal or weaker response to small moving targets in to the control group which respond similar to lobula plate tangential cells (LPTCs). Although some of the neurons in this control group may belong to another lobula complex neuron group.

We further classified the STMDs based on receptive field size. The receptive field of a visual neuron is the part of the visual field in which that neuron responds. Receptive field sizes below 25° were classified as small field STMDs (SF-STMD) and those above as large field STMDs (LF-STMD). The LPTCs were not split in to two groups despite varying receptive field sizes.

When comparing the primed and unprimed response, paired for each cell, the primed response tends to be higher than the unprimed for the SF-STMDs (Fig 8.4A). The lines between the dots in Fig 8.4A-C indicate that the trials between the dots come from the same cell. We see that the lines from unprimed to primed tend to point in a positive direction indicating a positive paired difference. This difference is shown in Fig 8.4D and we see that the difference is significant ($P=9.4*10^{-4}$) for the SF-STMDs according to the two sided Wilcoxon signed rank test for zero median (Matlab 2019b).

The LF-STMDs in Fig 8.4B do not facilitate in the same direct way as SF-STMDs. Instead the facilitation may be masked by an activity dependent inhibition. We think this inhibition is relatively lower in the primed neurons compared to other neurons. Further analysis of this can be found in Paper 2.

The LPTCs, which were used as control cells, are a well-studied subtype and we believe most of the cells in our LPTC group are actual LPTCs. Strictly speaking however, our LPTC group consist of neurons with part of its morphology (likely axon) located in the lobula complex, and response to a small moving target but an

equal or stronger response to a large moving bar. The LPTCs did not show small target motion facilitation.

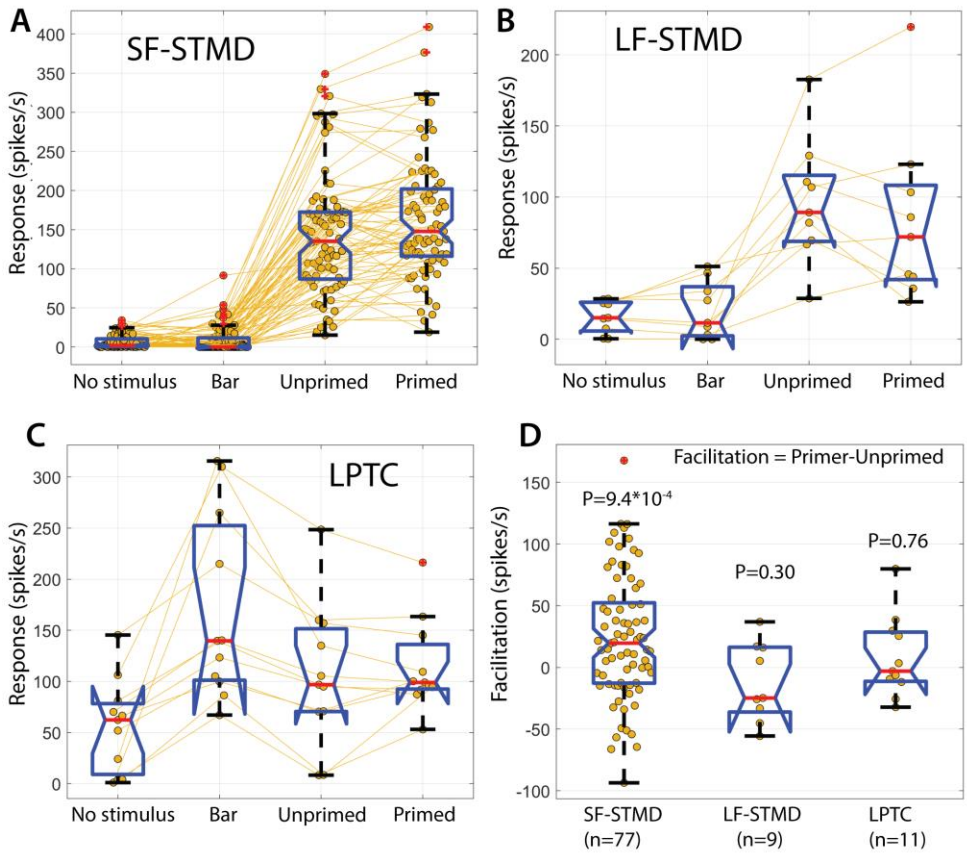

Fig 8.4. Boxplots of spike rates for neuron subtype identification and demonstration of response facilitation. (A-D) Box plots (Matlab R2016b built-in boxplot function) showing median (red line), notches, 25th and 75th percentiles (edge of boxes), whiskers (extreme data points). **(A-C)** Three neuronal subtypes showing spike rate response to No stimulus, moving bar and target (unprimed and primed) used to identify subtypes small field STMD, large field STMD and LPTC. Unprimed and primed responses are then used to measure response facilitation **(D)**. No stimulus responses are measured from the pre and post stimulus periods. Bar and target responses are measured using the analysis window indicated in green in Fig 8.3B and C. **(D)** Shows response facilitation as a difference between the primed and unprimed stimulus response.

8.4 A distractor target modulates selective attention

In the selective attention experiment I tested the effects of a distractor on the response of an unprimed test target going through the receptive field. Fig 8.5 shows that, when compared with T1 unprimed, both distractor trials display significantly

reduced responses ($P_{primed}=1.7*10^{-4}$ and $P_{unprimed}=3.9*10^{-6}$) according to the two sided Wilcoxon signed rank test for zero median.

Contrary to our expectation the primed distractor was not more distracting than the unprimed. Our hypothesis was that a primed distractor would recruit facilitation and thus be more likely to bias selective attention toward the distractor. If anything, the opposite is observed, albeit with weak significance (P=0.086). Interestingly, some of the individual trials (Fig 8.5B) had zero response indicating that the neuron was ignoring a highly relevant stimulus inside of its receptive field during the spike analysis period. This is most likely due to another neuron selectively attending the distractor target and thus inhibiting the response of the recorded neuron. Interestingly, a cluster of 15 responses can be found in the 300-400 spikes/s range in the primed distractor trials Fig 8.5B (Paired, T2 primed). The corresponding number of unprimed distractor trials (Pair unprimed) in this area is 3. This indicates that the primed distractor leads to more trials with enhanced attention to the target in the receptive field, as if the facilitation/attention was easily transferred between the neurons. More visualizations and control trials can be found in Paper 2.

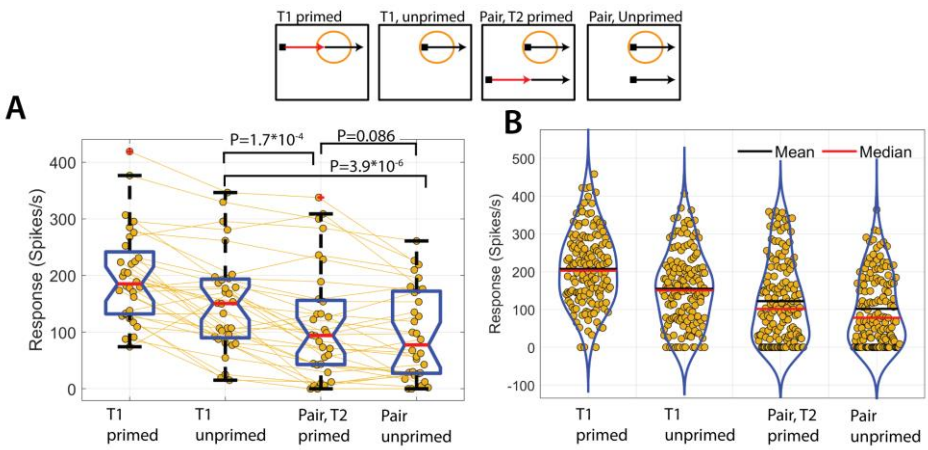

Figure 8.5. A distractor target modulates selective attention. (A) First the T1 primed trial is shown for comparison, then T1 unprimed is shown as the test without distracter. Finally the two distractor trials are shown including a primed and unprimed trial. Each dot consists of 3-8 repeated trials from the same cell. **(B)** Shows a violin plot of the distribution of individual trial responses. Each dot is thus here a trial and not a cell as in **(A)**.

8.5 Discussion and conclusions: facilitation in STMDs

The observation of facilitation in the primed visual stimulus seems to be apparent in the SF-STMDs when seen in contrast to the controls. This is exciting since the analysis of the predictive facilitation field of the dragonfly (Wiederman et al., 2017) started out by finding these types of measurements of facilitation with limited

predictive testing (Nordström et al., 2011; Dunbier et al., 2012). The facilitation could be used for predictive estimation of the immediate or far future position of the object. This paper shows evidence of the immediate predictive estimation. It has yet to be tested if the facilitation remains and spreads as in the dragonfly centrifugal STMD1 (CSTMD1) if the target disappears for 0-500 ms after priming and then re-appears. One hypothesis is that the CSTMD1 achieve this feat in conjunction with SF-STMDs in a network. The SF-STMD morphology acquired this paper is thus exciting since it enables future computational modelling of SF-STMDs that could be combined with LF-STMDs.

Future studies could also simply extend the current visual stimulus. Stimulus including priming and probe jumps to various other locations could tell us the shape of the facilitation in the different temporal stages. For example, a jump backwards could tell us about the directionality of the facilitation and whether the putative wave of facilitation is ahead or around the target position.

To avoid adaptation one could jump to the either side of the primer trajectory. However, this is not entirely obvious since adaptation could be part of the mechanism controlling the facilitation. It is challenging to make jumps in small field cells, since the receptive field is inherently small, and a jump could easily misplace the target with too short spiking period to analyze. Many parts of these extended experiment ideas would thus be better suited for large field STMDs. There are however plenty of attention experiments that are suitable for small field cells, such as those in the selective attention experiments of this chapter that show a distractor outside of the receptive field. This type of experiment is challenging in the large field cells due to the receptive field often covering large parts of the stimulus screen.

The selective attention experiment shows that a distractor can reduce the response, presumably by grabbing the attention of a neuron leading to inhibition of other neurons. The distractor is 20 ° away from the other target, and thus the effect is due to long range inhibition rather than local inhibition. Interocular long range inhibition in the CSTMD1 has previously considered as a mechanism of selective attention (Bolzon et al., 2009) and as previously described, CSTMD1 has strong of evidence for its involvement in selective attention (Wiederman and O'Carroll, 2013; Lancer et al., 2019). The selective attention experiment also indicates that the facilitation build-up from priming may sometimes be transferred to another neuron.

This paper shows evidence for facilitation in hoverflies and that there is a connection to selective attention. More experimentation is needed to discern the exact mechanism connecting facilitation and selective attention. One hypothesis is that non-linearly amplifying synapses such as the NMDA receptor is used in a winner-take all network. This hypothesis is tested in Paper 8 and will not be described here. Another hypothesis is that the specific morphology of STMD neurons together with NMDA synapses are mechanisms of facilitation and selective attention. This is investigated in Paper 3 and 4.

9 Computational simulations of NMDAR based facilitation in a hybrid STMD model (Paper 3 & 4)

This chapter describes both Paper 3 and 4. In this project the aim is to model object tracking generated facilitation in the dragonfly.

A hybrid model (Fig 9.1) was designed consisting of two parts. The first part was based on a previously published bioinspired object tracking algorithm called the ESTMD model (Wiederman et al., 2008; Bagheri et al., 2017) that also publicly shared the source code for the model. This model was then connected to a novel NEURON simulator model (biophysically plausible) using morphology of a BSTMD1 neuron (dendritic tree) and passive properties (Shoemaker, 2011). NMDA synapses were placed on the dendritic tree. In the latest version of the model we have included a spiking mechanism (voltage gated sodium and potassium channels) to compare the model to *in vivo* recordings of actual a BSTMD1 neuron. Together, the bioinspired and the biophysically plausible model constitute a hybrid model for a relatively large part of the insect brain. More details about the model can be found Paper 3 & 4.

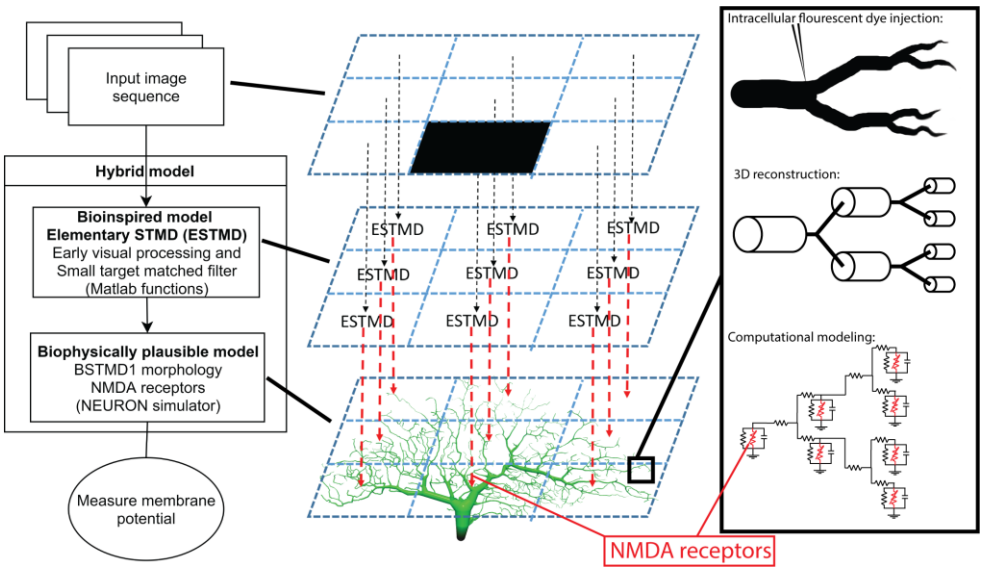

Fig 9.1. Illustration of the hybrid model. To the left is an overview of the model and what type of processing and mechanisms each component consists of. In the middle column is simplified explanation of how an input image gets translated on to the dendritic tree. To the right is a zoomed in illustration of the dendritic tree and how it was simulated, from dye injected neuron, reconstructed cable (cylinders) model, to mathematical compartment (electrical circuit) model.

Three experiments were executed in Paper 4 and only the first experiment was executed in Paper 3. Paper 4 is essentially a large extension of Paper 3. Thus, the focus of this chapter is on Paper 4.

The first experiment can be seen in the supplementary video S1 from Paper 4 and consists of three types of trials. In the first, a target (small black square) appear on a random location every 1 ms. In the second trial, the target travels on short (50ms) paths after which it jumps to a new random location and re-starts the short path. The third trial consists of a target moving continuously on a long trajectory (500ms). When NMDA receptors are included, the model responds stronger to continuously traveling targets compared to random and short paths starting from random positions. When NMDA receptors are replaced with “regular” (double exponential) excitatory synapses, the response between continuous and short is not separable. The random stimulus is filtered out already in the abstract model due to an activation threshold.

The second experiment (Fig 9.2) compares the response of a primed versus unprimed target (primer+probe experiment). A spiking mechanism (Hodgkin Huxley) was added to the model and the response (Fig 9.2B) was compared to that of *in vivo* BSTMD1 recordings (Fig 9.2A). The results show that the model does respond similarly to the BSTMD1 *in vivo* data. The response is however not identical, which is not to be expected between two different BSTMD1 neurons.

In the third experiment, the facilitation field was measured by systematically drifting a target over on a grid covering the visual field. The experiment was then repeated but with the target being preceded by a 500 ms primer locked to the same path position. We show that it is possible to generate a small facilitation spotlight.

The results Paper 3 & 4 show that the NMDA receptor, or any other receptor with similar nonlinearly amplifying properties and/or long rise and decay times, is a plausible candidate for the role of generating facilitation during the neural processes of tracking targets in insect brains. Paper 4 describes and discuss the results relating to the other experiments and compares the results from the BSTMD1 versus non-STMD neuron morphology.

Future computational studies of the STMD system have many potential hypotheses to test. One pathway is to test more specific dendritic computational functions or input combinations (Fromherz and Gaede, 1993; Du et al., 2017; Gidon et al., 2020).

With these papers we have followed up on the previous study (Shoemaker, 2011), and further established the NMDA receptor as a possible candidate mechanism for facilitation during object tracking. Further investigation in to the effects of BSTMD1 morphology on facilitation can be found in Paper 4.

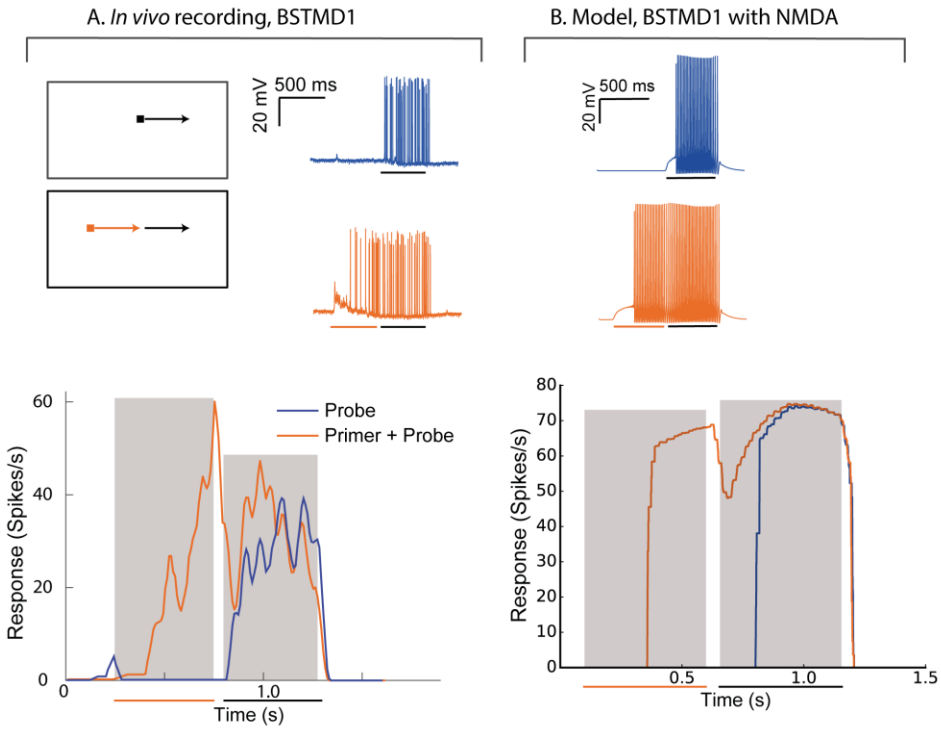

Fig 9.2. Demonstration of response facilitation in recordings from an *in vivo* BSTMD1 and model BSTMD1 with NMDA receptors. Compares *in vivo* intracellular recordings of BSTMD1 **(A)** with the hybrid BSTMD1 model with NMDA receptors **(B)**. The experimental protocol was the primer+probe experiment illustrated in the top left of A with a spike trace to the right. In the bottom is an average of 15 repetitions of the experiment showing the spike rate over time. The *in vivo* recordings and the simulations both have a higher Primer+Probe (orange) spike rate compared to the Probe (black) spike rate, during a short period after the onset of the Probe.

10 General discussion and conclusion

The thesis Papers aim to collectively find evidence for the neural mechanisms underlying visual selective attention in dipteran flies and dragonflies. Paper 1 highlighted the insect brain preparation methods that optimizes imaging quality. This in turn optimized the amount of neuronal morphology details that could be acquired, such as the SF-STMD in Paper 2. Paper 2 demonstrated evidence showing that STMDs use facilitation to enhance target motion response over time and that LPTCs do not facilitate target motion even though they respond to it. The paper also links facilitation to selective attention and shows that a primed distractor is not more distracting than an unprimed.

Paper 3 & 4 show that facilitation, similar to that which can be observed *in vivo*, can be generated using a NMDA receptor model and that this can be done irrespective if a dragonfly STMD or blowfly LPTC morphology was used. However, less synaptic gain was necessary for the BSTMD1 morphology to generate facilitation compared to the LPTC morphology (shown in Paper 3), indicating that the BSTMD1 neuron may be optimized for the task of tracking targets.

In conclusion, evidence has started to accumulate for the involvement of a facilitation (NMDA-like) synapse in hoverfly visual object tracking (Fig 10.1). Future studies could further investigate if there are any other synapses or axonal ion channels than NMDA that could fulfil this task.

In the following sections I attempt to further push the discussion, conclusions/speculations and future research to the edge.

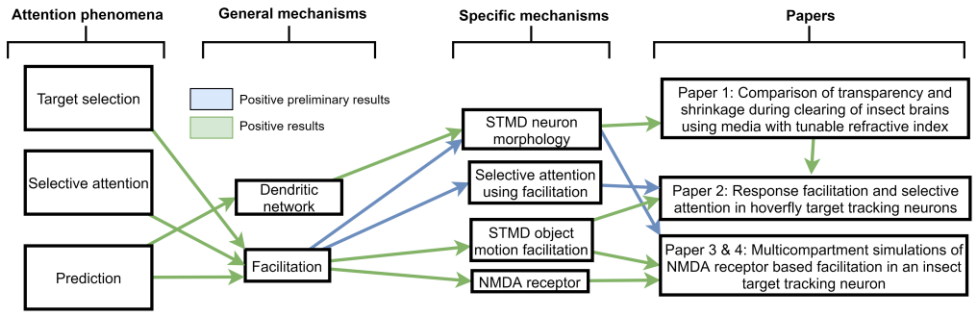

Fig 10.1. Illustration of hypothesis and results relation between behavior task, general/specific neural mechanism and project. The color of the arrow indicates the outcome of the results. The arrows to the Papers indicate if the measurement or implementation was successful whereas the arrows between the attention phenomena, general & specific mechanisms (three columns to the left) indicate if there was a successful implication or not. The blue arrows are used when the evidence was based on few data points or needs further investigation for more conclusive evidence.

10.1 The implications of facilitation on functional/conceptual attention models

In Knudsen's functional model of attention, he has separated the neural representation, competitive selection and working memory into three different functional units. The dragonfly/hoverfly lobula is or is likely the neural representation, the place where competitive selection takes place and where the working memory is stored. There may well be other parts for this too but one possibility is that due to the size constraints of an insect brain, the functional units became compressed through evolution and development and situated in the same neurons, in different layers in the same brain area or nearby brain areas. In contrast, the vertebrate brains may have solved it in a more separated processing manner.

Another set of models to discuss are the conceptual selective attention models used in cognitive psychology, such as the theory of perceptual load (Lavie, 1995) where selection is early in difficult tasks, and late in easy tasks. Regarding this models validity for insects performing target tracking, I think the stimulus needs to be more challenging. One example could be multiple frequency tagged small targets moving on a cluttered background such as the black-white clouds used in (Nordström et al., 2006). This idea could serve to inspire the development of future projects. Another variable is the appearance of the target, which could be changed from the current (black square) to a conspecific, predator or other flying insect.

10.2 Thoughts on automation in neurophysiology and neuroanatomy

In computer science and engineering it is very common to automatize tasks when possible. When I started working with neurophysiology and neuroanatomy I was surprised by how many of the tasks had the potential to be automated. While professors may joke about the acquisition of a PhD student being a method for automatizing science, the situation remains highly un-optimized. The reluctance to automate seem to stem from a fear of being left with system requiring programming skills, and many of the professors, albeit often capable, do prefer to not have to do programming. Also, I believe that it is an underestimation of the gains of automation. Although the financial gains may be limited, freeing up repetitive tasks for PhD students would give more time for creativity and an overview of the experiments not otherwise possible since one has to dive in to the specific tasks. I suspect that this will be a more natural development as more people with engineering background enter the biological fields or long term employment of Research engineers are established. As NEURO-biologists we should lead this development due to automation being related to artificial intelligence and that in turn being more or less related to how brains work.

10.3 Selective attention models in hoverflies & dragonflies

Determining the cognitive models that hoverflies, dragonflies or other insects use is not trivial. Although there has been some single cell evidence for selective attention (Wiederman and O'Carroll, 2013; Lancer et al., 2019) and an attentional spotlight (Wiederman et al., 2017) in the dragonfly, one cannot rule out that there might be parallel tracking of multiple targets followed by later selection as opposed to early selection. This problem may gain clarity by recording from many neurons at once using multi-unit extracellular recording systems such as those provided by Neuralynx or OpenEphys. Two photon calcium (or voltage) imaging could also be used, but the need for extensive averaging in this method makes it challenging since attention is something that likely change from trial to trial. Recording local field potentials has the problem of spatial resolution and may record signals from different areas which makes it hard to say something about the processing stages needed for these cognitive selective attention models.

10.4 Visual experiments inspired by vertebrate experiments

One point of including a lot of vertebrate background information in this thesis is that one can gain inspiration for designing new insect experiments. For example, from the study on an attentional gate in the visual cortex described in chapter 3.3.2 (Moran and Desimone, 1985) we are introduced to the concept of combining ineffective and effective stimulus in the receptive field of a neuron. In the insect brain (lobula) the STMD cells are generally much harder to record from than the cells that respond to more or equal to bars, and gratings (wide-field neurons) compared to small targets. One could therefore design a stimulus showing an effective stimulus (bar or grating) and an ineffective (or less effective) distractor (a small target). This could answer questions related to the involvement of LPTCs in attentional processing.

10.5 The ethics of using insects in research: the need for a method to quantify consciousness

This section serves to discuss, speculate, question and motivate research on animals from an ethical point of view and with a main focus on insects.

Life begins at the cellular level, so being alive is not an important ethical factor considering how we treat plants and cell cultures. Being conscious is considered as the main property when discussing ethical rules for usage of animals in experimental research (Burghardt, 2009). Consciousness can be defined as "any experience" of the world or the self and is dependent on brain activity (Tononi, 2012). The neural correlates of consciousness are still poorly understood but is actively being researched (Koch et al., 2016).

When discussing and determining ethical rules for use of animals in research, a measurement that quantifies "the amount of consciousness" that a certain animal species possess would be ideal, since it is a major component in the discussion (Burghardt, 2009). In the following text I further speculate on this measurement of consciousness:

For simplicity, although there is no strong evidence (Edelman et al., 2005; Boly et al., 2013; Harley, 2013), let us assume consciousness is a metric with a value that each species can be associated with. Humans would have the highest known consciousness value, perhaps (arbitrarily) followed by monkeys, dolphins. At the other end of the consciousness spectrum we have perhaps worms, nematodes and

finally plants. At a certain threshold of consciousness, certain ethical research rules would apply.

The complexity of the brain could perhaps be an objective measurement of the complexity of the system. One obvious follow-up question would then be: Is a complex network of interacting plants conscious? I think no system of interacting components should be excluded for potential measurement, as long as the measurement is objective.

Applying feelings to the equations would complicate things, and in reality this is what many people do with for example horses, dogs and cats. How conscious is a cow compared to a cat? I would argue that is on a similar level, yet we consume one of them and condemn anyone who eats a cat as cruel.

Eating and doing research are different things, but one could argue that eating animals for pleasure is worse than animal research that may lead to improved biomedical understanding and potentially saved lives or reduced suffering.

The hard thing is to make the measurement of the consciousness using the brain (Koch et al., 2016). Ironically we need to do more neuro-experiments on insects and other animals to be able to figure out how to measure consciousness.

However, as always with animals one should apply the 3R:s (Reduce, reuse, recycle). This is not officially needed however when working with insects, since an insect-handling-course is not required by the universities, which is usually mandatory when working with vertebrates such as mice. Given the interesting and complex behaviors and neural mechanisms that can be seen in insects (Barron and Klein, 2016), I think there is need for such a course but adapted for insects.

10.6 Final words

The aim of understanding the neural mechanism of selective attention is not trivial, and I think it requires a multimodal approach as the one in this PhD thesis. This approach enables the researcher to look at the whole system and selectively work on the most promising hypotheses. Having a helicopter perspective enables many project ideas to be generated. However, research demands, focus and completion of projects. This required me to focus on some projects and temporarily ignore others, evident by the List of papers and Papers not contained in this thesis (and chapter 6). In some ways I was part of a selective attention experiment myself.

This thesis provides a group of evidence pieces on the neural mechanisms of selective attention along with frameworks and ideas on which future studies can be constructed. I also included a set of speculations and experimental ideas and hope this gave the reader some new perspectives and ideas.

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