Neurons against Noise

Neural adaptations for dim light vision in hawkmoths

Stöckl, Anna

Neurons against Noise

Neural adaptations for dim light vision in hawkmoths

ANNA STÖCKL
DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY 2016

Neurons against Noise

Neural adaptations for dim light vision in hawkmoths

Anna Stöckl

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Science, Lund University, Sweden.
To be defended in the Blue Hall, Ecology Building, Sölvegatan 37, Lund, Sweden.
2nd of December 2016, 10.00 o'clock.

Faculty opponent

Prof. Simon Laughlin
University of Cambridge

Organization	Document name
LUND UNIVERSITY Department of Biology Sölvegatan 35, SE-22362 Lund, Sweden	DOCTORAL DISSERTATION
	Date of issue
Author(s) Anna Stöckl	Sponsoring organization

Title Neurons against Noise: Neural adaptations for dim light vision in hawkmoths

All animals perceive the world through their senses, which form the basis for their decisions and motor actions. However, when these all-important senses reach their limit and cease to provide reliable information, the animal's survival is threatened. Among the senses, vision is brought to its limits on a daily basis, because its signal strength is diminished as night falls, and increases again as the sun rises. In this thesis, I investigated adaptations that enable the visual system of hawkmoths, a group of insects, to cope with the low light intensities they face at night. I have focused on neural adaptations, manifested in the processing of visual neurons, in contrast to anatomical adaptations, such as modifications of the eye. I showed that neural adaptations exist in the motion vision system of hawkmoths, in the form of integration of visual information in space and time. Furthermore, I demonstrated that a combination of such spatial and temporal summation increased sensitivity and information content in dim light (Paper I). The amount of spatial and temporal summation matched the ecological needs of different hawkmoth species, as well as their anatomical adaptations for visual sensitivity: night active species, and species with less sensitive eyes had more extensive spatial and temporal summation than day-active species and species with very sensitive optics (Paper II). Furthermore, I identified and characterised candidate neurons that carry out spatial and temporal summation in the brain of hawkmoths (Paper III). Finally, I quantified the effects of temporal summation on the ability of hawkmoths to track flowers in hovering flight at different light levels, and showed that a subset of the observed behavioural phenomena could be explained by temporal processing in the nervous system (Paper IV). Taken together, this work has provided detailed insight into how neural processing can increase visual reliability in dim light. The results presented are not only relevant to hawkmoths, since neural summation is also expected to increase visual sensitivity in other species of nocturnal insects, and can be compared to similar mechanisms in vertebrates. Furthermore, this work is instructive for the development of artificial visual systems, for which insect brains have proven to be a successful biomimetic model.

Key words: vision, insect, neural processing, dim light, spatial and temporal summation, hawkmoth, motion vision

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN and key title		ISBN 978-91-7753-064-0 (print) 978-91-7753-065-7 (online)
Recipient's notes	Number of pages	Price
	Security classification	

Signature  Date 25.10.2016

Neurons against Noise

Neural adaptations for dim light vision in hawkmoths

Anna Stöckl

LUND
UNIVERSITY

Cover illustration by Anna Stöckl

Faculty of Science
Department of Biology

ISBN 978-91-7753-064-0 (print)
ISBN 978-91-7753-065-7 (online)

Printed in Sweden by Media-Tryck, Lund University
Lund 2016

"It's a dangerous business, Frodo, going out your door. You step onto the road, and if you don't keep your feet, there's no knowing where you might be swept off to."

J.R.R. Tolkien

Contents

List of Papers

I. **Stöckl**, A., O'Carroll D., Warrant E. (2016) Neural summation in the hawkmoth visual system boosts contrast sensitivity and information rate in dim light. *Curr Biol.* 26: 821–826. doi:10.1016/j.cub.2016.01.030

II. **Stöckl**, A., O'Carroll D., Warrant E. (2016) Matching neural processing to visual ecology: spatial and temporal summation in motion vision of hawkmoths active at different light levels. (*in preparation*).

III. **Stöckl**, A., Ribi W., Warrant E. (2016) Adaptations for nocturnal and diurnal vision in the hawkmoth lamina. *J Comp Neurol*. 524: 160–175. doi:10.1002/cne.23832

IV. **Stöckl**, A., Kihlström, K., Chandler, G.S., Sponberg, S. (2016) Behavioural adaptations for flower tracking in hawkmoths. *Phil Trans B.* (in press). doi:10.1002/cne.23832

Author contributions

I. Conceptualization, E.J.W., D.C.O.; Methodology, A.S., D.C.O., E.J.W.; Investigation, A.S.; Formal Analysis, A.S., D.C.O.; Original Draft, A.S.; Review & Editing, A.S., D.C.O., and E.W.; Visualization: A.S.

II. Conceptualization, A.S., E.J.W., D.C.O.; Methodology: A.S., D.C.O., E.J.W.; Investigation, A.S.; Formal Analysis, A.S.; Original Draft, A.S.; Review & Editing, A.S., D.C.O., and E.J.W.; Visualization: A.S.

III. Conceptualization: AS, WR, EW. Acquisition of data: AS. Analysis and interpretation of data: AS, WR, EW. Drafting of the article: AS, EW. Critical revision of the article: AS, WR, EW. Statistical analysis: AS.

IV. Conceptualization: S.S. and A.S.; Acquisition of data: A.S., K.K., S.C.; Analysis and interpretation of data: A.S. and S.S.; Drafting of the article: A.S. Critical revision of the article: A.S., S.S., K.K., S.C.

Articles not contained in this thesis

V. **Stöckl**, A., Heinze, S. (2015) A clearer view of the insect brain - combining bleaching with standard whole-mount immunocytochemistry allows confocal imaging of pigment-covered brain areas for 3D reconstruction. *Front Neuroanat*. 9. doi:10.3389/fnana.2015.00121

VI. **Stöckl**, A., Heinze, S., Charalabidis, A., el Jundi, B., Warrant, E., Kelber, A. (2016) Differential investment in visual and olfactory brain areas predicts behavioural performance in hawk moths. *Sci Reports*. 6: 26041. doi:10.1038/srep26041

VII. **Stöckl**, A., Smolka, J., O'Carroll, D., Warrant, E. (2017) Hawkmoths sacrifice spatial resolution to increase sensitivity in dim light. *Integr Comp Biol. (in preparation*)

VIII. Dahake*, A., **Stöckl***, A., Sane, S., Kelber, A. (2017) The role of vision and antennal mechanosensors in flight control of a diurnal hawkmoth. (*in preparation*)

IX. **Stöckl**, A., O'Carroll D., Warrant, E. (2017) A large variety of wide-field motion sensitive neurons in the hawkmoth visual system suggests modulatory functions. (*in preparation*)

X. **Stöckl**, A. et al. (2017) The effect of wing damage on flight control in the hawkmoth *Macroglossum stellatarum*. (*in preparation*)

Acknowledgements

My dearest **hawkmoths**. Who would have thought our relationship would ever grow into the adoration I feel for you? On our first encounter I could barely touch you, and only because the urge to not embarrass myself in front of my new supervisors was greater than my inner revulsion for insects of all sorts, I would eventually hold you in my hand. And look at us now – I rear your babies at home like my own, feed you first before I eat (ok, sometimes…), and to see you flying in the wild makes my day. In hindsight, how could I have resisted your charm? You fashionably show off my favourite colours, and your caterpillars poop in the shape of little stars!!! Thank you, for being such wonderful study objects, and giving me insight into your lives and brains.

I would like to thank my supervisors, who have guided me through this PhD, and who provided me with role models for how to be a great scientist and a great person.

Eric, how can I put in words the incredible support you have provided throughout this work. You have given me so much freedom to develop, follow my passions, and research interests. Whatever I wanted to do, I named it, and you helped me to put my plans into action. I really appreciate the trust you put in me, and how you helped me to become an independent researcher. Whenever I really needed to talk to you, you would make time, and if not physically in place, Skype me from down-under, or down the hill. Few can give a pep talk as good as you can, and make you wonder what you were worried about in the first place. I will not forget your incredible generosity and hospitality, and hope I can retaliate in the future, living up to the great example that you have set.

David, I am eternally grateful that you shared your extensive knowledge of insect neurophysiology with me. This thesis would not have got very far off the ground without your practical instructions on electrophysiology when I came to Lund, your insights into the hawkmoth visual system, and our many discussions of results and future projects (to which I learned to carry a notebook, because I can definitely not think as fast as you can come up with important things to remember). It was a lucky instance for me when you relocated to Lund, because not only could I make much more use of you as a resource and supervisor, but I also gained a research group that I felt very integrated in. I hope to continue learning from you in the future – and with time needing to scribble fewer and fewer notes.

Almut, you have become somewhat of an unofficial co-supervisor during my thesis. You have such a great share in this work, because without your introduction into

the wondrous lore of hawkmoth breeding, and your generous sharing of *Macroglossum* pupae, a great part of the of this project would not exist. I am also very grateful that you always had an open ear to my questions and worries, and have given me so much helpful advice. Most of all, I really enjoyed our collaborative projects, which were initiated from the many fruitful conversations we had at lunch, in doorways and corridors, and I hope they will continue in the future.

Marie, thank you so much for being a wonderful co-supervisor, and for being so understanding of the struggles, doubts and fears one does experience during a PhD. I have scheduled my next panic session for in 2 years from now ;-) Thank you also for inspiring me to continue following my science communication ambitions – I hope we will have a chance to conduct a project together one day.

Vision Groupies (main and honorary members). I thought a long time about how to appropriately thank you all for the support you have given me throughout my time here– both personally and scientifically. Because I don't dare to even attempt mentioning you in any order of "impact" (sorry, I know this is hard for you, impact factor junkies), I decided to go by the order I met you in (approximately).

Dan, who initially recruited me to the Vision Group, thank you for your support as my examiner, and for guiding this group with just the right mixture of humour and rigour that makes it so special. **Miriam**, thank you for so many enjoyable conversations (and some good old gossip!) shared during nights of recording and days in the office. **Emily** for many a good laugh, and a serious discussion at pub and lunch. **Damian** for being a great office mate, and **Maren** for sharing her science communication passion. **Meire**, **Therese**, **Nellie**, my dearest ladies, the wonderful time we had in my first summer here I will never forget, and I am so glad to have won you as my friends to discuss everything that burns on our tongues and soul! Thank you **Aravin** for teaching me some of your Indian dance moves! **Peter**, thank you for remaining patient with my rants about Sweden, and convincing me to see the great sides of this country and always helping me with any question I had. **Johanna**, thank you for sharing what moves you and listening to my stories, and giving a great example that it is worthwhile to take the time to find your passion. Į sveikatą **Mindaugas**, for many a good philosophical argument when drunk.

Carina, **Eva**, **Olle,** I really appreciate how much you work behind the scenes to keep everything running noiselessly in the lab, thank you so much! Great thanks to **Ylwa** and **Katarina**, for running all my administrative deeds so smoothly, and to **Stefan**, **Lars** and **Anders** in the workshop.

Duuu **Jochen**, thank you for always having a good answer to the many questions I have brought to your office. **Basil**, I do miss the early days of washing brains and

recording alongside you, always a good chat and a laugh and so much great advice! If I had to name one thing you taught me, it is that perseverance pays off, so I shall try, try and try, try and tryyyyy – I'll succeed at last. **Lana, Inga** who gave me interesting insights into the strange world that is fashion – it couldn't be clearer I am a hopeless case. Thank you to **Ronald**, **Rachel**, **Erik, Rolf** and everyone I shared many a nice lunchtime with. **Sissel** thank you for including me in your wisdom about how things work in the biology department. Life would have been so much harder without you. **Stanley**, thank you for all your support, both on insect brains and earthly matters. And for showing me that strong-willed (maddening!) optimism can really help you move mountains (or at least insanely ambitious research projects). **Tini**, my dearest one, thank you so much for being so honestly interested in people's joys and despairs, and opening your house to me for many a wonderful evening!

Nele, neighbour! Sometimes science brings people together who might never have met otherwise – but I am so glad to have found such a wonderful friend in you. We really did share laughter and pain (Muskelkater!!). **Cynthia**, thank you for tolerating all my terrible politically incorrect jokes! And for being a wonderful officemate, who helped me through rough times, and shared so many of the good times with me. Go raibh maith agat, **John**, for reminding me that Ireland is not part of Great Britain. And for sharing your extensive knowledge of history (made up and real!). **Mike**, thank you for your hospitality and photographic advice! And for letting me spoil Charlie. **Charlie**, thank you so much for your unconditional love (of my dog treats). You can make a smile appear on the grumpiest of days! **Atticus**, king of cake, thank you for being so incredibly generous. And for tolerating my absolute ignorance of the fantasy world (hey, at least I like John Williams). **Tomasz**, without you, I would have continued to embarrass myself not knowing the difference between Marvel and – what was the other one? Thank you for great discussions on graphic design. **Carola**, thank you for alleviating my fears of dangerous South American countries. **Gavin**, thank you for your support with my Marie Curie application. **David**, dankschä' for a great mixture of political discussions, funny Austrian accents and a brilliant gusto when it comes to cakes and deserts.

Klara, thank you so much for your support on my various projects - your hard work and great organisation of the many moth related things are greatly appreciated! **Ekku**, thank you for insights into the soul of my next host-country. **Anna** H. and **Julia**, I really admire you for making research work in Panama, and remain calm. I aspire to obtain similar levels of Zen. **Anna** B., for tolerating my frequent visits to your office and my poor capacity to detect your irony. **Miia**, **Cara**, **Laura**, **Kevin**, honorary members of the Vision Group, thank you for livening up pub, for many

dinner and barbecue invitations, and for reminding me that there is a life outside University. **Andrea**, thank you so much for sharing many a good exchange on the topics that move us, during long office hours and walks back home. I hope we can make up for the far too few occasions we had for some wine and chats in the future. **Elisa**, mille grazie for sharing your ephys insights and enthusiasm with me, and marvelling at my impeccable Italian!

A big thank you to my wonderful **collaborators**, who have greatly contributed to this work. Thank you **Willi**, for coming to Lund and teaching me how to conduct Golgi stainings, for ongoing support with this capricious technique and great insights into insect neuroanatomy. Thank you **Mark**, for the supply of *Manduca* pupae, without which an important part of this project would be missing, for inspiring scientific discussions, and for your great hospitality when I visited Cleveland. **Simon**, thank you for opening up the world of system identification to me. I have learned so much from our collaboration, and I am really grateful for the long and fruitful exchanges that bridged between our two worlds.

I would like to thank my **family**, for making my stay here in Sweden here so much more enjoyable – by visiting me and taking me places I would have never seen without them, and by bringing me many things from Germany that helped me feel like home (wine, nuts, throat-well tea, my beautiful punch bowl, wine, a Lantana plant, did I mention the wine?). I would also like to thank my Foster family, which I had the great luck to be adopted by during this time! Thank you all so much for your support, and for the moth hunting base-camps in Sorède and Grünstadt!

James, you know, I think not only myself, but every member of the Vision Group should thank you for your contribution. Because you are the reason that, for the most part, I could be my happy cheerful self at work: you absorbed much of the anger, frustration, doubts, and fears that surfaced during this PhD, calmed me down again and helped me to be positive. If I had to pay a psychiatrist for all the hours we talked about PhD related matters, even my nice Swedish salary couldn't have paid for it! Thank you so much for sharing this time with me, being my bouncing wall for scientific ideas, making sure I have a comforting place to come home to after work, taking me out on long skates to clear my head, and for enriching the culinary scope of my work lunches beyond pasta with pesto!

If I forgot someone, please feel very much included in my acknowledgements, and let me know of my crime, so I can give you the appreciation you deserve (alternative forms of payment are cake and drinks)!


thank you
danke
dziękuję
obrigado
shukran
tak
cheers mate
go raibh maith agat
ačiū
kiitos
toda
tack
grazie
gracias
dankie
merci

Summary

All animals perceive the world through their senses, which form the basis for their decisions and motor actions. However, when these all-important senses reach their limit and cease to provide reliable information, the animal's survival is threatened. Among the senses, vision is brought to its limits on a daily basis, because its signal strength is diminished as night falls, and increases again as the sun rises. In this thesis, I investigated adaptations that enable the visual system of hawkmoths, a group of insects, to cope with the low light intensities they face at night. I have focused on neural adaptations, manifested in the processing of visual neurons, in contrast to anatomical adaptations, such as modifications of the eye. I showed that neural adaptations exist in the motion vision system of hawkmoths, in the form of integration of visual information in space and time. Furthermore, I demonstrated that a combination of such spatial and temporal summation increased sensitivity and information content in dim light (**Paper I**). The amount of spatial and temporal summation matched the ecological needs of different hawkmoth species, as well as their anatomical adaptations for visual sensitivity: night active species, and species with less sensitive eyes had more extensive spatial and temporal summation than day-active species and species with very sensitive optics (**Paper II**). Furthermore, I identified and characterised candidate neurons that carry out spatial and temporal summation in the brain of hawkmoths (**Paper III**). Finally, I quantified the effects of temporal summation on the ability of hawkmoths to track flowers in hovering flight at different light levels, and showed that a subset of the observed behavioural phenomena could be explained by temporal processing in the nervous system (**Paper IV**). Taken together, this work has provided detailed insight into how neural processing can increase visual reliability in dim light. The results presented are not only relevant to hawkmoths, since neural summation is also expected to increase visual sensitivity in other species of nocturnal insects, and can be compared to similar mechanisms in vertebrates. Furthermore, this work is instructive for the development of artificial visual systems, for which insect brains have proven to be a successful biomimetic model.

Sammanfattning

Alla djur uppfattar världen med sina sinnen, vilka ger information till beslut och rörelser. Tyvärr har sinnena begränsningar och när de upphör att ge pålitlig information kommer djurets överlevnad att hotas. Synsinnet till exempel utmanas dagligen, eftersom dess signalstyrka minskar när mörkret faller och ökar igen när solen går upp. I denna avhandling undersökte jag synsystemets anpassningar hos svärmare, en grupp insekter som klarar av mycket låga ljusintensiteter under natten. Jag fokuserade mina studier på neurala anpassningar som yttrar sig i informationsbearbetning hos synneuroner, i motsats till anatomiska anpassningar, såsom modifikationer i ögat. Jag kunde visa att neurala anpassningar förekommer i synsystemet hos svärmare i form av integrering av visuell information i tid och rum. Dessutom visade jag att en kombination av spatial och temporal summering ökar ljuskänsligheten och informationsinnehållet på ett supralinjärt sätt (**Publikation I**). Mängden spatial och temporal summering matchade de ekologiska behov som olika arter av svärmare har, samt deras anatomiska anpassningar för synkänslighet (**Publikation II**). Jag identifierade också nervceller som utför spatial och temporal summering i hjärnan hos svärmare (**Publikation III**). Slutligen kvantifierade jag effekterna som temporal summering har på svärmares förmåga att spåra blommor i rörelse och visade att en del av det observerade beteendet kan förklaras med temporal bearbetning i nervsystemet (**Publikation IV**). Sammanfattningsvis ger detta arbete en detaljerad inblick i hur neural summering kan öka ljuskänsligheten i svagt ljus. Dessa resultat är inte bara relevanta för svärmare, utan även för andra insekter och djurgrupper. Dessutom är detta arbete lärorikt för utvecklingen av artificiella synsystem, som insekters syn visat sig vara en framgångsrik biomimetisk modell för.

Zusammenfassung

Ihre Sinne erschließen Tieren und Menschen die Welt und die Informationen, die sie bereit stellen, bilden sie die Basis für alle gezielten Bewegungen und Handlungen. Wenn jedoch ein Sinn an seine Grenzen kommt und keine verlässlichen Informationen mehr liefert, gefährdet er das Überleben seines Besitzers. Besonders ein Sinn wird täglich an seine Grenzen gebracht: der Sehsinn. Die Lichtintensität, und damit die Grundlage für das Sinnessignal, steigt und fällt mit dem stetigen Wandel von Tag und Nacht. In der vorliegenden Arbeit habe ich Anpassungen im visuellen System von Schwärmermotten untersucht, die es dieser Gruppe von Insekten ermöglichen, auch bei den geringsten nächtlichen Lichtintensitäten aktiv zu sein. Dabei konzentrierte ich mich vor allem auf neuronale Anpassungen zur Erhöhung der Sensitivität, also Anpassungen der Nervenzellen und der neuronalen Signalverarbeitung, im Gegensatz zu anatomischen Anpassungen wie zum Beispiel Modifikationen des Auges. Ich konnte zum ersten Mal in nachtaktiven Insekten nachweisen, dass neuronale Anpassungen existieren, und dass sie durch die räumliche und zeitliche Integration visueller Signale die Sensitivität des visuellen Systems von Schwärmermotten erhöhen (**Publikation I**). Die Stärke der Integration hing dabei von den Lebensumständen verschiedener Schwärmerarten und den anatomischen Voraussetzungen ihres visuellen Systems ab: nachtaktive Arten mit weniger sensitiven Augen integrierten Information stärker in Zeit und Raum als tagaktive Arten und Arten mit sehr sensitiven Augen (**Publikation II**). Des weiteren identifizierte und charakterisierte ich Nervenzellen, die wahrscheinlich für die räumliche und zeitliche Integration von visuellen Signalen im Schwärmergehirn verantwortlich sind (**Publikation III**). Abschliessend untersuchte ich die Auswirkungen von zeitlicher Signalintegration auf das Vermögen der Schwärmer, Blüten im Schwebeflug zu verfolgen. Dabei konnte ich zeigen, dass eine solche Integration die Fähigkeit, schnelle Flugmanöver durchzuführen, reduziert (**Publikation IV**). Zusammenfassend hat die vorliegende Arbeit wichtige Einsichten geliefert, wie die Verlässlichkeit von schwachen visuellen Signalen durch räumliche und zeitliche Integration erhöht werden kann. Die präsentierten Ergebnisse sind nicht nur für Schwärmermotten relevant, sondern auch für andere nachtaktive Insekten, und können außerdem mit ähnlichen Strategien in Wirbeltieren verglichen werden. Darüber hinaus sind sie instruktiv für die Entwicklung künstlicher visueller Systeme, für die Insekten in der Vergangenheit erfolgreich Model gestanden haben.

"The only things we perceive are our perceptions."

George Berkeley

I. The scope of this thesis

We humans and all other animals perceive the world through our senses. While not everyone might agree with Berkeley's idealist view of the world, it is a fact that the only way we receive information about the physical world around us is through our sensory organs. And thus, our view of the world is shaped—and limited—by the properties of our senses. Sensory information provides the basis for an animal's decisions and motor actions.

But what happens, when these all-important senses reach their limit and cease to provide reliable information? The answer is straightforward: the animal does not receive the information on which its survival depends, and is faced, in the worst case, with death. To avoid this scenario, animal senses have undergone a long trajectory of evolutionary optimisations, both in terms of selectivity and sensitivity, to make sure that they provide rich and reliable information under various conditions. Nevertheless, many animals live in environments in which (some of) their senses are challenged regularly. One sense in particular is brought to its limits on a daily basis by the way our solar system is organised— interestingly, it is the one that many animals rely on most strongly: vision. Its signal strength is diminished as night falls, and increased again, as the sun rises, day in and day out.

In this thesis, I investigated adaptations that extend the sensitivity of the visual system to cope with the low light intensities it faces at night. I focused my studies on neural adaptations, manifested in the processing of visual neurons, in contrast to anatomical adaptations, such as modifications of the eye.

In general, neural adaptations differ from anatomical ones in that they can achieve their purpose without alterations to the physical characteristics of the sensory organ in question. Say you want to build an auditory device which detects sounds of a certain pitch, that is a certain pressure-wave frequency. You could build a device with a membrane that resonates specifically at this frequency, and measure the resonance of this membrane. Alternatively, you could build a device with a membrane that resonates at many frequencies, and select the frequency of interest using a software tool. This second option is equivalent to a neural solution to the task, and has the advantage of keeping the physical aspect of the device (the

receiving membrane) more general. This makes it possible to receive other frequencies and perform other tasks as well. While a device with a physical solution might be more robust, and simpler to construct, it is less versatile. Thus, using neural processing, the animal's sense organ can remain more general, while specific aspects of a sensory percept are shaped by neural circuits which filter and process the sensory information. In reality, anatomical and neural adaptations are not "either-or" scenarios, but are integrated with each other to optimise the sensory percept with respect to the animal's specific needs.

Neural processing in the brains of insects, which increases the reliability of visual information in dim light, provides an ideal system to study such neural adaptations. While a great body of work has investigated anatomical adaptations for dim light vision in insects, neural ones have long been proposed, although not quantified to date. As a study model, I chose to use hawkmoths; which are a very visual group of insects that rely on sight for their superb flight control. This group contains species active both during the day and at night, thus making it possible to compare their visual systems with respect to specific adaptations for dim light vision.

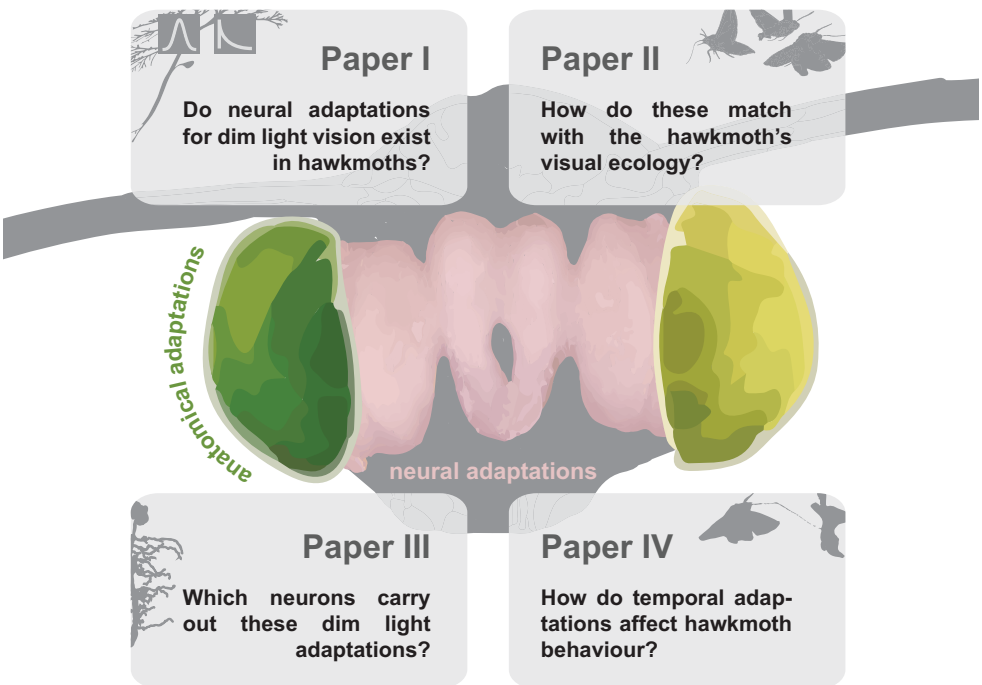


Fig. 1 Summary of the main projects of this thesis.

My thesis comprised several projects (Fig. 1). First, I established the existence of neural adaptations for dim light vision with physiological measurements in a nocturnal hawkmoth species, and quantified the extent to which they improve signal reliability in dim light (Chapter IV, Paper I). Second, I investigated how these neural adaptations compared in species active at different times of day, which therefore have different constraints on their visual systems, as well as how they integrated with anatomical adaptations, to understand the general strategies behind their evolution (Chapter V, Paper II). Thirdly, I focused on the identity of the neurons that are responsible for the neural adaptations underlying dim light vision (Chapter VI, Paper III), to eventually enable more detailed studies of their properties and the neural processing they provide. Finally, I investigated what consequences dim light adaptations had for the behaviour of hawkmoths under different light intensities, using the example of flower tracking (Chapter VII, Paper IV).

"Mehr Licht!"

Allegedly the last words of Johan Wolfgang von Goethe

II. The challenges for vision in dim light

Low light intensities—be it at night or in the deep sea—challenge the visual systems of animals in a number of ways, which are discussed in the following section, before taking a closer look at why animals nevertheless rely on vision in dimly lit habitats.

Photon shot noise

The most fundamental challenge for vision (or any kind of light detection) in dim light arises from the physical nature of light itself. To understand the limits of vision, we have to consider the particle nature of light. Light particles, photons, interact in a quantal fashion with the photopigments in the photoreceptors, which detect light in the eyes of animals (Lillywhite, 1977). Photoreceptors are capable of responding to single photons of light. In the invertebrate literature, these electrical responses are known as "photon bumps" (Lillywhite, 1977, Yeandle, 1958). Photons arrive at any given surface in a stochastic manner (De Vries, 1943, Rose, 1942), and thus the number of photons arriving within a given area fluctuates over time.

This uncertainty in photon arrival and absorption is called photon shot noise, and it is the absolute limit for vision. Irrespective of how sensitive light detectors are, if the photon shot noise is too high, it is impossible to reconstruct the original image from the signal that the detectors receive. The fluctuations in photon arrival follow known stochastic rules, so-called Poisson statistics (De Vries, 1943, Rose, 1942). The photon fluctuation, or noise, can be measured as the variance in the number of photon arrivals (N). According to Poisson statistics the variance can be calculated as the square root of the number of photons arriving per area and time. The signal-to-noise ratio, which is the 'detection criterion' needed to reliably infer the original image, reduces to the square root of the signal (De Vries, 1943, Rose, 1942):

$$\mathrm{SNR} = \mathrm{N} / \sqrt{\mathrm{N}} = \sqrt{\mathrm{N}} \quad (1)$$

Thus, the noise is much higher for smaller photon catches in relative terms, than for larger photon catches. In other words, the reliability of signals in dim light is much lower than in bright light, and this is a major reason why nocturnal vision is challenging.

Consider an image, say a black flower on a white background, which signifies that the flower absorbs all incident photons, while the background absorbs none. Many photons are reflected from the white background, but none from the perfectly black flower (Fig. 2). When the light intensity is high, it is straightforward for the eye (or us as an observer of the figure) to distinguish the flower from the background. But as the light intensity falls, fewer and fewer photons are reflected from the background and reach our eye. Averaged over time, we can measure a constant low light intensity, but at any given point in time, discrete photons are sparsely distributed over the background, not representing the underlying image as well as at high light intensities. When the photons are distributed too sparsely and noisily to represent the difference between the flower and the background, the observer cannot discriminate the flower any more, and spatial vision breaks down.

Fig. 2 The effect of photon shot noise.
At high light intensity, many photons are reflected from the white background, but none from the black flower, and the clear difference between the number of photons reflected from the background and from the flower enables the observer to discriminate the object from the background. As the light intensity decreases (panels from left to right), fewer and fewer photons are reflected from the background and the black flower becomes less and less discernible.

Receptor noise

The discussion so far considers a perfect sensor, which detects every single photon that reaches it. Eyes are far from being perfect sensors. First of all, only a fraction of the photons that hit the eye travel to the retina. But what is more, the

photoreceptors themselves are limited in their detection accuracy by noise, as first demonstrated by Barlow (1956). The arrival of each photon triggers a biochemical cascade in the photoreceptor, which transduces the photon energy into a chemical and subsequently into an electrical signal, called a photon bump. All the elements of this transduction cascade are subject to thermal noise, which means there can be 'false alarms' when parts of the transduction cascade are activated by thermal energy instead of photon energy.

These thermal responses are indiscriminable from real photon responses (Fig. 3). Therefore, **thermal noise** poses the ultimate limit to the reliability of photon detection in the photoreceptor and constitutes the physiological limit for vision in dim light (Aho et al., 1988). In insects, this so-called dark noise is relatively low (around ten false alarms every hour at 25°C in locusts (Lillywhite & Laughlin, 1979)), while it is considerably higher in vertebrates (around 360 per hour at 20°C in toads (Baylor et al., 1980)).

Not only can there be 'false positive' photoreceptor responses, but in addition the amplitude, latency and shape of the photoreceptor response to single photons can vary, because of imprecisions in the transduction cascade (Lillywhite & Laughlin, 1979). This type of receptor noise is called **transducer noise**. Transducer noise has been estimated to equal the contribution of photon shot noise in locust receptors at low light intensities, and has been shown to have an even greater contribution at higher light intensities (Lillywhite & Laughlin, 1979).

Fig. 3 The effect of thermal noise
Receptor noise adds to visual unreliability. Here depicted is the effect of thermal noise - the detection of 'false positive' photon absorption events, created by triggering the photo-transduction cascade with thermal rather than photon energy. As false positive detections increase (from left to right), the black flower becomes less discriminable from the background.

Visual sensitivity

The visual system can only provide spatial vision as long as it can discriminate different levels of intensity, and thus discriminate objects and patterns from one another and from the background. In order to discriminate different intensity levels, the difference in received signal between the levels needs to be greater than the noise associated with each signal detection (Snyder et al., 1977). Thus, the higher the noise in the visual system, caused by photon shot noise and receptor noise, the lower the number of intensity levels that can be discriminated – until eventually none can be discriminated, and (spatial) vision breaks down. In order to remain reliable, the visual system has to collect as many photons as possible per visual integration time and spatial unit.

A measure for the ability of a visual system to capture light is the optical sensitivity. The higher it is, the more light an eye can capture. For extended scenes, it can be expressed as (Land, 1981, Warrant & Nilsson, 1998):

$$S = \left(\frac{\pi}{4}\right)^2 A^2 \left(\frac{d}{f}\right)^2 \left(\frac{kl}{2.3 + kl}\right) \tag{2}$$

A is the diameter of the aperture, d is the photoreceptor diameter, f is the focal length of the eye, and $kl/(2.3 + kl)$ is the fraction of light absorbed, with k being the absorption coefficient of the photoreceptor and l its length. By multiplying the optical sensitivity (equation (2)) by the average light intensity, one obtains the average number of photons absorbed during one integration time Δt of the photoreceptor (Warrant & Nilsson, 1998):

$$N = \frac{\pi}{4} A^2 \, \Delta t \, 1.13 \, \Delta\rho^2 \, \tau \, \kappa \int \left(1 - e^{-kR(\lambda)l}\right) I(\lambda) \, d\lambda \tag{3}$$

κ is the quantum capture efficiency of the transduction cascade, and τ is the fraction of incident light transmitted by the optics of the eye. The solid angular subtense of the photoreceptor's (Gaussian) visual field $1.13 \, \Delta\rho^2$ has replaced the solid angle of visual space viewed by a photoreceptor $(\pi d^2)/(4f^2)$ in equation (2). $\Delta\rho$ is the half-width of the receptive field, otherwise known as the acceptance angle of the photoreceptor. The integral term represents the number of photons absorbed by a photoreceptor viewing a radiance spectrum of quantal intensity $I(\lambda)$ with a visual pigment that has a normalised absorption spectrum $R(\lambda)$, where λ is wavelength (Kelber, 2002, Warrant, 1999, Warrant & Nilsson, 1998).

Trade-off between photon catch and resolution in space and time

Visual sensitivity is inversely related to the resolution of the eye in space and time. A closer look at equation (3) shows that the number of photons N scales with the photoreceptor integration time Δt and the photoreceptor acceptance angle $\Delta \rho$. The longer the integration time, and the wider the acceptance angle, the higher the photon catch, and thus visual sensitivity. The longer the integration time of photoreceptors, however, the lower the temporal resolution of the receptors, and a similar trade-off also applies to the acceptance angle: the wider it is, the lower is the spatial resolution of the eye. Thus, the finer the temporal or spatial scale at which photons are sampled in the visual system, the lower the photon count, and thus the lower the visual sensitivity. Therefore, a trade-off exists between sensitivity and resolution, which different animal species resolve according to their ecological requirements for sensitivity or resolution in space and time (Warrant et al., 1999).

Why do animals use vision in dim light?

If vision is so strongly challenged by low signal and high noise in dim light, why do animals still rely on it under these conditions, often even as their primary sensory system? To answer this question, we first need to ask: why would it be evolutionarily favourable for animals to be active when one of their major sensory systems is strongly challenged? The question actually contains the answer already: it is evolutionarily favourable to be active in dim light, because one of the major sensory systems of animals is strongly limited by the lack of available signals necessary to perceive the world around them. Thus, the first animal species which overcame these challenges, while all other animals relying on vision were restricted to other times of day, or ocean depths, could conquer a habitat with little predation, and little competition for food resources. Thus, survival greatly benefited from being able to expand activity into ecological niches that were not filled with competitors and predators (Wcislo et al., 2004). While many predators and competitors have followed the initial dim light pioneers into their ecological niches, extending the limits of vision is connected to considerable investments and trade-offs, and thus, these niches remain less populated than those in which vision can be used with less restrictions.

But why do animals rely so strongly on vision, even in dim light, rather than switching to other senses? The answer lies in the unrivalled depth of information the visual sense provides. Vision has the potential to provide remote, high-resolution spatial information about an animal's surroundings, reaching from only millimetres to distances of many light-years (when viewing the starry sky). This information is crucial for tasks that require information about objects with no direct contact to the animal, such as obstacles, predators or prey. One behaviour in particular that cannot be executed with any other passive sense than vision (active senses are discussed below), is flight (Davies & Green, 1994). Flying animals need to obtain rapid and remote information about their three dimensional environment, to detect obstacles well ahead of their current position, as well as changes in their own body position with respect to the environment (Srinivasan et al., 1999). Tactile senses, which can provide walking animals with all the necessary information to safely negotiate their environment (Harley et al., 2009), are not far-reaching enough and require contact with the stimulus, which make them unfeasible for flight control. Auditory information can be used to locate a sound source precisely (Payne, 1971)), yet will not help a flying animal to negotiate silent obstacles. In addition to providing remote spatial information, the visual domain exclusively contains information important for locomotion and navigation, such as absolute orientation cues (sun position, or the pattern of polarised light (el Jundi et al., 2014)).

Tasks other than flying (especially food, prey and predator detection) can also be carried out by other senses than vision. A food source can be found and evaluated by gustation and olfaction, many predators can be detected using hearing, somatosensation and olfaction, communication with con-specifics can act via auditory or olfactory signals, and the immediate surroundings can be explored using tactile senses. Thus, flying animals that feed from flowers by approaching them on the wing, such as hummingbirds or hawkmoths, require vision for foraging behaviour, as does a nocturnal dung beetle that uses the polarisation pattern of the sky to maintain a stable heading direction to roll its food safely away from competition at the dung pile (Dacke et al., 2003). A ground dwelling and foraging animal can rely stronger on other senses, to navigate and forage, such as the flightless kiwi birds, whose eyes are distinctly smaller in relation to their body size than those of many flying birds, and whose brain dedicates less neural tissue to vision but more to olfaction and somatosensation in comparison (Martin et al., 2007). Nevertheless, many animals, both flying and non-flying, often rely primarily on vision even for those tasks for which other senses would have been adequate,

because vision provides such a depth of information, and can be used for a wide range of tasks.

In the discussion so far, only passive senses, which receive their input signal from the environment, have been considered. However, some animals have evolved active senses, which send out a signal that interacts with their environment, obtaining information about this environment in the perturbations created by these interactions. Examples for active senses are the electrolocation system of weakly electric fish (Chacron, 2007), and the echolocation systems of cetaceans and bats (Thomas et al., 2004). These senses can indeed provide remote information with high spatial detail, just as vision can. And they are predominantly used by animals living in conditions with poor visual information, taking over the function of vision (Nelson & MacIver, 2006). However, the scanning signals of active sensory systems require energy to produce (though possibly less than generally expected (Nelson & MacIver, 2006)), and also make the animal more conspicuous to predators, which could detect the scanning signals – two possible reasons why they are not more widespread.

Thus, many nocturnal and deep-sea animals use vision as a dominant sense to provide information about their environment in dim light, despite the considerable challenges their visual systems face. How do they overcome these challenges, and make sure the information their eyes obtain is reliable enough for the behavioural tasks that require it?

"Long is the way and hard, that out of Hell leads up to light."

John Milton

III. Adaptations to increase sensitivity in dim light

Vision at night suffers from the low ratio of signal (photon catch) to noise, as detailed in Chapter II. Thus, in order to improve vision at night, the signal to noise ratio has to be increased, either by increasing the number of photons reaching each visual unit or through physiological processes, reducing the effect of noise.

Adaptations to increase photon catch

Equation (3) shows a number of ways to maximise the number of photons each visual channel detects at a given integration time: (1) Modifications of the optics and shape of the eye that increase the aperture diameter (A), or decrease the focal length (f). (2) Anatomical modifications of the photoreceptors that increase their light capture efficiency (κ), their diameter (d) or length (l). (3) Physiological modifications increasing the photoreceptor integration time (Δt).

(1) Modifications of the optics

Optical adaptations maximise the number of photons that are focused on the retina, which can be achieved by increasing the aperture of the eye, and decreasing its focal length. This relationship between aperture and focal length with respect to sensitivity is encompassed in the F-number: $F = f/A$. Eyes with lower F-numbers focus a wider cone of light onto the photoreceptors, thus increasing sensitivity (Warrant & McIntyre, 1991).

Nocturnal animals with **camera type eyes** (Fig. 4A) therefore generally have larger lenses and wider pupils (which are the effective aperture of the eye) than their diurnal relatives, as well as shorter focal lengths, as can be seen in the eyes of nocturnal birds (Hall & Ross, 2007) and nocturnal primates (Kirk, 2004). The nocturnal spider *Dinopis subrufus* achieves its remarkable sensitivity with the largest

lenses of any terrestrial arthropod (Blest & Land, 1977), as well as with a relatively short focal length, resulting in a very low F-number of 0.6.

Compound eyes, which are the most common eye type among insects and crustaceans, are composed of many 'little eyes' or ommatidia, which constitute the visual units of the eye. Each ommatidium contains a corneal lens, the facet, which together with the underlying crystalline cone focusses light onto the photoreceptors. There are 8 photoreceptor (retinula) cells in a typical ommatidium, which are arranged in a circle and each possess a structure composed of photosensitive microvilli, the rhabdomere. These face inwards and form the rhabdom.

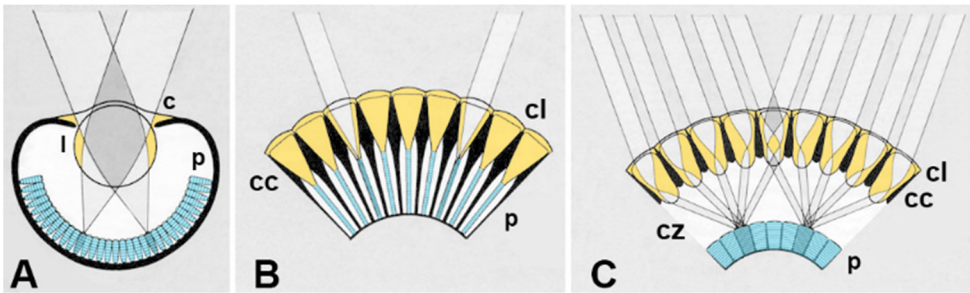


Fig. 4 The three most common eye designs in animals.
A Camera eye. Light is focused by the cornea and lens on to the photoreceptors in the retina. This eye design is the only one found in vertebrates and is also common in molluscs and arachnids. **B** Apposition compound eye. Light is focused by the cornea and cystalline cone in each ommatidium onto the underlying rhabdom. Each ommatidium therefore views a 'pixel' of the overall image. This eye design is commonly found in diurnal insects. **C** Superposition compound eye. Light is focused by the corneas and crystalline cones of a large number pf ommatidia across the clear zone (cz) onto one rhabdom. Therefore, this eye design is typical for nocturnal insects, which require improvements in relative photon catch. Courtesy of Dan-Eric Nilsson, from (Warrant, 2004).

In ***apposition compound eyes*** (Fig. 4B), the ommatidia are optically separated from each other by a sheath of screening pigment. Thus, each facet represents the aperture of an apposition eye. Because of the small size of individual facets, apposition compound eyes have limited sensitivity and are mostly found in diurnal animals, with a few notable exceptions including nocturnal mosquitoes (Land et al., 1997), the tropical halictid bee *Megalopta genalis* (Greiner, Ribi & Warrant, 2004, Warrant et al., 2004), the wasp *Apoica pallens* (Greiner, 2006) and nocturnal ants (Menzi, 1987, Moser et al., 2004, Narendra et al., 2011). As in camera type eyes, the optical sensitivity of an apposition compound eye increases as a function of

increasing aperture and decreasing focal length. Nocturnal mosquitoes (Land et al., 1999), for example, have fewer, but larger facets than their diurnal relatives, thus improving light capture, though at the expense of spatial resolution. In order to retain spatial acuity, the apposition eyes of nocturnal sweat bees are larger relative to body size than those of their diurnal relatives, to achieve similar resolution with wider facet apertures (Greiner, Ribi & Warrant, 2004, Jander & Jander, 2002).

Insects with a nocturnal life style usually possess ***superposition compound eyes*** (Fig. 4C). In this eye type, the pigment that separates the ommatidia in apposition compound eyes can be withdrawn, which leaves a wide clear zone between the crystalline cones and the retina. The crystalline cones refract or reflect light (depending on the type of superposition eye), such that multiple facets focus light from one point in space to the same rhabdom (Exner, 1891, Nilsson, 1989). Thus, the effective aperture of this type of eye comprises all facets with a shared optical axis for one point in space. We can therefore extend equation (3) by n_f, the number of facets contributing to the superposition aperture, and hence obtain the overall aperture area of the superposition eye as $\pi/4\ D^2 * n_f$ where D is the facet diameter.

Optical superposition can make a large difference to sensitivity: the nocturnal hawkmoth *Deilephila elpenor* possesses superposition optics in which an effective average of 576 facets contribute to the superposition aperture, while in its diurnal relative *Macroglossum stellatarum* only 114 facets contribute (Warrant et al., 1999). Both species have quite similar rhabdom diameters, rhabdom lengths and facet diameters, though *M. stellatarum* has a 50% shorter focal length, actually resulting in a higher sensitivity per ommatidium than *D. elpenor*. The fact that *D. elpenor* has an optical sensitivity (equation (2)) that is nearly double that of *M. stellatarum* (*D. elpenor*: 26 μm^2 sr, *M. stellatarum* 11 μm^2 sr) is due to a difference in the superposition aperture. By comparison, the sensitivity of an apposition compound eye of comparable size with a similar interommatidial angle would be approximately 0.05 μm^2 sr.

Superposition compound eyes might appear to be an "evolutionary twist" that has partly overcome the trade-off between resolution and sensitivity, because they allow for much greater sensitivity while retaining the same theoretical acuity as comparable apposition eyes. However, while diffraction-limited superposition compound eyes, which attain the maximum acuity that is theoretically achievable (Land, 1984), do seem to exist in diurnal insects, the superposition compound eyes of many nocturnal species possess significant spherical aberration, which reduces

their acuity well below the theoretical maximum (Caveney & McIntyre, 1981, McIntyre & Caveney, 1985, Warrant & McIntyre, 1990).

Tapetal sheath.

There is one drawback, however, with reducing the F-number of a compound eye to increase sensitivity. In the ideal case, light entering the rhabdom is totally internally reflected at the boundary of the rhabdom and its surroundings, thus making the rhabdom function as a light guide without significant loss of light. For this to work, light has to strike the rhabdom at an angle steeper than 10°, which is only guaranteed for eyes with F-numbers higher than 2.8 (Warrant & McIntyre, 1993). If the F-number is lower, light can leave its rhabdom and contaminates the signals of neighbouring ommatidia, thus degrading image quality. Most apposition eyes have F-numbers higher than the critical value, and are therefore not affected by this stray light problem, while most superposition eyes, due to their larger apertures, have F-numbers considerably below the critical value (Warrant & McIntyre, 1991).

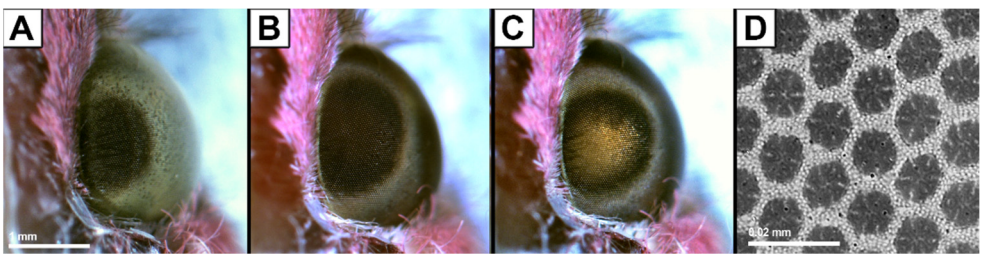


Fig. 5 Superposition pupil and tapetal sheath.
The superposition pupil of the hawkmoth *D.elpenor* light adapted (**A**), dark adapted for 5 minutes (**B**) and dark adapted for 30 minutes (**C**). The size of the pupil increases as the eye dark-adapts, and with it the intensity of the eye glow. The eye glow is caused by a tapetum comprised of air-filled trachea, which surround the photoreceptors (note the flower-shaped rhabdoms) in the retina (**D**).

How can stray light be removed in eyes of low F-number? One solution would be to absorb it with screening pigment that encircles the rhabdom, but this would result in a loss of photons and would therefore be disadvantageous to a nocturnal eye. The solution many nocturnal insects employ is to reflect the light back into the rhabdom using a reflective tapetal sheath (Land, 1984, Warrant, 1999, Warrant & McIntyre, 1991). For example, the nocturnal hawkmoth *D. elpenor* has an F-number as low as 0.72. Light shone into its dark adapted pupil results in a bright

reflected eye glow, a clear indication of a tapetum. In hawkmoths, the tapetum is constructed from air-filled tracheal tubes that tightly surround the rhabdoms in the retina (Fig. 5D).

Dynamic optical adaptations

Many specialisations for dim light vision can adapt to the ambient light intensity dynamically. Photoreceptors have a limited dynamic range, maximally three log units (Walcott, 1975), but many eyes operate over broader ranges of light intensity. One means by which eyes can increase their dynamic range is to control the amount of light entering them, using a pupil. In vertebrate camera eyes the iris works as a pupil by contracting to restrict the amount of light entering through the lens (Walls, 1942). In arthropod compound eyes, the pupil is composed of migrating screening pigment granules, which move to produce a dark-adapted and a light-adapted state. In apposition eyes, pigment typically moves radially towards and away from the rhabdom. It tightly encircles the rhabdom in the light-adapted state and moves away from it in the dark-adapted state (Ribi, 1978). In the light-adapted state the pigment absorbs stray photons and additionally sharpens spatial acuity (Land & Osorio, 1990). In superposition eyes, the pigment of secondary pigment cells moves longitudinally instead of radially. In the light-adapted state it separates neighbouring ommatidia, decreasing the pupil size, while it retracts around the crystalline cones in the dark-adapted state to reveal the clear zone essential for superposition optics (Warrant & McIntyre, 1996) (Fig. 5A-C).

(2) Anatomical modifications of the photoreceptors

Anatomical modifications to the photoreceptors in addition to the eye itself can increase photon catch (equation (3)): longer photoreceptors can pack in more membrane microvilli containing photopigment, and thus increase the absorption area and with it the photon catch. This is especially important, because the absorption efficiency k of every single photopigment is relatively low (given as 0.0067 μm^{-1} in arthropods and 0.035 μm^{-1} in vertebrates), and thus only by stacking membranes with photopigments can efficient absorption be achieved. The fraction of absorbed white light γ is therefore directly proportional to the length of the photoreceptors l: $\gamma \propto kl/(2.3 + kl)$ (Warrant & Nilsson, 1998).

In parallel to the length of the photoreceptor, increasing the area of the photoactive membranes increases photon catch. Thus, photoreceptors with greater diameters—and in the case of insects, greater rhabdom diameters d —have greater visual sensitivity. While in camera eyes the diameter and length of the photoreceptors do not affect the anatomy of the eye's optics (d only affects the maximum packing

density of photoreceptors, and thus the maximum spatial resolution), in compound eyes the diameter of the rhabdom, as well as the length of the photoreceptors, are intricately linked with the anatomy of the entire eye, and thus often co-vary with the aperture of the facet lenses and the focal length of the eye.

(3) Physiological modifications of the photoreceptors

Dark-adapted photoreceptors respond more slowly than light-adapted ones: their latency (time-to-peak) increases with decreasing light levels, and their responses become broader, as shown in the dark adapted photoreceptors of flies (Dubs, 1981). By decreasing their response dynamics, photoreceptors can increase their integration time, that is the time during which photons are sampled to give a response. By sampling over longer intervals, also called temporal summation, more photons are collected, and thus a more reliable response is produced (Laughlin, 1981, Lythgoe, 1979, Snyder, 1979, Snyder, 1977, Warrant, 1999, Warrant & McIntyre, 1996).

While the photoreceptor integration time in the dark-adapted state in many nocturnal insects is longer than in the light-adapted state and also longer than in many of their diurnal relatives, it is still relatively fast at about 40 to 50 ms. Very long integration times have been measured in nocturnal toads (1.5s (Donner, 1989)) and in a deep-sea crustacean (160 ms (Moeller & Case, 1995)). Such long integration times lead to severe blurring of a moving object (similar to the effects of temporal summation, see Fig. 7), which makes them unsuitable to flying animals, or animals that need to chase fast moving prey.

In addition to temporal adaptations, the electric responses of photoreceptors to individual photon detections, the so-called photon bumps, have been shown to be considerably bigger in nocturnal species than in their diurnal relatives (de Souza & Ventura, 1989, Frederiksen et al., 2008, Heimonen et al., 2006, Howard et al., 1984, Laughlin, 1996, Pirhofer-Walzl et al., 2007, Weckström et al., 1993). This results from a higher gain in the transduction cascade of their photoreceptors, which transduces the same incoming signal (one photon) into a larger electrical signal. While this mechanism does not counter-act photon shot noise or thermal noise (as it also increases the gain on 'false positive' detections due to thermal activation of the transduction cascade), it increases the likelihood that incoming photons are reliably translated into a physiological response, which can then be synaptically transmitted.

Neural summation

As discussed above, the challenge to vision is set by both the low signal, as well as the relatively high rate of noise. Adaptations to increase overall visual sensitivity at night can thus result from both increases in photon catch, and adaptations to reduce the adverse effects of noise. Neural adaptations operate on the visual signals acquired by the photoreceptors in the eye to increase their signal to noise ratio.

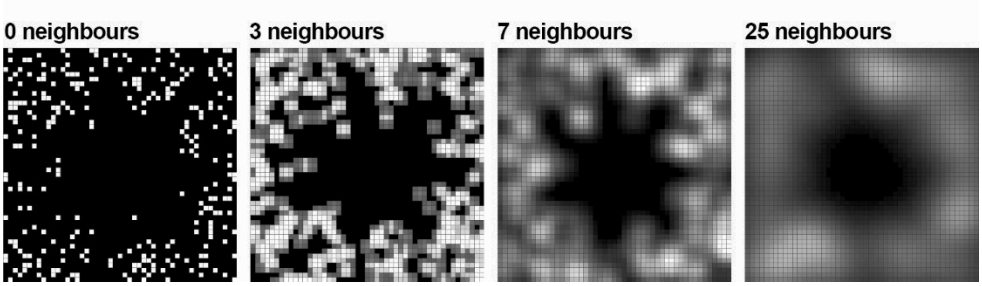


Fig. 6 The effect of spatial summation.
Spatial summation of visual signals over neighbouring channels can help to increase the reliability of vision, but when summing over many channels, spatial resolution decreases. Starting with a noisy image, spatial summation over increasingly wider receptive fields (radius of summed area expressed in neighbouring pixels indicated above each panel) initially improves image quality and lets the flower appear clearly again. When the summation becomes too extended, spatial detail is lost and the flower is converted to a black circle. However, even though spatial detail is lost, for some tasks it can be beneficial to detect the presence of an object (whatever its shape) rather than to not detect anything at all. The extent of spatial summation therefore has to be matched to the amount of spatial detail required for the task at hand.

The discrepancy between the optical sensitivity and visual performance in some nocturnal insects has sparked the suggestion that additional neural processing increases the sensitivity of the animal for certain visual details beyond that provided by the eye and retina: the night-active bee *Megalopta genalis*, for example, has optical adaptations that make their eyes about 30 times more light sensitive than the eyes of the honeybee *Apis mellifera*. However, *M. genalis* flies at light intensities that are several orders of magnitude lower than those at which the honeybee is active (Greiner, Ribi & Warrant, 2004), thus suggesting additional mechanisms to increase the overall sensitivity of their visual system.

These mechanisms include neural summation of visual information in space and time. By summing signals over several neighbouring visual units, reliability

increases, because the noise is uncorrelated, while the signal is correlated in neighbouring channels and therefore increases upon summation (Laughlin, 1981, Lythgoe, 1979, Snyder, 1979, Snyder, 1977, Warrant, 1999, Warrant & McIntyre, 1996). Correspondingly, summing signals in time removes noise that is uncorrelated in the time domain, while the signal, which is correlated (at least over limited periods of time) is amplified. This temporal summation is similar to the adaptations found in photoreceptors, just that it does not affect the number of photons that are captured per visual integration time directly, but acts at a later stage of visual processing.

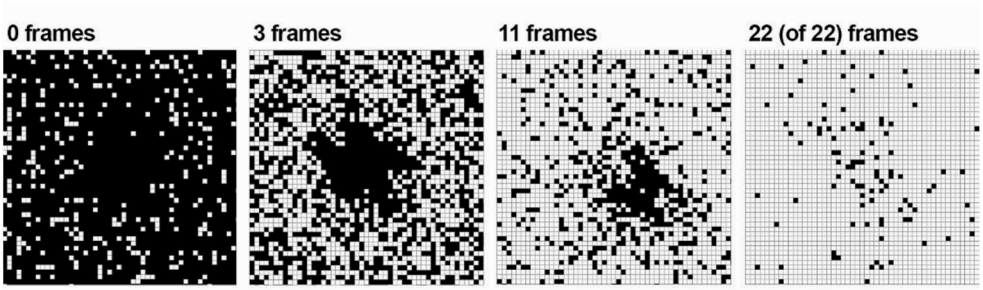


Fig. 7 The effect of temporal summation.
Temporal summation can increase visual reliability by summing visual signals over a longer time span. The panels from left to right show the original image of a flower moving from the top to the bottom of the scene (first panel), temporal summation over 3 frames, 11 frames and 22 frames (of a total of 22). Moderate temporal summation aids in object recognition (second panel), though spatial detail is lost in motion blurr. If the integration window becoems too long with respect to the speed of the object, the visual object degrades (third panel) and eventually vanishes completely (fourth panel). Temporal summation must therefore be carefully matched to the image speed expected on the sensor.

In order to incorporate the effect of spatial and temporal summation in the capacity of an animal to capture photons from a light source of a given intensity I (Warrant, 1999), we can extend equation (2) with a term for spatial summation ($\Delta\rho_s$ represents the half-width of a Gaussian integration filter, which is implemented as a multiple of the inter-ommatidial angle $\Delta\varphi$), and a term for temporal summation (Δt_s represents the time constant of the temporal summation filters):

$$N = \frac{\pi}{4}\,(D)^2\,n_f\,(\Delta t + \Delta t_s)\,1.43\left(\frac{\Delta\rho_s}{\Delta\varphi}\right)^2\,1.13\,\Delta\rho^2\tau\,\kappa\left(\frac{kl}{2.3+kl}\right)I \qquad (4)$$

Neural summation represents a distinct example of the trade-off between sensitivity and resolution: while spatial and temporal summation increase the signal to noise ratio, they reduce resolution in space (Fig. 6) and time (Fig. 7), respectively.

Thus, the level of spatial or temporal summation is expected to be adapted to the animal's requirements in terms of spatial and temporal acuity (van Hateren, 1993b, Warrant, 1999): animals that experience high temporal frequencies (for example, if they are fast-flying), should rely more strongly on spatial summation, thus maintaining high temporal resolution, while animals that do not move very fast can compromise their temporal resolution in favour of retaining higher spatial acuity. Moreover, in eyes with photoreceptor acceptance angles that are larger than their interommatidial angles (visual field overlap), the visual acuity does not suffer significantly from spatial pooling, if the extend of pooling is matched to the receptive fields of the photoreceptors (Snyder, 1979).

In vertebrates, there is a very widespread and clear architecture for spatial summation in the retina, particularly in the rod system and in the retinal periphery. Hundreds to thousands of rods converge onto one ganglion cell (which is the representation of one 'pixel') in order to improve sensitivity. Correspondingly, vision in the periphery, where the most extensive spatial summation takes place, is the least spatially resolved in the retina. In contrast, vision in the fovea is most resolved: here very few photoreceptors converge onto each ganglion cell (Hallett, 1963, Zuidema et al., 1981).

While suffering from the same trade-off between sensitivity and resolution as adaptations in the eye and retina, neural summation can be implemented in specific visual pathways, and thus does not affect the entire visual system, as optical and retinal adaptations do. There can be different levels of spatial and temporal summation, each matched to the specific requirements for sensitivity, as well as spatial and temporal acuity, of each sub-system. Thus, unlike adaptations of the eye or retina, spatial and temporal summation can be adjusted to the specific requirements and constraints of each visual pathway, thus retaining the highest possible acuity, while providing the sensitivity each system requires.

"I get by with a little help from my friends."

The Beatles

IV. Quantifying spatial and temporal summation in the hawkmoth motion vision system

Summing information in space and time can increase the reliability of the visual signal, and thus constitutes a neural adaptation to improve visual sensitivity in dim light (Chapter III). While behavioural evidence (Dvorak & Snyder, 1978, Pick & Buchner, 1979) and theoretical models (Laughlin, 1981, Lythgoe, 1979, Snyder, 1979, Snyder, 1977, Warrant, 1999, Warrant & McIntyre, 1996) pointed towards the existence of such adaptations in insects, they had not been physiologically quantified. A major reason for this was that candidate neurons for spatial or temporal summation were unknown, and thus it was not clear which neurons to target for physiological investigations. While candidate neurons for spatial summation have subsequently been identified anatomically ((Greiner, Ribi & Warrant, 2004, Greiner et al., 2005), Chapter V), they have proven challenging to record from. Summation by these cells – the lamina monopolar cells – has so far only been recorded in day-active flies (Dubs et al., 1981), the only physiological evidence for spatial summation in the insect visual system at the time of the current study.

Recording spatial and temporal summation in the motion vision system of hawkmoths

Because of the challenges in identifying and recording from candidate neurons for spatial and temporal summation, an indirect approach was used to quantify these neural adaptations. I measured the spatial and temporal properties of the photoreceptors of a nocturnal hawkmoth species (*Deilephila elpenor*), and compared them to those of output neurons of their motion vision system (Fig. 8). The reasoning was that if spatial and temporal summation occurred in the pathway

between input (photoreceptors) and output (motion neurons), it should be revealed by differences in their spatial and temporal properties (which should show reduced spatial and temporal resolution due to neural summation).

I conducted this study in the wide-field motion vision system of hawkmoths for a number of reasons: as discussed in Chapter II, most animals have to rely on vision to control flight, even in very dim light. Thus, we can expect the flight control system of hawkmoths to be optimally adapted for the dimmest light conditions these animals are active in. In all insects, flight control is based on optic flow information, provided by the wide-field motion vision system (Srinivasan et al., 1999). Furthermore, this system is one of the best studied visual pathways in insects, with well characterised output neurons, which are large and readily accessible to electrophysiological recordings.

Wide-field motion-sensitive neurons have previously been described in the brains of hawkmoths (O'Carroll et al., 1996, O'Carroll et al., 1997, Theobald et al., 2010, Wicklein & Varjúe, 1999), and their responses strongly resemble the well-studied lobula plate tangential cells in flies (Hausen, 1982a, Hausen, 1982b, Hausen & Wehrhahn, 1989, Hengstenberg, 1982, Hengstenberg et al., 1982, Krapp, 1999, Weber et al., 2010), for which a great body of information is available. Finally, computational models of the wide-field motion pathway exist (Borst & Euler, 2011), and can be used to quantify the exact extent of the spatial and temporal summation in the system (O'Carroll & Warrant, 2011).

Motion vision and models to quantify neural summation

Wide-field motion information (optic flow) is used in insects for the estimation of flight speed from translational image motion (Srinivasan et al., 1991, Srinivasan et al., 1996), and the estimation of roll and yaw from rotational image motion (Goetz, 1975, Kern & Egelhaaf, 2000, Srinivasan et al., 1999). In addition, the travelled distance can be estimated using optic flow (Srinivasan, Zhang, Altwein & Tautz, 2000). Moreover, optic flow perception is also essential for collision avoidance, (Mronz & Lehmann, 2008, Tammero & Dickinson, 2002), gap negotiation (Srinivasan & Zhang, 2000), landing (Borst, 1990, Srinivasan, Zhang, Chahl, Barth & Venkatesh, 2000, Tammero & Dickinson, 2002), and position stabilization when hovering in front of flowers (Farina et al., 1995, Kern, 1998, Kern & Varjú, 1998).

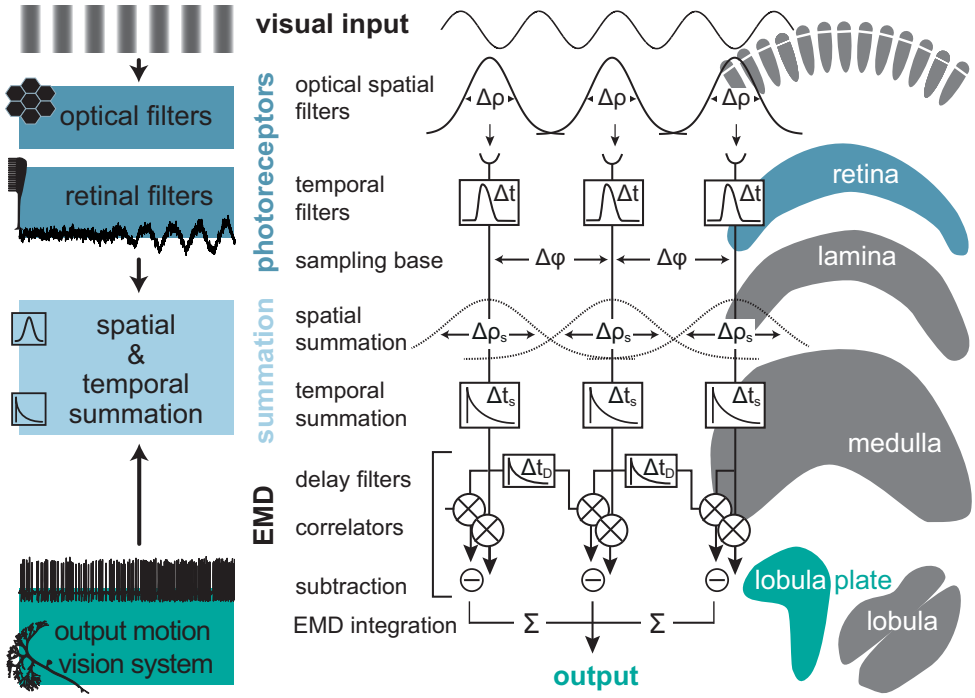


Fig. 8 Quantifying spatial and temporal summation in the hawkmoth motion vision system. In order to quantify the amount of neural summation, I recorded from photoreceptors in the retina and from wide-field motion-sensitive neurons in the lobula plate, while stimulating the visual system with moving sinusoidally-modulated patterns of black-and-white stripes (known as "gratings"), which were presented on an LCD screen. Comparing the spatial and temporal response characteristics of the photoreceptors to those of the motion-sensitive neurons allowed us to extract the additional spatial and temporal processing of visual signals that occurs in the motion pathway between the retina and the wide-field motion neurons. I used a computational model to estimate the extent of spatial and temporal summation. The parameters describing the spatial acuity – the half-width of the photoreceptor's spatial receptive field ($\Delta\rho$), set by the optics of the eye – as well as temporal acuity – the photoreceptor's integration time (Δt) – were obtained from the physiological recordings. The interommatidial angle $\Delta\varphi$,representing the spatial sampling base, was obtained from histological sections of the eye. Spatial summation is implemented as a Gaussian filter (half-width $\Delta\rho_s$), and temporal summation as an exponential filter (time constant Δt_s). The signal then passes through an elementary motion detector (EMD) with a first order delay filter (time constant Δt_D), and finally, multiple EMD outputs are integrated to produce the output signal, which can then be fitted to the physiologically obtained motion responses.

In order to detect a directional movement, two points in space and time have to be compared. All models for directional motion vision require at least (1) two input lines that detect the brightness values of neighbouring points in the image, (2) an asymmetry with respect to the temporal filtering of the input in order to compare the two inputs at different time points, (3) a non-linearity at the stage where the two inputs are combined again (Borst & Egelhaaf, 1989, Clifford & Ibbotson, 2002, Borst & Euler, 2011).

The motion model assumed to be implemented in insects is called the Hassenstein-Reichardt detector (Hassenstein & Reichardt, 1956, Reichardt, 1961). An elementary motion detector (EMD) is thought to consist of two mirror symmetric sub-units, each sampling from a neighbouring point in space (Fig. 8). The outputs of the two sub-units are carried in two arms, one of which is delayed in time. They are correlated with each other, so that the delayed signal from sub-unit one is multiplied with the un-delayed signal from sub-unit two and vice-versa. The two correlated signals are subtracted to produce the final output of the correlator. The preferred direction of motion is the one that the correlator responds to with a positive output, while it responds with a negative output in the reverse or "null" direction. Since optic flow is a global movement of the environment, signals are averaged over many individual EMDs to obtain a quantitative estimate of optic flow– a task that is thought to be executed by the lobula plate tangential cells (Single & Borst, 1998, Straw et al., 2008).

As described in O'Carroll & Warrant (2011), I expanded the classical motion correlator core with peripheral filtering, to more realistically describe the visual signal entering through the eyes and retina (Fig. 8). I obtained the spatial and temporal parameters necessary for these filters from physiological recordings of the photoreceptors at various light intensities. Furthermore, the model contained additional spatial and temporal filters, which represented the spatial and temporal summation. Their parameters could be estimated by fitting the result of this model with the responses obtained physiologically from wide-field motion sensitive neurons at the same range of light intensities used for the photoreceptor recordings. In this manner, a quantitative estimate of spatial and temporal summation, based on physiological measurements, in the hawkmoth motion vision system at various light intensities could be obtained, without having to identify and record from the actual neurons generating spatial and temporal summation.

Spatial and temporal summation in the hawkmoth visual system improve sensitivity and information content in dim light (Paper I)

I found substantial levels of spatial and temporal summation in the motion vision system of the nocturnal hawkmoth *D. elpenor* (Fig. 9). There were high levels of temporal summation (TS) at all light intensities, which increased further as light intensities decreased to the lowest tested values. Spatial summation (SS), however, remained low for a wide range of light intensities, and only increased to values affecting the spatial acuity of the responses at intensities equivalent to dim moonlight and starlight levels. Moreover, I quantified the effects of the measured spatial and temporal summation on contrast sensitivity and information content at the light intensities examined physiologically. In order to do this, I added the levels of photon shot noise present at these intensities to the motion vision model, and analysed the resulting sensitivity and information content of the output responses, comparing models with as well as without (NO) different combinations of spatial and temporal summation.

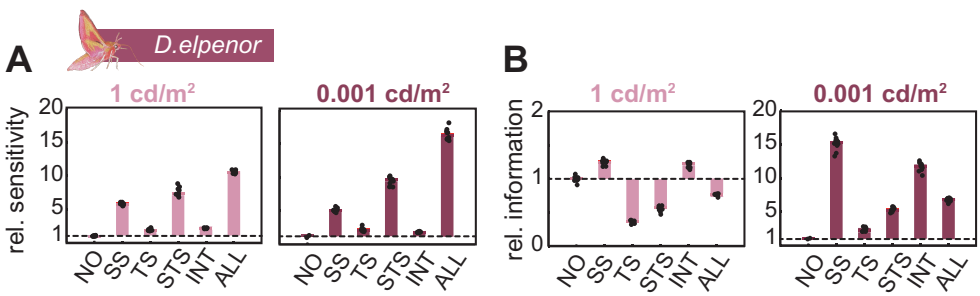


Fig. 9 Spatial and temporal summation extent, sensitivity and information rate in dim light. The modelled effect on contrast sensitivity (**A**) and information rate (**B**) of spatial summation (SS), temporal summation (TS), and the two combined (STS), integration of 30 EMDs without previous summation (INT) and a combination of all these (ALL), on the motion vision pathway. Results were normalised to the output of the model without summation and EMD integration (NO) at 1 and 0.001 cd/m^2 (bright moonlight and starlight intensities). Noise was added to reflect the system's photon shot noise at these light intensities (as determined from the LCD screen used for stimulation). All data obtained represent the responses of the motion vision system to stimuli changing in temporal frequency, with a constant spatial frequency.

We could show that the motion vision system would not have sufficient sensitivity to function at the two lowest light intensities without neural summation (Paper I). It was also noteworthy that spatial and temporal summation improved sensitivity in a supralinear manner, that is their combined contribution was greater than the sum of their individual contributions (STS). This finding is interesting from an evolutionary perspective, because it would suggest a great incentive to invest in such neural sensitivity enhancement — as minor increments can lead to benefits far greater than their individual contributions would suggest.

Sensitivity was further improved by integrating the output of many individual EMDs (Fig.8, INT), as wide-field motion sensitive neurons in the lobula plate do. Interestingly, this integration was most effective when combined with spatial and temporal summation (ALL), while on its own it only marginally improved sensitivity. This finding makes a strong case for previous theoretical arguments that spatial and temporal summation should occur as early as possible in the visual system—to improve the visual signal before it enters into further non-linear processing (as found in most pathways in the visual system), and information is un-retrievably lost (van Hateren, 1992b, van Hateren, 1993a, van Hateren, 1992c, van Hateren, 1993b).

When a very weak visual signal enters the motion correlators, with such high levels of noise that their outputs do not contain any motion information, summing over many individual EMDs will not improve the reliability of the combined signal (because there is no information left in the individual EMD responses). If, however, spatial and temporal summation act on the incoming signal before the correlators, and reduce the noise in the input channels so that the motion correlators can produce motion estimates with some information about the underlying signal, integrating many individual EMDs will improve the reliability of the final response.

Finally, it was interesting to note that these models also showed that neural summation in fact increased the information content of the output response in very dim light - not just its contrast sensitivity (Fig. 9B). One might initially have thought that spatial and temporal summation might reduce the information content, because they reduce resolution in both space and time, and thus the possible bandwidth of the signal. However, the information content of the motion vision system depends not only on its resolution, but also on its sensitivity, that is the signal strength available across the spectrum of spatial and temporal frequencies. If there is not sufficient sensitivity, and hence the signal is smaller than the noise, then there is no information in the response, no matter how high the resolution is. This result may demonstrate why it is that we often find that the trade-off between

sensitivity and resolution is resolved evolutionarily in favour of sensitivity in nocturnal animals: the information content of the visual system can be maximised with neural summation, despite the reduction in resolution.

"There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved."

Charles Darwin

V. Neural and anatomical adaptations for dim light vision match the visual ecology of hawkmoths

After establishing that spatial and temporal summation exist in the visual system of a nocturnal hawkmoth species, and that these neural adaptations indeed improve visual sensitivity, I asked how general this strategy was in other hawkmoth species with different sensitivity requirements, and furthermore how it tied in with their anatomical adaptations for vision in dim light.

Hawkmoths as a model species for visual adaptations

The family of hawkmoths (*Sphingidae*) constitutes a fantastic model system in which to compare adaptations for different light intensities. While it contains many nocturnal and crepuscular species, the family also has some diurnal members, and thus spans the whole range of light environments. More importantly, many hawkmoth species share a very similar lifestyle, hovering in front of flowers to suckle nectar (Pittaway, 1993), which means that these species share a dependence on vision for survival. Furthermore, the hovering flight has selected for very similar general body types, which allows stable hovering (Henningsson & Bomphrey, 2013). Finally, there are many species that even share the same habitat, and thus the same sensory environment (Pittaway, 1993). Together, all these similarities ensure that differences in sensory adaptations, and especially in visual adaptations, are likely due to differences in the light intensities these species are active under, rather than general differences in their ecology and lifestyle. Moreover, hawkmoths have a very similar brain layout (El Jundi et al., 2009, Stöckl et al., 2016), and their wide-field motion sensitive neurons share similar characteristics (O'Carroll et al., 1996, O'Carroll et al., 1997, Theobald et al., 2010, Wicklein & Varjúe, 1999), suggesting that they derive from the same underlying neural circuits.

For this study, and the subsequent comparative studies (Chapter VI and VII), we chose to investigate the diurnal *Macroglossum stellatarum*, the crepuscular-nocturnal *Manduca sexta* and the nocturnal *Deilephila elpenor* (Fig. 10). *M. stellatarum* and *D. elpenor* are both European species, have overlapping habitats and even share some of the same food flowers (Pittaway, 1993). *M. sexta* is native to North and Middle America, and while generally sharing a similar lifestyle, does not have the same food plants and habitat as the other two species. Furthermore, the three species have different anatomical prerequisites in terms of their visual system and visual sensitivity: *M. sexta* is by far the largest of the three species, and also has much bigger eyes, with twice the facets of the nocturnal species, and more than 4 times as many as the diurnal species. The nocturnal species takes an intermediate position in body and eye size, while the diurnal species is the smallest of the three, and also has the smallest eyes (Fig. 10). Thus, comparing the three species not only allows for a comparison of adaptations to the light environment, but also between different anatomical architectures for dim light vision.

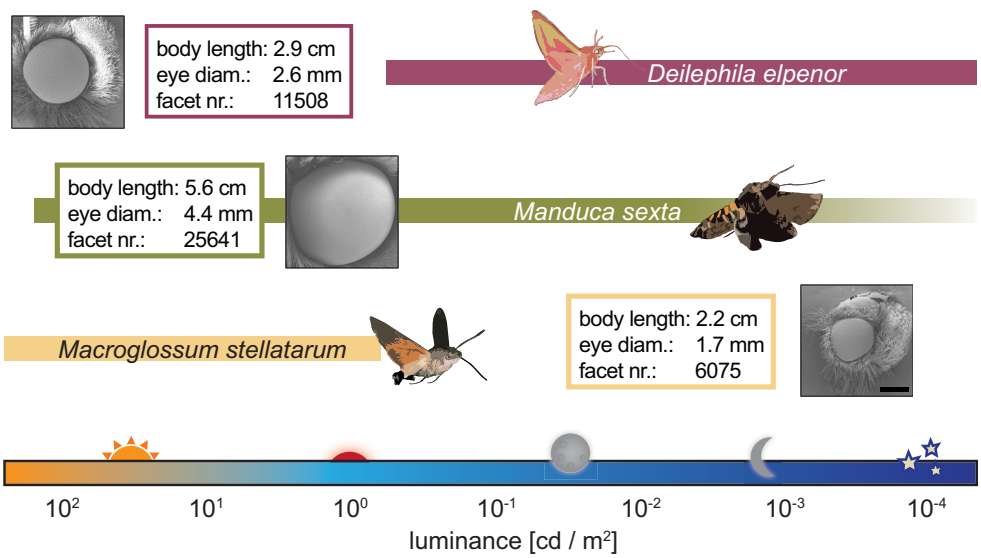


Fig. 10 The three hawkmoth species used for comparative studies.
This project compares three hawkmoth species active at different light intensities: The nocturnal *Deilephila elpenor,* the diurnal *Macroglossum stellatarum* and the crepuscular-nocturnal *Manduca sexta*. The three species have different body and eye sizes (averages from Paper III). Scale bar on the scanning electron micrographs is 2 mm.

Spatial and temporal summation match the visual ecology of hawkmoths (Paper II)

In order to quantify how neural summation was adjusted to the natural light environments of the three hawkmoth species, we compared the extent of spatial and temporal summation in their motion vision system, using the methods described in Chapter IV. We expected the diurnal species to have the lowest levels of spatial and temporal summation, as they are active in bright light, and thus have no need to implement strategies to improve sensitivity, but can maximise spatial and temporal resolution in their visual system. The crepuscular and nocturnal species, one the other hand, were expected to show distinctly higher levels of neural summation, and thus lower spatial and temporal resolution that the diurnal species.

First of all, the contrast sensitivity of the wide-field motion sensitive neurons in the three species was scaled as expected given their natural visual environments (Fig. 11A): while all species had rather similar contrast sensitivities at the highest measured light intensity (which corresponded to early sunset, and thus was already dim for the diurnal species, but so bright for the nocturnal and crepuscular, that their pupils were closed), the contrast sensitivity of the diurnal species rapidly decreased with decreasing light intensity, and its neurons did not respond at intensities below moonlight levels. The contrast sensitivity of the crepuscular and nocturnal species, however, increased initially with decreasing light intensity, due to the opening of the pupil, and remained high as far down as dim moonlight levels, after which it drastically decreased in both species. There was no distinct difference in contrast sensitivity between the crepuscular and nocturnal species. As expected, the diurnal species had the highest spatial and temporal acuity at the brighter light intensities—suggesting their visual system is optimised for acuity rather than sensitivity. The nocturnal species had the lowest spatial and temporal acuity, while the crepuscular species was intermediate in terms of both (Paper II).

The extent of temporal summation was closely matched to temporal acuity (Fig. 11C): the diurnal species had the lowest level of temporal summation, the crepuscular species was intermediate, and the nocturnal species had the highest level—as we had expected. In all three species, the level of temporal summation increased as light levels decreased. In the spatial domain, the effects were more complex. This was, in part, because of the tight link between effective retinal illumination (controlled by the state of the pupil) and the level of spatial summation. While in the diurnal species, the extent of spatial summation increased as a function of decreasing light intensity, in the crepuscular and nocturnal species

summation decreased initially as the effective light intensity on the retina increased with the pupil opening, and then increased again at even lower light intensities. Overall, the maximum extent of spatial summation matched the visual ecology of the diurnal *M. stellatarum* and nocturnal *D. elpenor*: the nocturnal species had the greatest extent of summation, while the diurnal species had the lowest.

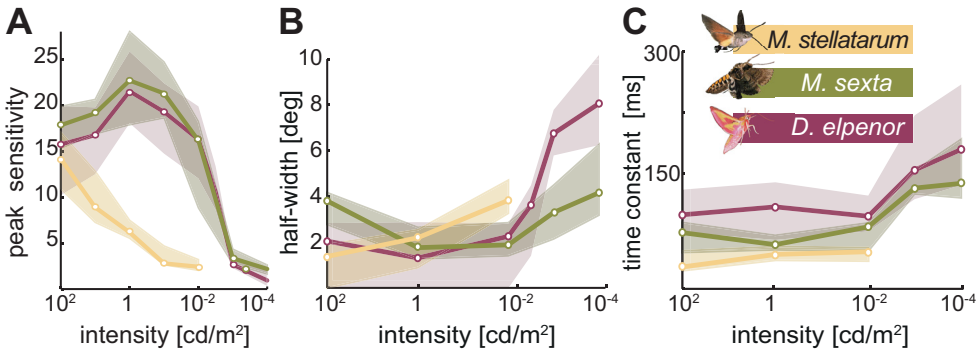


Fig. 11 Neural summation in three hawkmoth species
A Peak contrast sensitivity of motion-sensitive neurons at different light intensities. **B** Model estimates of the angular half-width $\Delta\rho_s$ of the best-fitted spatial low-pass filters performing spatial summation and of time constants Δt_s of the best-fitted temporal low pass filters performing temporal summation (**C**) in each species at different light levels. Shaded regions around the average represent the inter-quartile range. See Fig. 8 for model details.

It was rather surprising, however, that the crepuscular *M. sexta* had relatively low levels of spatial summation, which did not exceed those of the diurnal species. Considering that this species' contrast sensitivity was similarly high as that of the nocturnal species, we expected high levels of spatial summation (an explanation for how the high sensitivity was achieved is discussed in the next section). Here I discuss why evolution might have selected for higher spatial resolution (and thus less spatial summation) in the motion vision system of *M. sexta*—considering that the nocturnal *D. elpenor* seems to thrive with a motion vision system of lower spatial acuity. *M. sexta,* however, flies roughly twice as fast than *D. elpenor* in free flight (Henningsson & Bomphrey, 2013), which correlates with its considerably greater body size. The nocturnal species, in fact, flies rather slowly, even compared to other species of the same body size and weight (Henningsson & Bomphrey, 2013).

Thus, if both species had similar spatial resolution, and could therefore detect an obstacle in their flight path at the same distance, *M. sexta* would only have half the

time to respond and correct its course, because it approaches the obstacle at twice the speed. A higher resolving power compared to the nocturnal species might therefore enable *M. sexta* to have adequate reaction times despite flying quickly. It is important to note that the looming network (Fotowat & Gabbiani, 2011), rather than the wide-field motion pathway investigated here, is responsible for obstacle avoidance in flying insects, and hence the constraints on spatial acuity might primarily apply to the former and to the visual periphery. To what extent these constraints trickle down to the motion vision system remains to be investigated.

It is also conceivable that *M.sexta* retains higher spatial acuity in their motion vision system to allow for higher temporal resolution (since they fly faster, which might generate more high temporal frequency visual input), while still retaining sensitivity to slow image speeds, which are thought to be required to control the hovering flight hawkmoths perform (O'Carroll et al., 1996, O'Carroll et al., 1997). Since the perceived velocity is a combination of temporal and spatial frequencies, low velocity tuning can be achieved by a combination of low spatial and temporal frequency tuning, as well as a combination of high spatial and temporal frequency tuning: both species' peak velocity tuning (the ratio between their peak temporal and peak spatial tuning, Paper II) at 0.001 cd/m^2 was around 20 deg/s, despite the spatial and temporal peak tuning in *M.sexta* being double that in *D.elpenor*.

While there are formalisms to obtain estimates of optimal spatial and temporal receptive fields (van Hateren, 1992a, van Hateren, 1992b, van Hateren, 1992c), as well as the optimal spatial and temporal summation under noisy conditions (Klaus & Warrant, 2009), realistic estimates require knowledge of the spatial and temporal properties of the visual scenes the animals perceive. The average image statistics of terrestrial natural scenes are well studied (Balboa & Grzywacz, 2003, Simoncelli & Olshausen, 2001, van der Schaaf & van Hateren, 1996), and are often used to approximate the visual input an animal receives, but they can differ greatly between habitats (Balboa & Grzywacz, 2003), and also depend on how closely (Torralba & Oliva, 2002), and how rapidly, flying animals negotiate their environments. Thus, observations of freely flying hawkmoths, ideally in their natural habitat, are required to reconstruct the spatial and temporal image statistics their wide-field motion system operates on. With this information, the optimal spatial and temporal acuity of their visual system, as well as optimal levels of spatial and temporal summation, could be determined theoretically, and compared with their physiologically quantified equivalents. This would allow us to determine how the quantified differences in spatial and temporal summation match with the species' requirements not only in terms of sensitivity, but also in terms of spatial and temporal acuity.

The balance between neural and anatomical adaptations

In addition to the extent of spatial and temporal summation, I also compared how neural adaptations were balanced with the adaptations of the eye and retina to achieve optimal sensitivity. In all three hawkmoth species, the spatial resolution of their motion neurons approached the limits set by their eyes in bright light, thus maximising potential spatial acuity (Fig. 12A). At low light intensities, spatial resolution decreased below the limits set by the eye, as a result of spatial summation (Fig. 12B). This was rather different in the temporal domain, where all species had a lower temporal resolution than their photoreceptors provided even at high light intensities, which was lowered further at the dimmer intensities (Fig. 12). These findings suggest that spatial summation functioned as a dynamic dim light adaptation: it increased the sensitivity of the visual system when needed, while its levels were low to optimise spatial acuity when there was sufficient light. Temporal summation, however, did not seem to be limited to enhancing sensitivity, because then we would expect it to have had a lower impact in brighter light.

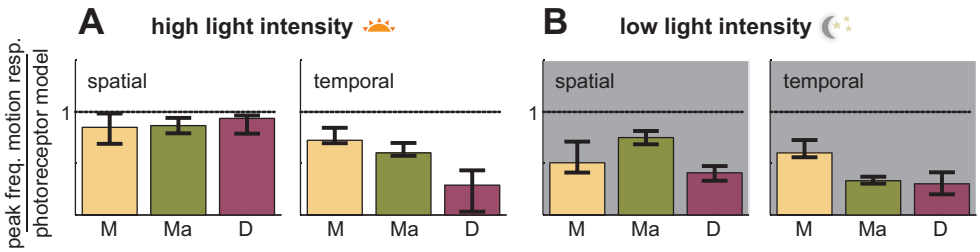


Fig. 12 The balance of peripheral adaptations and neural summation
The ratio between the peak spatial and temporal frequencies of the motion-sensitive neurons and those obtained from a motion model (Fig. 8), whose spatial and temporal properties were set entirely by the photoreceptors (i.e. higher-order summation was absent). This ratio was calculated for all three species, both at high effective retinal light intensities (**A**, 100 cd/m^2 *M. stellatarum,* 1 cd/m^2 *D. elpenor, M. sexta*) and at low ones (**B**, 0.01 cd/m^2 *M. stellatarum,* 0.001 cd/m^2 *D. elpenor, M. sexta*). A ratio of 1 means that the spatial and temporal tuning of the motion neurons was set by the photoreceptors, while a ratio less than 1 implies higher-order summation which decreased spatial and temporal resolution. Bars show averages and quartiles.

There is evidence that the motion vision system of insects is tuned to optic flow patterns experienced in their natural habitats, and that hawkmoth wide-field motion neurons are tuned to particularly low frequencies to match the flow patterns experienced during hovering (O'Carroll et al., 1996, O'Carroll et al., 1997). The

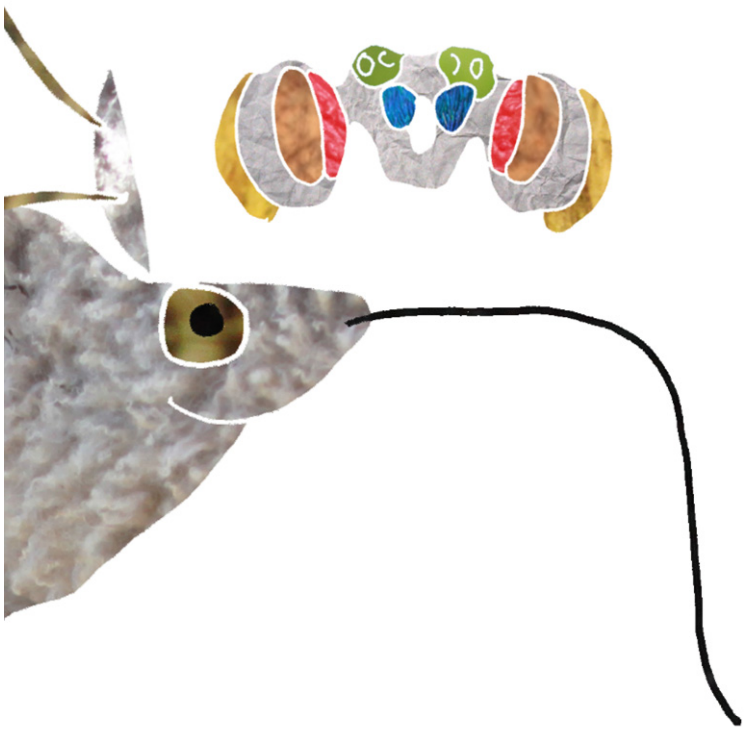
temporal summation observed in three hawkmoths species in this study might therefore tune their visual system to the optic flow they experience during hovering, in addition to improving visual sensitivity. The fact that the photoreceptors responded much faster than wide field motion neurons also opens up the possibility that the other visual pathways in hawkmoths respond distinctly faster than motion neurons. It would be intriguing to test this, for example in looming neurons (Wicklein & Strausfeld, 2000), which, if detecting predators or obstacles, should respond with the highest temporal acuity possible.

While the nocturnal and crepuscular species both had similar contrast sensitivity across all light intensities, the crepuscular species had distinctly lower levels of both spatial and temporal summation, and therefore a smaller contribution of neural processing to sensitivity. This suggests that their eyes must be more sensitive than those of the nocturnal species, in order to reach the same overall sensitivity. Indeed, we found this to be the case: the crepuscular *M. sexta* absorbs a three times higher proportion of photons per visual integration time of a given light intensity I (34.2 μm^2 sr s I) than the nocturnal *D. elpenor* (11.3 μm^2 sr s I), when accounting only for the properties of the optics and photoreceptors (equation (3). The superior sensitivity of *M. sexta* was mainly due to its enormous superposition pupil - a feat that is only physically possible because of the greater eye size and higher facet number, which in turn is only sustainable because *M. sexta* itself is a much larger species. We might therefore conclude that the nocturnal and crepuscular species follow different evolutionary strategies with respect to anatomical and neural adaptations for dim light vision: because of its superior eye size, which allows for higher optical sensitivity, the crepuscular species can afford to have lower levels of neural summation, therefore compromising its spatial and temporal resolution less, and still achieving similar levels of overall sensitivity as the nocturnal species, which has to compensate for the lower optical sensitivity (due to a smaller eye and pupil size) with increased neural summation.

In summary, there are probably a number of factors that set the optimal levels of spatial and temporal summation in insects (and in any other animal groups where these neural strategies exist), among which are requirements for sensitivity (set by the species' natural light environment), the range of spatial and temporal frequencies that each animal's visual system has to be most sensitive to, as well as the physical pre-requisites of each species in terms of optical sensitivity. How these factors are weighted depends upon the ecological needs, and the specific evolutionary history, of each species, and may be better understood with greater insight into the natural requirements of the different visual pathways of these animals.

"It is not down on any map; true places never are."

Herman Melville



VI. Where do spatial and temporal summation take place?

While it was not necessary to know the identities of the neurons carrying out spatial and temporal summation in the motion vision system, in order to quantify the extent of neural summation, their identity is crucial for more detailed investigations of the neural properties of these processing strategies. There is a great body of evidence pointing towards a candidate neuron for spatial summation, but the neurons carrying out temporal summation have so far remained elusive.

Do lamina monopolar cells carry out spatial summation?

Several lines of evidence point to the lamina as the location of spatial summation, and the lateral dendrites of the lamina's main relay neurons, the lamina monopolar cells (LMCs), as its substrate. Perhaps the strongest evidence comes from physiological recordings of anatomically unidentified lamina neurons (physiologically classified as LMCs), which showed that these neurons produced more single photon responses than they would have had they only been connected to photoreceptors processing information within their own retinotopic sub-unit (Dubs et al., 1981). Theoretical considerations support the lamina hypothesis, because they suggest that spatial filtering should best take place as early as possible in the visual system, before any information is irreversibly lost in non-linear processing (Srinivasan et al., 1982, van Hateren, 1992b, van Hateren, 1993a, van Hateren, 1992c, van Hateren, 1993b) (see also Chapter IV).

The lamina is the first brain area in the optic lobes of insects (Fig. 13A). Its main cell type, lamina monopolar cell, receives information directly from the axons of photoreceptors (Trujillo-Cenóz & Melamed, 1966), thus forming the first synaptic relay in the visual pathway. They send information on to the second optic neuropil, the medulla. The cell-bodies of LMCs form the outer layer of the lamina, while one central prolongation from each cell body extends through the lamina proper and

forms the main axon, from which collaterals branch in the external plexiform layer of the lamina (Cajal & Sánchez, 1915, Strausfeld, 1970, Strausfeld & Blest, 1970).

The lamina is organised into so called 'cartridges' (Strausfeld, 1976), reflecting the retinotopy of the insect visual system. Cartridges are repetitive elements of neurons, each of which receives input from the retinula cells of one ommatidium (or in dipteran neural superposition eyes, of all retinula cells sharing the same visual axis). In addition to LMCs, the lamina contains the fibres of those photoreceptors, which terminate in the lamina (short visual fibres) and those that cross the lamina to terminate in the medulla (long visual fibres). Each cartridge also contains the dendrites of amacrine cells, which are confined to the lamina and form lateral connections between neurons, as well as the projections of neurons that connect the medulla and lamina (T-cells).

The structure of the lamina is strongly conserved across insects (Shaw & Moore, 1989), and the same general types of neurons are found in all major groups, including hymenopterans (bees (Ribi, 1975b), ants (Ribi, 1975a), sweat bees (Greiner, Ribi, Wcislo & Warrant, 2004)), dipterans (*Calliphora*, *Eristalis* and *Syrphus* (Strausfeld, 1970), fruitflies (Fischbach & Dittrich, 1989)), blattarians (cockroach, (Ribi, 1977)), hemipterans (backswimmers, (Wolburg-Buchholz, 1979)), odonata (dragonflies, (Meinertzhagen et al., 1983)) and lepidopterans (butterflies, (Ribi, 1987), hawkmoths (Strausfeld & Blest, 1970) and skipper-butterflies (Shimohigashi & Tominaga, 1999)).

LMCs have become the candidate neurons for spatial summation because of their morphology. Their lateral dendrites show varying degrees of length in different species, contacting different numbers of neighbouring cartridges, thus making them well suited to perform spatial summation (Warrant, 2004). It has furthermore been shown that the LMC collaterals are short and stay within their cartridge in most diurnal insect species investigated (e.g. housefly *Musca* (Strausfeld, 1970), dragonfly *Sympetrum* (Meinertzhagen et al., 1983), cabbage butterfly *Pieris* (Strausfeld & Blest, 1970)). In many nocturnal insects, however, LMCs have wide-ranging collaterals, as found in hawkmoths (Strausfeld & Blest, 1970), waterstriders (Wolburg-Buchholz, 1979) or cockroaches (Ribi, 1977).

Studies on closely related insect species, where one can test the possibility that differences in LMC morphology reflect differences in lifestyle rather than general differences between insect orders, support this hypothesis: the nocturnal sweat bee *Megalopta genalis* has much longer LMC collaterals than its diurnal near-relative *Lasioglossum leucozonium* (both family *Halictidae* (Greiner, Ribi & Warrant, 2004)), correlating with the need for visual sensitivity.

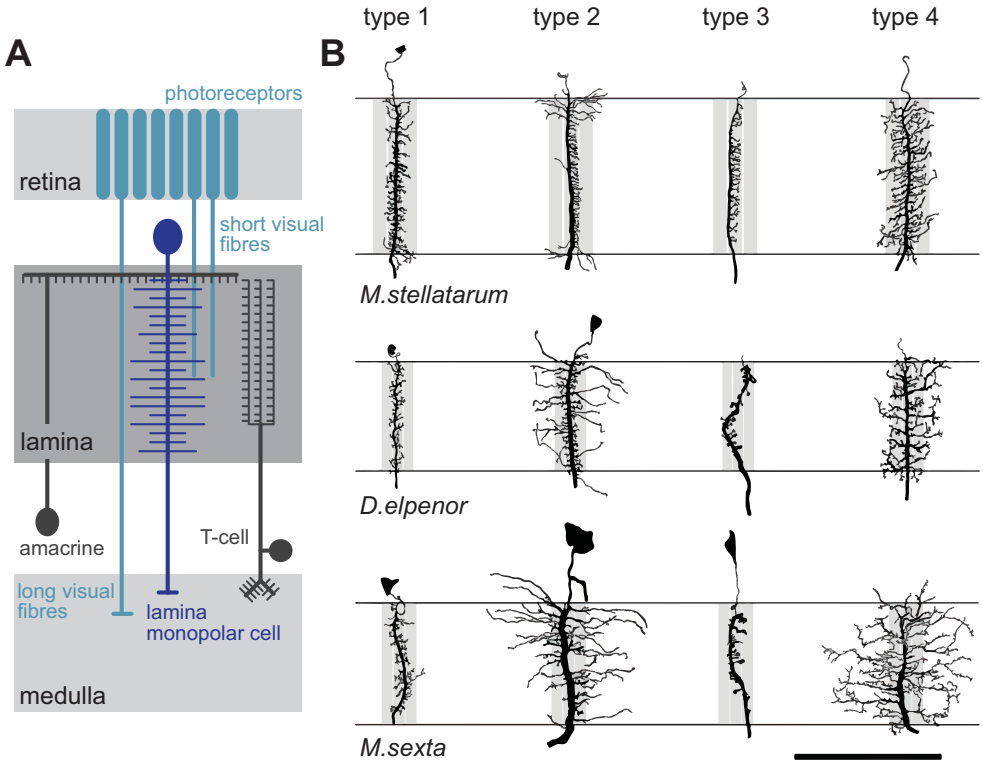


Fig. 13 Lamina monopolar cells.
A. The lamina of insects contains lamina monopolar cells as its main relay neuron, which receive information from photoreceptor axons, and send information to the medulla. A sub-population of photoreceptors terminate in the lamina (short visual fibres), while others cross it to terminate in the medulla (long visual fibres). In addition, the lamina contains the dendrites of amacrine cells, which are confined to this brain area, and establish lateral connections between cell types. So-called T-cells connect the medulla and lamina. **B** The four types of lamina monopolar cells (LMCs) in the diurnal *M. stellatarum* (upper row), the nocturnal *D. elpenor* (middle row) and the crepuscular *M. sexta*. Dimensions of three cartridges (the "home" cartridge and two neighbours) are indicated in light grey. Scale bar 10µm.

Do amacrine cells carry out spatial summation?

The evidence identifying LMCs as the spatial summation candidate neurons is mainly based on anatomy, however, and thus remains inconclusive. What is more, some insect species do not fit with the general pattern of LMC collateral extent in diurnal and nocturnal species: many diurnal lepidopterans (e.g. the skipper

butterfly *Parnara guttata* (Shimohigashi & Tominaga, 1999) and the butterfly *Papilio aegeus* (Ribi, 1987)) have LMCs that protrude into neighbouring cartridges, even though they have no need to increase visual sensitivity. It is thus suggested that their collaterals might carry out lateral inhibition. Furthermore, despite the electrophysiological evidence for spatial summation in the lamina of the house fly, most LMCs types in flies have processes restricted to one cartridge (Fischbach & Dittrich, 1989, Strausfeld, 1970) – with the possible exception of L4 (Strausfeld & Campos-Ortega, 1973) - and thus it remains questionable whether the spatial summation that was physiologically quantified in house fly LMCs actually originated in these neurons (Dubs, 1981).

The fact that LMC responses reveal spatial summation does not necessarily provide evidence that these cells actually sum information in space – they might have received spatially integrated visual information from other cell types in the lamina, which are the actual source of spatial summation. The second cell type in the lamina that would have the anatomical potential for lateral summation of visual signals are the amacrine cells, because their dendrites extend over several cartridges in all species where they have been identified (Fischbach & Dittrich, 1989, Strausfeld, 1970, Strausfeld & Blest, 1970). Unfortunately, amacrine cells do not stain very reliably or regularly in Golgi impregnations of insect brains (discussed in (Strausfeld, 1970, Strausfeld & Blest, 1970), and therefore there is no quantitative data-base for the morphology of amacrine cells in diurnal and nocturnal species, such as exists for LMCs.

In the end, decisive evidence for the identity of the neurons carrying out spatial summation can only be obtained by finding a model species with quantified levels of spatial summation in a visual pathway, where candidate cell types in the lamina can be specifically eliminated (anatomically or physiologically) and the resulting spatial summation quantified. These types of investigations, however, require identified lamina cell types, and genetic access to label and eliminate them – a feat that is currently only possible in the fruit fly model.

Hawkmoth lamina monopolar cells have the anatomical potential to perform the physiologically observed spatial summation (Paper III)

I characterised the anatomy of lamina monopolar cells in three hawkmoth species with different diel activity patterns: the nocturnal *D. elpenor*, the crepuscular

M. sexta and the diurnal *M. stellatarum* (Fig. 13B). Very much like in other insects, there were differences in the dendritic extent of LMCs between species, differences which correlate with their need for increased sensitivity, and thus spatial summation: the diurnal species had the shortest LMC collaterals, while the crepuscular and nocturnal species had LMCs with collaterals reaching dozens of neighbouring cartridges (Fig. 13B). The different types of lamina monopolar cells in the hawkmoth species investigated also had an interesting property that is rare outside the order Lepidoptera: they had vastly different dendrite ranges. Thus, if the dendrites were performing spatial processing, some LMC types would be able to integrate from wider areas than others.

If the LMC types we characterised were contributing information to different visual pathways to varying degrees, as has been described for different LMC types in flies (Behnia & Desplan, 2015), it would be anatomically possible that they contribute different spatial properties to these pathways. Unfortunately, we do not know about the homology between the LMC types we distinguished in hawkmoths (or any other Lepidoptera) and those of fruit flies, for which the visual circuitry is being unravelled, and thus this hypothesis has to remain a speculation for now.

Since we obtained physiological estimates of the amount of spatial summation in the motion vision system of all three hawkmoth species (Chapter V), we could go one step further than the pure anatomical comparison, and ask whether the LMC anatomy would actually allow the extent of spatial summation we observed physiologically. We thus compared the estimated spatial summation (Gaussian kernel) with the dendritic extent of LMCs (in degrees of visual space) for all three species (Fig. 14). Across all species it was very striking that the summation kernel in bright light matched the dendrite distributions of LMCs 1 and 3 in all species very well, and also matched the distribution of the peak of shorter dendrites in LMC types 2 in all species (Fig. 14, light blue kernels). These matches suggest that if the LMC dendrites collected visual signals with similar weight for each dendrite, they could produce the spatial processing observed in the motion vision system of all three hawkmoth species in bright light with astonishing accuracy.

In reality, the situation is likely to be more complicated, as signals reach the lamina in discrete spatial locations through the axons of the photoreceptors, and not in a continuous fashion as the models would suggest, and moreover, it is unlikely that dendrites of vastly different length, and thus potential synapsing surface, all have the same effective weight. However, it is striking how well the dendrites of the different LMC types matched the required spatial processing profiles in bright light.

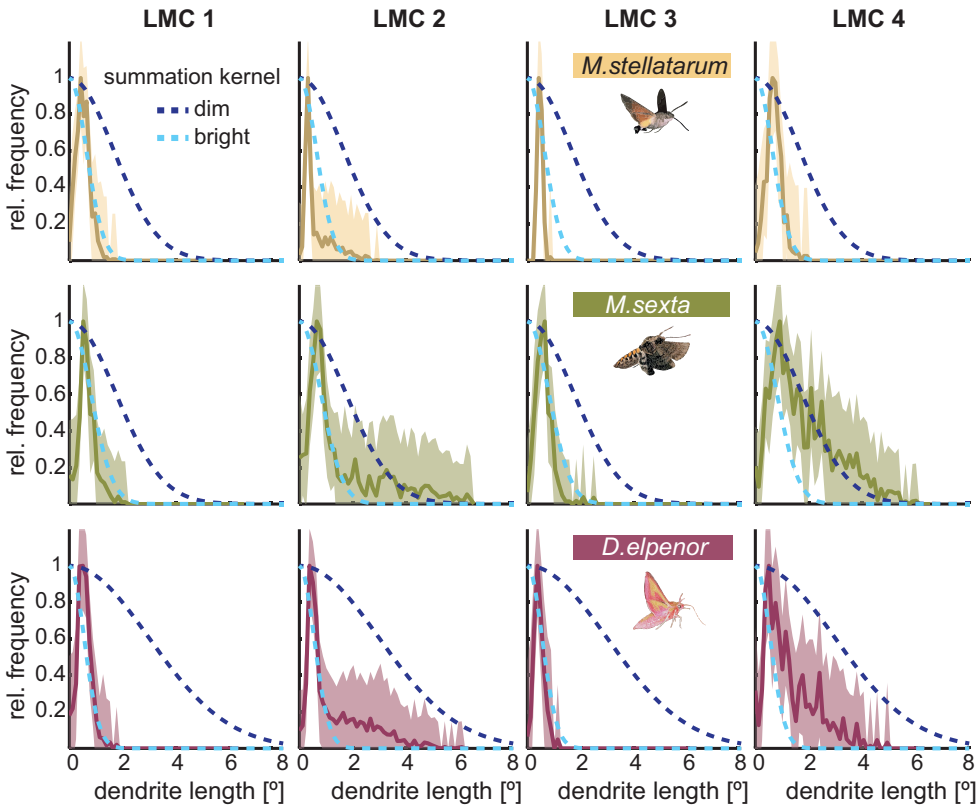


Fig. 14 Dendritic extents of lamina monopolar cells in the three hawkmoths species.
Dendrite length was normalised to visual angles by dividing by the average cartridge diameter, and multiplying by the average inter-ommatidial angle. Lines show averages, shades standard deviation. Gaussian summation kernels estimated from the responses of wide-field motion-sensitive neurons are overlaid: *light blue* corresponds to the brightest tested intensity (300 cd/m^2), and *dark blue* to the dimmest tested for each species (0.01 cd/m^2 for *M. stellatarum* and 0.0001 cd/m^2 for *M. sexta* and *D. elpenor*).

When focusing on low light intensities (Fig. 14, dark blue kernels), the spatial summation estimated from the motion responses in each species could not be carried out by LMC types 1 and 3 in all species, as their dendrites did not extend over a visual angle wide enough to provide the necessary spatial integration. In *M. sexta,* the spatial kernel fell well within the range of dendrites of LMCs 2 and 4 – and again, their dendrite distributions matched the filter so closely, they could achieve similar spatial processing if the dendrites had equal weights. In *M. stellatarum* and *D. elpenor,* however, the dendritic distributions of LMC types 2 and 4 did not resemble the spatial summation filters. In both species, LMC type 4

especially fell short of covering the length required to implement the summation filters observed in these species under dim light conditions, while LMC types 2 came close. However, their dendrites would need synapses with much higher weight on the distal ends of the longest dendrites, compared to the shorter ones, to achieve the observed spatial filters. An involvement of these LMC types in spatial summation becomes more likely when we consider that the spatial integration need not necessarily follow a Gaussian distribution (a filter chosen in the model for its simplicity). Similar results could be achieved with a more step-like filter, which does not range quite as wide as the outer flanks of the Gaussian kernels, and therefore could be implemented by the dendrites of type 2 (and possibly type 4) LMCs.

While I discussed earlier that it is hard to provide conclusive evidence of the identity of neurons producing spatial summation with physiological recordings alone, the LMCs of the three hawkmoth species have one great advantage for such studies: their vastly different morphology in terms of dendritic extends. If one could correlate the specific dendritic extends of different LMC types with matching spatial acuity (ergo, higher acuity in type 1 and 3, and lower acuity in type 2 and 4 LMCs), using physiological recordings and dye injections to identify LMC types, the evidence would be rather strong in favour of LMCs carrying out spatial processing. If the different LMC types had rather similar spatial profiles, it is likely that other types of neurons (possibly amacrine cells) generate spatial summation through synaptic connections onto the LMCs. Thus, apart from genetic model systems like fruit flies, hawkmoths actually are a very suitable model system to investigate the origin of spatial summation in the lamina.

Which neurons carry out temporal summation?

Unlike for spatial summation, no candidate neurons for temporal summation have been identified as yet. This is mainly because there is no anatomical correlate of the temporal properties of neurons. Furthermore, physiological recordings from neurons carrying the visual signals from photoreceptors to the wide-field motion output neurons have proven to be very challenging, and therefore remain sparse. However, it is possible to restrict the search area for candidate neurons: all lamina monopolar cells which have been recorded from in insects so far, show strong high-pass characteristics (de Souza et al., 1992, Dubs, 1981, Laughlin & Hardie, 1978, van Hateren, 1992b), thus, speeding up the signals they receive from photoreceptors, rather than slowing them down, as one would expect for neurons conducting temporal summation. We could confirm these findings for neurons in

the lamina of all three hawkmoths species (Fig. 15 shows an example from *M. stellatarum*). Thus, it is unlikely that LMCs are the source of temporal summation in any insect species, including hawkmoths.

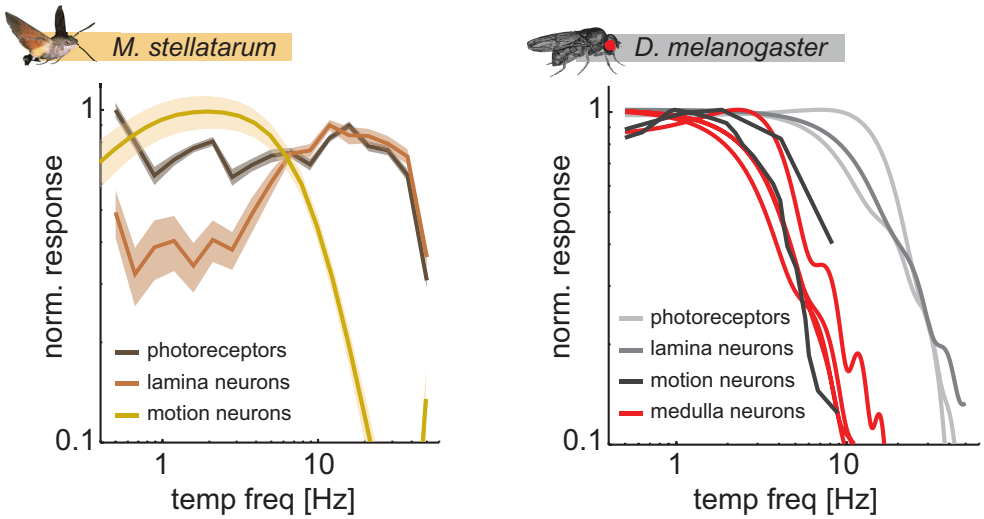


Fig. 15 The temporal properties of neurons in the motion-vision system.
A depicts the normalised response of photoreceptors, lamina monopolar cells and wide-field motion sensitive neurons to sinusoidal gratings of various temporal frequencies at 1 cd/m^2 in *M. stellatarum*. Note the very low frequency tuning of the wide-field motion neurons, compared to the other two cell types, indicating that temporal summation, shaping the low tuning of motion neurons, has to occur downstream of the lamina monopolar cells. **B** shows the same cell types in the motion vision system of *Drosophila melanogaster* (data sources: photoreceptors (Gonzalez-Bellido et al., 2011, Wardill et al., 2012), lamina monopolar cells (Wardill et al., 2012), wide-field motion neurons (Chiappe et al., 2010, Haag et al., 2004)). In addition, the medulla neurons Mi1, Tm1, Tm2, Tm3 are shown (Behnia et al., 2014), whose temporal properties make them exceptionally well suited as the source of temporal summation. Note that the physiological data in *D.melanogaster* was obtained under different experimental conditions and therefore is likely less comparable than the hawkmoth data.

The fact that LMCs constitute the start of distinct visual pathways (Behnia & Desplan, 2015), and that they are not the source of temporal summation, suggests that temporal summation might not be ubiquitous to all visual streams, and not occur at the earliest possible stage of visual processing. It is therefore very likely that we have to investigate temporal summation within the specific visual pathways, which might possibly result in different temporal characteristics across visual tasks. Accepting this hypothesis, we should investigate temporal summation within the motion vision pathway, downstream of the LMCs. Reason (and our modelling

results, Chapter IV) would suggest that the temporal summation stage should be located before the actual motion correlators, because information that is lost due to noise in the correlation step cannot be retrieved by neural summation afterwards.

Recent success in dissecting the motion circuitry in fruit flies helps us to limit our search for candidate neurons to the medulla, and specifically to 4 different types of medulla neurons, which constitute the motion pathway between the LMCs, and neurons which already possess directional motion responses (T4 and T5 (Maisak et al., 2013)). Mi1, Tm1, Tm2, and Tm3 are medulla and trans-medulla neurons, which receive information from the LMCs, and make connections with the fully directionally selective T4 and T5 (Bausenwein & Fischbach, 1992, Takemura et al., 2013). These neurons have been suggested to be responsible for the motion correlation step (possibly in combination with their post-synaptic targets) in the fly motion pathway (Behnia et al., 2014), which suggests that any temporal summation should either occur before, or as part of, the delay mechanism. They also have the desired properties of neurons conducting temporal summation, in that they are distinctly slower in their responses than either photoreceptors or LMCs, and rather closely match the temporal properties of their wide-field motion neurons (Fig. 15B).

Thus, the recent advances in dissecting the wide-field motion vision circuitry in *Drosophila* have brought to light prime candidates for temporal summation. Currently, it is nearly impossible to identify and physiologically access homologues of these neurons in other insect species, especially those with nocturnal lifestyles and high levels of temporal summation such as hawkmoths. The expansion of genetic tools (Huang et al., 2016, Perry et al., 2016), and genetic information (i5KConsortium, 2013, Wimmer, 2003, Yin et al., 2016), which were previously only available for *Drosophila,* to other insect species brings hope that these neurons will become accessible in other insects in the future, and it will be possible to study the cellular details of temporal summation.

"Time flies like an arrow; fruit flies like a banana."

Anthony G. Oettinger

VII. How does neural summation affect behaviour in dim light?

A central question in neuroscience is how neural activity generates behaviour. Ultimately, the nervous system does not exist for its own purpose, but to collect information through the senses, process it, and then generate an output that controls muscles to execute a specific behaviour. These behaviours determine the survival and reproductive success of an animal, and thus affect the evolutionary selection of the specific neural circuits that underlie them. Therefore, in order to understand the purpose of a specific neural response, we need to understand its effect on the behaviour of the animal. In this project I asked how adaptations for vision in dim light shape the behaviour of animals active at night, compared to animals active in bright conditions.

Neural adaptations shape behavioural sensitivity

The effect of neural summation on visual sensitivity in hawkmoths has been impressively shown in behavioural experiments, where the nocturnal *D. elpenor* had to choose between targets of different colour, by flying towards them and touching the selected one with their proboscis, in light levels as dim as starlight (Kelber et al., 2002). The lowest intensity tested in these experiments, where moths not only needed to discriminate colours, but also to fly safely towards the targets and hover in front of them, was also the lowest intensity tested in our physiological study, where we showed that only neural summation enabled *D. elpenor* to see at these light intensities (Paper I). Thus, these behavioural experiments showed impressively that *D. elpenor* can behaviourally perform to the same intensity limits as observed in its visual physiology, and what is more, that this performance is enabled by neural summation. Similar observations have been made in nocturnal bees (*Megalopta genalis*), which fly at light intensities where the sensitivity that their eyes and retina provide would not be sufficient to obtain reliable signals (Greiner et al., 2005,

Theobald et al., 2007, Warrant et al., 2004), thus suggesting that this performance is made possible by neural summation. Also in walking animals, the effect of neural summation has been observed: cockroaches (*Periplaneta americana*) perform optomotor responses at dim starlight intensities, even though their individual photoreceptors do not provide enough information necessary for this task (Honkanen et al., 2014), thus strongly suggesting that their signals are summed in space or time or both.

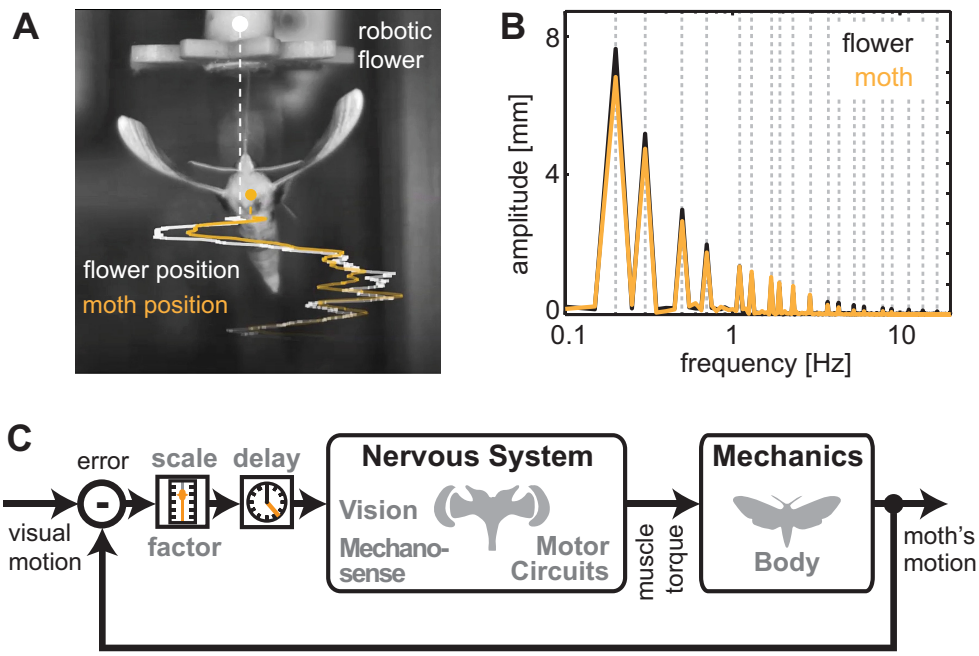


Fig. 16 System identification of hawkmoth flower tracking.
Moths tracked and fed from robotic artificial flowers (**A**, example from *D. elpenor*) whose movement trajectories were created by a combination of sinusoids of different temporal frequencies (**B**). The Fourier transformation of the tracked flower shows the stimulus frequencies and amplitudes (**B**), which were chosen to give equal velocities across frequencies. A system identification approach was used to describe the closed-loop behaviour of a moth's flower tracking (**C**). The inner part of the closed loop contains the nervous system (sensory and motor circuits) and the flight mechanics (body and wings). A simple time delay (delaying the moth's response with respect to the perceived visual input), as well as a scaling factor (scaling the response to the perceived error between the flower's and the moth's motion), can be added to the inner part of the loop, to model simple adaptations of the nervous system.

Neural summation affects the spatial and temporal acuity of behaviour

As discussed in Chapter III, adaptations for dim light vision trade off sensitivity for resolution: longer integration times in photoreceptors and temporal summation both reduce temporal acuity, while wider photoreceptor acceptance angles, as well as spatial summation, both reduce spatial acuity. Thus, are reductions in spatial and/or temporal resolution observed in insect behaviour?

A number of studies have indeed shown a reduction in spatial and temporal acuity of behaviour with decreasing light intensity, as a result of neural summation. Walking and tethered flies, for example, respond to higher spatial and temporal frequencies in bright light, than in dim light, when presented with rotating grating stimuli (Dvorak & Snyder, 1978, Pick & Buchner, 1979). The same observations have been made for tethered walking cockroaches (Honkanen et al., 2014). In freely moving, and especially flying, animals it is harder to quantify the spatial and temporal resolution of their behaviour, because flying animals can select their sensory environment to some degree: they can fly closer to or further away from objects, as well as fly faster and slower, and therefore change the spatial and temporal properties of the stimuli they perceive. Despite these difficulties, assays on bumble bees have shown that they decrease their flight speed as light levels decrease (Reber et al., 2015). Moreover, the decrease in flight speed was dependent on optic flow information, thus suggesting that reduced temporal acuity of the visual system was responsible for the change in flight speed, rather than a general luminance measuring mechanism (da Silva et al., 2016). The bumble bee experiments were performed in flight tunnels with patterned walls, in which the distance of the bees to the walls can be controlled by the properties of the patterns (Kirchner & Srinivasan, 1989) – and thus some control can be exerted over the spatial structure of the environment.

Another experimental paradigm where a tight control over the stimulus can be retained is flower tracking in hawkmoths. Hawkmoths, when suckling nectar from a flower through their proboscis while hovering, have to tightly follow the movement of the flower while keeping a constant distance, in order to keep their proboscis in contact with the nectary. Sponberg et al (2015) have used this behaviour to construct a setup in which the movement of an artificial flower is tightly controlled, to test the temporal acuity of the moth's tracking response by moving the flower at different temporal frequencies. They could show that the hawkmoth *Manduca sexta* had a higher tracking error at higher temporal

frequencies in dim light – exactly the pattern that would be expected if the visual system increased its sensitivity through temporal summation. Moreover, they quantified the temporal change in the response between the two light intensities with a system identification approach (Fig. 16).

System identification is the process of generating mathematical models of dynamical systems based on measured data – in this case a mathematical model of the moth's flower tracking response, based on the perceived relative movement of the flower with respect to the moth's movement (sensory error). This mathematical model makes it possible to manipulate the incoming sensory error, which the responses are based on – for example using temporal filters – and thus to model the equivalent of neural processing. Using this approach, Sponberg et al (2015) showed that a simple temporal delay captured the differences in flower tracking responses in bright and dim light surprisingly well. Since the only experimental difference between two modelled conditions was light intensity, this suggests that there is a luminance dependent time delay in the hawkmoth flower tracking system. The most likely place for such a delay would be the visual system – and concretely, the temporal properties of photoreceptors, as well as neural summation downstream of the photoreceptors.

System identification reveals temporal changes in flight control (Paper IV)

I used this system identification approach, to test the flower tracking performance of the diurnal *M. stellatarum* and the nocturnal *D. elpenor* in the same way. This enabled us to compare our results to those in *M. sexta*, as well as to our physiological data (Paper II). As in *M. sexta,* we observed luminance dependent changes in behaviour in the two other hawkmoth species that were well explained by a simple temporal delay (Paper IV). However, while in *M. stellatarum* the response in bright light was delayed with respect to the dim light response (just as in *M. sexta*), it was the opposite in the nocturnal *D. elpenor:* the bright light response was delayed with respect to responses in two dimmer light intensities, resulting from moths tracking higher temporal frequencies more accurately in dim than in bright light.

We went on to compare these findings to our physiological results from the wide-field motion system. While the neural basis for visual flower tracking is not known, it must have a motion component to determine the relative movement between the moth and flower, and thus requires elementary motion detectors. Furthermore,

experiments suggest an involvement of the wide-field motion vision system in this behaviour (Farina et al., 1995, Kern, 1998, Kern & Varjú, 1998). Even if a separate motion pathway, such as shown to support target tracking in flies (Fox et al., 2014, Fox & Frye, 2014), was the basis of this behaviour, it likely requires the same elementary motion detectors – and since we argue that spatial and temporal summation likely take place before the motion computation, this pathway might have the same spatial and temporal characteristics as the wide-field motion pathway.

At first sight, the physiological responses in *M. stellatarum* and *M. sexta* show very similar trends to the behavioural ones: in both species, the temporal characteristics of the motion neuron's responses became slower from brighter to dimmer light intensities, thus matching the reduction in power at high temporal frequencies in the behavioural responses. In a brute-force attempt to quantitatively compare the findings of the behavioural and physiological experiments, I applied the computational models extracting temporal summation from the physiological data (Chapter IV) to re-create the experimental paradigm of the behavioural experiments. The behavioural paradigm compares flight performance between two light conditions, and estimates the delay that needs to be applied to the brighter condition in order to obtain the performance at the dimmer condition. I mirrored this approach in the physiological model: I estimated the overall temporal delay filter at equivalent light intensities from the physiological data, using the photoreceptor characteristics measured at the bright light intensity for both light conditions. In this way, the estimated delay filter captured temporal changes along the entire pathway (both in the photoreceptors, and at higher centres performing additional temporal summation), similar to the behavioural experiments. The difference in the delay filter time constants from the physiological models at bright and dim light intensities was then compared to the estimated delay obtained from behaviour, to see if the physiological estimates were similar to the behavioural ones (Fig. 17).

Surprisingly, there was a close match between the physiologically and behaviourally estimated temporal delays in the diurnal species *M. stellatarum* (Fig. 17A). Flower tracking is a complex behaviour that requires visual input, but also likely mechanosensory input from the proboscis, which senses the mechanical displacements of the flower (Roth et al., 2016), as well as feedback from the mechanosensors in the antennae (Sane et al., 2007). Moreover, the mechanics of flight determine the frequency and amplitude range over which a moth can actually respond by correcting its hovering position – a moth will not be able to follow movements of the flower which are too fast or too big, even if its sensory system perceives them reliably. All these factors together make it rather unlikely that the

temporal properties of flower tracking accurately reflect the temporal properties of the visual system. This is why the close match between the behavioural and physiological predictions of temporal delays in the diurnal species was surprising. It does not necessarily mean that flower tracking in this hawkmoth species is solely determined by vision, but it suggests that it is limited in its temporal properties by vision – and therefore when vision becomes slower, flower tracking becomes slower to the same extent.

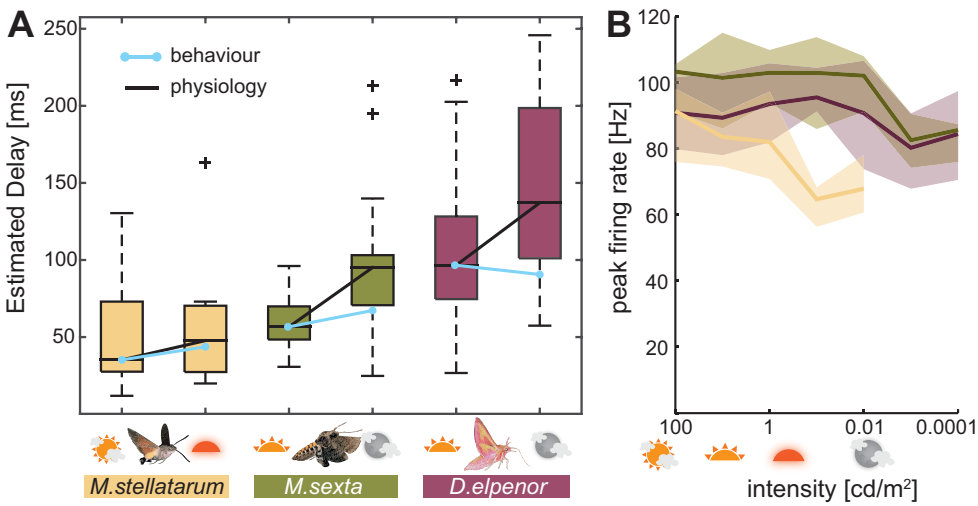


Fig. 17 Comparison of physiology and behaviour in hawkmoth flower tracking.
A Comparison of effective temporal delays between bright and dim light implemented in the visual system of the three hawkmoth species, estimated from behavioural (*blue,* Paper IV) and physiological (*black,* Paper II) experiments, using the computational model described in Chapter IV. **B** Peak firing rate of wide-field motion sensitive neurons in the three hawkmoth species. The firing rate was determined from responses to moving sinusoidal gratings of different spatial and temporal frequencies at 25%. Solid lines are median values, and shaded areas represent the inter-quartile range. The symbols on the intensity axis represent comparable light conditions in our behavioural experiments.

For *M. sexta* and *D. elpenor,* however, the physiological and behavioural estimates of temporal delays did not match well: the physiological delays were more than twice as large as the behaviourally estimated ones in *M. sexta*. In *D. elpenor,* the estimated time delays they even pointed in different directions: while the physiological responses became slower at lower light intensities, the behavioural

responses showed the opposite trend: moths responded to higher temporal frequencies at lower light intensities (Fig. 17A).

Thus, flower tracking in the two dim light active species seemed not to be limited by the visual system's temporal characteristics at the tested light intensities. Differences the in temporal delay estimates in *M. sexta* may be due to differences in the techniques and measurements applied, and might eventually be resolved, thereby explaining their flower tracking on the basis of properties of the visual system. However, it is hard to conceive how such an explanation would be possible in the case of *D. elpenor*, where physiology and behaviour show opposing trends. We therefore suggest that the motivational or activity state of the animal may play a role in flower tracking, in additional to the sensory input in *D. elpenor* (Paper IV). This species is normally not active in the bright intensity tested, but rests throughout the day, and only flies briefly to find a new hiding place when disturbed. Thus, it is conceivable that these light intensities induce a state of "rest" or "sleep" in *D. elpenor*, which modifies the properties of their sensory and/or motor systems, and thus affects the temporal flight performance. Similar hormonal modulation of visual physiology and behaviour have been documented amply in recent years (Chiappe et al., 2010, Jung et al., 2011, Lüders & Kurtz, 2015, Longden & Krapp, 2010, Suver et al., 2012, van Breugel et al., 2014).

While some of our results suggest a quantitative link between physiology and behaviour in the flower tracking paradigm, *D. elpenor* shows that we are far from understanding the system in its entirety. To really quantitatively link physiology and behaviour, we need to be able to take apart the individual components of flight control – the contribution from each sense, the transfer functions of the muscles, as well as of the flight mechanics, and the modulation induced by hormones, which currently are all treated as one block in the system identification model (Fig. 16).

Dim light adaptations outside the visual system?

In addition to quantifying changes in temporal flight performance within species, I also wondered whether differences between species could be captured by simple temporal delays – and these in turn be linked to the temporal differences between the three hawkmoth species' motion vision responses (Chapter V). And indeed, I did find striking differences in the temporal performance of the three species, where the diurnal species showed lower tracking errors and higher tracking gain at higher

frequencies than the crepuscular and nocturnal species (Paper IV). Could these differences be captured by simple temporal delays?

I applied the same system identification model as we applied within species, fitting a temporal delay from the response of one species to that of another at the same light intensity. However, unlike for the intra-species comparisons, we did not obtain good model fits between any of the species using a simple delay, suggesting that there is more to the differences in flight performance than simple temporal changes. We thus introduced a scale factor, which scales how strongly each species responds with corrective flight manoeuvres to the perceived relative movement of the flower, and observed that a combination of a scale factor and a delay produced very good fits between the nocturnal and diurnal species, and better fits to the crepuscular species than without the scale factor (though these fits still did not capture large parts of the response). Interestingly, the scale factor resulting from all model fits between the diurnal species, and either the nocturnal or crepuscular species, scaled the latter's responses by 50%, compared to those of the diurnal species. This suggests that the diurnal species reacted to relative flower movements twice as strongly as the crepuscular and nocturnal species did.

Such a scale factor could be implemented in the visual system of the three hawkmoth species, by means of visual neurons responding more strongly to motion in the diurnal, than in the crepuscular and nocturnal species. However, the firing rates of the wide-field sensitive neurons in the three species were quite similar, and if anything, lower in the diurnal species (Fig. 17B), suggesting that the difference in response strength was not likely implemented in the wide-field motion system. This still leaves a wide range of possibilities for the implementation of a scale factor, both on the sensory side, as well as between sensory and motor systems.

In terms of dim light adaptations, the scale factor is very interesting, because it is correlated with the reliability of visual information across our three moth species: the crepuscular and nocturnal species, which are faced with less reliable visual information due to the dim light conditions they operate in, scale down their corrective movements to relative flower motion, compared to the diurnal species which operates under conditions of very reliable visual information. It is therefore conceivable that this scale factor acts as a dim light adaptation, reducing the chance that moths erroneously correct their hovering position due to unreliable visual information – but at the same time also reducing the magnitude of correct adjustments of hovering position, and thus reducing the overall fidelity of flower tracking. It would be very interesting to test the gain of descending motor neurons which control steering in hawkmoths to motion stimuli in the three hawkmoth

species, to see whether a scale factor like in our model might be implemented in the flight control system of hawkmoths.

In general, hawkmoths are a very powerful model to bridge the gap in our knowledge between physiology and behaviour in flight control, because they have both a well accessible and characterised visual system and well quantifiable flight behaviours. We have here started with the first steps to integrate findings between these two domains in hawkmoths, but as I outlined throughout this chapter, many white spots on the map need to be filled in before this ultimate goal can be achieved.

"The night
Shows stars and women in a better light."

Lord Byron

VIII. A final perspective

In this thesis, I have investigated how neural summation improves sensitivity in dim light in the visual system of hawkmoths (Fig. 18). I could show that such adaptations exist, in the form of spatial and temporal summation. Furthermore, I quantified their effect, and demonstrated that a combination of spatial and temporal summation increased sensitivity and information content in the wide-field motion vision system in a supralinear way (Paper I). The degree of spatial and temporal summation was matched to the ecological needs of different hawkmoth species, as well as their anatomical adaptations for visual sensitivity (Paper II). Furthermore, I identified likely candidate neurons to carry out spatial and temporal summation in the brains of hawkmoths (Paper III). Finally, I quantified the effects of temporal summation on the flower tracking abilities of hawkmoths, and showed that a subset of the observed behavioural phenomena could be explained by temporal processing in the nervous system. I furthermore proposed a number of possible explanations for the variance not explained by visual processing (Paper IV).

Taken together, this work has provided detailed insight into how neural processing can increase visual reliability in dim light. These results are not only relevant to hawkmoths, as similar neural adaptations are expected to be implemented in other crepuscular and nocturnal insects, since insects in general have very conserved neural circuitry (Strausfeld, 2012), and are, therefore, likely to have very similar neural strategies to cope with the visual limitations of dim light. However, even beyond insects, the general principles of spatial and temporal summation are found in vertebrates and other animal groups (Warrant, 2008), which experience the same pressures to increase visual reliability in dim light. One area for which these results are especially applicable are in artificial visual systems, which often have a limited processing capacity when mounted on autonomous vehicles, drones or robots and are therefore limited in size and weight. The small size and limited processing power of insect brains can therefore serve as a fruitful inspiration for technical solutions for dim light vision (Warrant et al., 2014).

This work has also identified, and hypothesised about, a number of open questions concerning dim light adaptations. The first one is the identity of the neurons that

carry out spatial and temporal summation. Being able to identify and access these neurons physiologically would make it possible to understand how the different properties of neural summation I have observed come about.

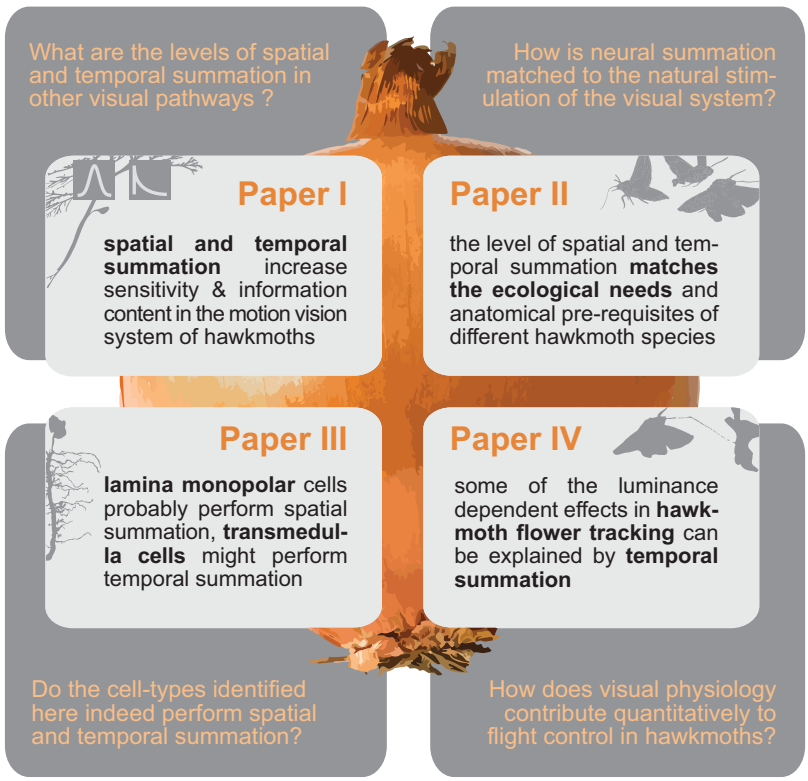


Fig. 18 Summary of the main results.
Main results of the four projects on *light grey* background, and further questions on *dark grey*.

One of these properties is the dynamic nature of neural summation, which increased in its extent at low light intensities. Furthermore, we have only investigated neural summation in one visual sub-system – wide-field motion. Different visual tasks require different levels of spatial and temporal acuity, and therefore it is likely that different visual pathways have different levels of spatial and temporal summation. As the neural circuits underlying separate visual tasks are unravelled step by step (Behnia & Desplan, 2015), and their relation to each becomes better understood, investigations can commence that compare neural summation across visual circuits. One recurring theme throughout this work is the

lack of available information about the properties of the natural stimuli hawkmoths (and other flying insects) experience, as they navigate their environments. While simple controllable stimuli such as the ones used in this work are powerful experimental tools, they are not what the visual system of these moths have evolved to interpret. In order to understand the fine-tuning of the spatial and temporal properties of the visual system, as well as spatial and temporal summation, information about the natural stimuli that it has evolved to respond to is crucial.

Apart from the field of dim light vision, this thesis has also ventured into a number of other research fields, and has outlined potential research trajectories. One of them is the evolutionary ecology of vision. Our comparison of neural summation between the three hawkmoth species raised the question: when is it evolutionarily adaptive to solve a problem with neural processing, rather than a "hardware" solution? In our particular case: when is it evolutionarily beneficial to increase sensitivity using neural summation rather than via improvements in optical sensitivity? Are there general strategies across insects, similar to the strategies we found in hawkmoths? While I have concentrated primarily on adaptations of the visual system in this thesis, other sensory systems can complement visual information to aid in flight control and foraging. How these sensory systems are balanced has begun to emerge in studies of hawkmoth flower tracking (Roth et al., 2016), as well as flower choice (Stöckl et al., 2016), and will remain an intriguing research question for the future.

The second field that this project branched into was motion vision in insects. Hawkmoths provide a fantastic model system, in addition to the classical dipteran model (Borst, 2009, Borst, 2014). Their motion vision system is organised in a similar way and classical computational motion models developed for flies work reasonably well for hawkmoths (O'Carroll & Warrant, 2011). In favour of hawkmoths, their neurons are much bigger than those of flies, and thus more accessible to physiological recordings, and they have unusual properties that have not been described in any other insect group, thus making them a useful group to test how well the results from the fly model are generally applicable to other insects, and to what degree these findings are restricted to the *Diptera*. One intriguing detail of the visual systems of hawkmoths is the distinct spatial extents of their different lamina monopolar cell types. How this affects the computation of motion, which is based on different pathways originating from different LMC types in flies (Joesch et al., 2010), is an intriguing question. Furthermore, the motion vision system of hawkmoths has the very peculiar property of temporal aliasing (the wide-field motion neurons are inhibited at high temporal frequencies), which has not been observed in dipterans. In order to explain it, additions to the standard models of

motion computation in insects (Borst & Egelhaaf, 1989, Clifford & Ibbotson, 2002), which are based on the Dipteran system, are required. New insights into the fly motion pathway might provide the key to this task (Behnia et al., 2014). Finally, while recording from wide-field motion sensitive neurons for Paper I, we characterised a whole range of neurons with wide-field motion characteristics, whose properties strongly suggest that they are modulatory neurons, targeting various neuropils in the optic lobes and central brain. Many of them have not been characterised in flies (as yet), and their function in hawkmoths is still unknown, providing plenty of research opportunities for the future.

The third field this thesis ventured into is flight control: hawkmoths have been used to investigate the mechanics and kinetics of flight, because of their impressive flight abilities, their size, and their hovering behaviour, all of which facilitate quantitative studies in free flight. Our research aligns with many similar ventures, which seek to understand the neural basis of behaviour, and the system identification of hawkmoth flower tracking might prove to be a fruitful approach. However, there is still some way to go before we fully understand this system, and are able to tie together the physiology and behaviour. Specifically, we need to understand which sensory systems contribute to flower tracking and flight control, and how they are integrated. Moreover, accurate models of the actual flight kinetics are needed, in order to separate the sensory contributions to flight control from the physical limitations, and, finally, to arrive at a model that contains individual modules for each of these elements, rather than one great "black box".

Thus, this quest for unravelling the neural summation strategies in the hawkmoth motion vision system has provided insights into the general strategies for dim light vision in insects, their neural substrates, as well as their behavioural consequences. It has also opened up many further questions, in this and related research areas. Just like peeling an onion, every layer removed revealed a new one. However, unlike peeling an onion, this process was great fun, deeply satisfying, and rarely triggered any tears.

References

Aho AC, Donner K, Hydén C, Larsen LO, Reuter T (1988). Low retinal noise in animals with low body temperature allows high visual sensitivity. *Nature* 334:348–350.

Balboa RM, Grzywacz NM (2003). Power spectra and distribution of contrasts of natural images from different habitats. *Vision Res* 43:2527–2537.

Bausenwein B, Fischbach KF (1992). Activity labeling patterns in the medulla of Drosophila melanogaster caused by motion stimuli. *Cell Tissue Res* 270:25–35.

Baylor DA, Matthews G, Yau KW (1980). Two components of electrical dark noise in toad retinal rod outer segments. *J Physiol* 309:591–621.

Behnia R, Clark DA, Carter AG, Clandinin TR, Desplan C (2014). Processing properties of on and off pathways for Drosophila motion detection. *Nature* 512:427–430.

Behnia R, Desplan C (2015). Visual circuits in flies: beginning to see the whole picture. *Curr Opin Neurobiol* 34:125–132.

Blest AD, Land MF (1977). The physiological optics of Dinopis subrufus L. Koch: a fish-lens in a spider. *Proc R Soc Lond B Biol Sci* 196:197–222.

Borst A (1990). How do flies land? *Bioscience* 40:292–299.

Borst A (2009). Drosophila's view on insect vision. *Curr Biol* 19:R36–R47.

Borst A (2014). Fly visual course control: behaviour, algorithms and circuits. *Nat Rev Neurosci* 15:590–599.

Borst A, Egelhaaf M (1989). Principles of visual motion detection. *Trends Neurosci* 12:297–306.

Borst A, Euler T (2011). Seeing things in motion: models, circuits, and mechanisms. *Neuron* 71:974–994.

Cajal SR, Sánchez D (1915). Contribución al conocimiento de los centros nerviosos de los insectos. *Trab Lab Inv Biol* 13:1–68.

Caveney S, McIntyre P (1981). Design of graded-index lenses in the superposition eyes of scarab beetles. *Philos Trans R Soc Lond B Biol Sci* 294: 589–632.

Chacron M (2007). Electrolocation. *Scholarpedia* 2:1411.

Chiappe ME, Seelig JD, Reiser MB, Jayaraman V (2010). Walking modulates speed sensitivity in Drosophila motion vision. *Curr Biol* 20:1470–1475.

Clifford CWG, Ibbotson MR (2002). Fundamental mechanisms of visual motion detection: models, cells and functions. *Prog Neurobiol* 68:409–437.

da Silva AC, Dacke M, Peixoto dos Santos C, Baird E (2016). Temporal summation affects optic flow estimates: A mechanism for flight speed reduction in dim light. *under review*.

Dacke M, Nilsson DE, Scholtz CH, Byrne M, Warrant EJ (2003). Animal behaviour: insect orientation to polarized moonlight. *Nature* 424:33.

Davies MN, Green PR (1994). *Perception and motor control in birds: an ecological approach.* Springer-Verlag. Berlin. Multiple sources of depth information: An ecological approach., pp. 339–356.

de Souza J, Hertel H, Ventura DF, Menzel R (1992). Response properties of stained monopolar cells in the honeybee lamina. *J Comp Physiol A* 170:267–274.

de Souza JM, Ventura DF (1989). Comparative study of temporal summation and response form in hymenopteran photoreceptors. *J Comp Physiol A* 165:237–245.

de Vries H (1943). The quantum character of light and its bearing upon threshold of vision. *Physica* 10:553–564.

Donner K (1989). Visual latency and brightness: An interpretation based on the responses of rods and ganglion cells in the frog retina. *Vis Neurosci* 3:39–51.

Dubs A (1981). Non-linearity and light adaptation in the fly photoreceptor. *J Comp Physiol A* 144:53–59.

Dubs A, Laughlin SB, Srinivasan MV (1981). Single photon signals in fly photoreceptors and first order interneurones at behavioral threshold. *J Physiol* 317:317–334.

Dvorak D, Snyder A (1978). The relationship between visual acuity and illumination in the fly, Lucilia sericata. *Z Naturforsch C* 33:139–143.

el Jundi B, Huetteroth W, Kurylas AE, Schachtner J (2009). Anisometric brain dimorphism revisited: Implementation of a volumetric 3D standard brain in Manduca sexta. *J Comp Neurol* 517:210–225.

el Jundi B, Pfeiffer K, Heinze S, Homberg U (2014). Integration of polarization and chromatic cues in the insect sky compass. *J Comp Physiol A* 200:575–589.

Exner S (1891). *Die Physiologie der facettirten Augen von Krebsen und Insecten*. Deuticke.

Farina WM, Kramer D, Varjú D (1995). The response of the hovering hawk moth Macroglossum stellatarum to translatory pattern motion. *J Comp Physiol A* 176:551–562.

Fischbach KF, Dittrich A (1989). The optic lobe of Drosophila melanogaster. I. A Golgi analysis of wild-type structure. *Cell Tissue Res* 258:441–475.

Fotowat H, Gabbiani F (2011). Collision detection as a model for sensory-motor integration. *Annu Rev Neurosci* 34:1–19.

Fox JL, Aptekar JW, Zolotova NM, Shoemaker PA, Frye MA (2014). Figure-ground discrimination behavior in Drosophila. I. Spatial organization of wing-steering responses. *J Exp Biol* 217:558–569.

Fox JL, Frye MA (2014). Figure-ground discrimination behavior in Drosophila. II. Visual influences on head movement behavior. *J Exp Biol* 217:570–579.

Frederiksen R, Wcislo WT, Warrant EJ (2008). Visual reliability and information rate in the retina of a nocturnal bee. *Curr Biol* 18:349–353.

Goetz K (1975). The optomotor equilibrium of the Drosophila navigation system. *J Comp Physiol* 99:187–210.

Gonzalez-Bellido PT, Wardill TJ, Juusola M (2011). Compound eyes and retinal information processing in miniature dipteran species match their specific ecological demands. *Proc Natl Acad Sci USA* 108:4224–4229.

Greiner B (2006). Visual adaptations in the night-active wasp Apoica pallens. *J Comp Neurol* 495:255–262.

Greiner B, Ribi WA, Warrant EJ (2004). Retinal and optical adaptations for nocturnal vision in the halictid bee Megalopta genalis. *Cell Tissue Res* 316:377–390.

Greiner B, Ribi WA, Warrant EJ (2005). A neural network to improve dim-light vision? dendritic fields of first-order interneurons in the nocturnal bee Megalopta genalis. *Cell Tissue Res* 322:313–320.

Greiner B, Ribi WA, Wcislo WT, Warrant EJ (2004). Neural organisation in the first optic ganglion of the nocturnal bee Megalopta genalis. *Cell Tissue Res.* 318:429–437.

Haag J, Denk W, Borst A (2004). Fly motion vision is based on Reichardt detectors regardless of the signal-to-noise ratio. *Proc Natl Acad Sci USA* 101:16333–16338.

Hall MI, Ross CF (2007). Eye shape and activity pattern in birds. *J Zool* 271:437–444.

Hallett P (1963). Spatial summation. *Vision Res* 3:9 – 24.

Harley CM, English BA, Ritzmann RE (2009). Characterization of obstacle negotiation behaviors in the cockroach, Blaberus discoidalis. *J Exp Biol* 212:1463–1476.

Hassenstein B, Reichardt W (1956). Systemtheoretische analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers Chlorophanus. *Z Naturforsch B* 11:513–524.

Hausen K (1982*a*). Motion sensitive interneurons in the optomotor system of the fly. *Biol Cybern* 46:67–79.

Hausen K (1982*b*). Motion sensitive interneurons in the optomotor system of the fly. *Biol Cybern* 45:143–156.

Hausen K, Wehrhahn C (1989). Neural circuits mediating visual flight control in flies. I. Quantitative comparison of neural and behavioral response characteristics. *J Neurosci* 9:3828–3836.

Heimonen K, Salmela I, Kontiokari P, Weckström M (2006). Large functional variability in cockroach photoreceptors: Optimization to low light levels. *J Neurosci* 26:13454–13462.

Hengstenberg R (1982). Common visual response properties of giant vertical cells in the lobula plate of the blowfly Calliphora. *J Comp Physiol* 149:179–193.

Hengstenberg R, Hausen K, Hengstenberg B (1982). The number and structure of giant vertical cells (VS) in the lobula plate of the blowfly Calliphora erythrocephala. *J Comp Physiol* 149:163–177.

Henningsson P, Bomphrey RJ (2013). Span efficiency in hawkmoths. *J R Soc Interface* 10:20130099.

Honkanen A, Takalo J, Heimonen K, Vähäsöyrinki M, Weckström M (2014). Cockroach optomotor responses below single photon level. *J Exp Biol* 217:4262–4268.

Howard J, Dubs A, Payne R (1984). The dynamics of phototransduction in insects. *J Comp Physiol A* 154:707–718.

Huang Y, Liu Z, Rong YS (2016). Genome editing: From drosophila to non-model insects and beyond. *J Genet Genomics* 43:263–272.

i5KConsortium (2013). The i5k initiative: advancing arthropod genomics for knowledge, human health, agriculture, and the environment. *J Hered* 104:595–600.

Jander U, Jander R (2002). Allometry and resolution of bee eyes (Apoidea). *Arthropod Struct Dev* 30:179–193.

Joesch M, Schnell B, Raghu SV, Reiff DF, Borst A (2010). On and off pathways in Drosophila motion vision. *Nature* 468:300–304.

Jung SN, Borst A, Haag J (2011). Flight activity alters velocity tuning of fly motion-sensitive neurons. *J Neurosci* 31:9231–9237.

Kelber A (2002). Pattern discrimination in a hawkmoth: Innate preferences, learning performance and ecology. *Proc Biol Sci* 269:2573–2577.

Kelber A, Balkenius A, Warrant EJ (2002). Scotopic colour vision in nocturnal hawkmoths. *Nature* 419:922–925.

Kern R, Varjú D (1998). Visual position stabilization in the hummingbird hawk moth, Macroglossum stellatarum L. I. Behavioural analysis. *J Comp Physiol A* 182:225–237.

Kern R, Varjú D (1998). Visual position stabilization in the hummingbird hawk moth, Macroglossum stellatarum L. II. Electrophysiological analysis of neurons sensitive to wide-field image motion. *J Comp Physiol A* 182:239–249.

Kern R, Egelhaaf M (2000). Optomotor course control in flies with largely asymmetric visual input. *J Comp Physiol A* 186:45–55.

Kirchner WH, Srinivasan MV (1989). Freely flying honeybees use image motion to estimate object distance. *Naturwissenschaften* 76:281–282.

Kirk EC (2004). Comparative morphology of the eye in primates. *Anat Rec A Discov Mol Cell Evol Biol* 281A:1095–1103.

Klaus A, Warrant EJ (2009). Optimum spatiotemporal receptive fields for vision in dim light. *J Vis* 9:18.1–1816.

Krapp HG (1999). *in* Lappe M, ed., Neuronal Processing of Optic Flow. Vol. 44 of *International Review of Neurobiology*. Academic Press. pp. 93 – 120.

Land M (1981). *Handbook of Sensory Physiology*. Springer. Optics and Vision in Invertebrates, pp. 471–592.

Land M (1984). The resolving power of diurnal superposition eyes measured with an ophthalmoscope. *J Comp Physiol A* 154:515–533.

Land MF, Gibson G, Horwood J (1997). Mosquito eye design: conical rhabdoms are matched to wide aperture lenses. *Proc R Soc Lond B Biol Sci* 264:1183–1187.

Land MF, Gibson G, Horwood J, Zeil J (1999). Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *J Comp Physiol A* 185:91–103.

Land MF, Osorio DC (1990). Waveguide modes and pupil action in the eyes of butterflies. *Proc Biol Sci* 241:93–100.

Laughlin SB (1981). *Handbook of Senory Physiology*. Springer. chapter Neural Principles in the Peripheral Visual Systems of Invertebrates, pp. 133–280.

Laughlin SB (1996). Matched filtering by a photoreceptor membrane. *Vision Res* 36:1529–1541.

Laughlin SB, Hardie RC (1978). Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. *J Comp Physiol* 128:319–340.

Lüders J, Kurtz R (2015). Octopaminergic modulation of temporal frequency tuning of a fly visual motion-sensitive neuron depends on adaptation level. *Front Integr Neurosci* 9:36.

Lillywhite P (1977). Single photon signals and transduction in an insect eye. *J Comp Physiol* 122:189–200.

Lillywhite PG, Laughlin SB (1979). Transducer noise in a photoreceptor. *Nature* 277:569–572.

Longden KD, Krapp HG (2010). Octopaminergic modulation of temporal frequency coding in an identified optic flow-processing interneuron. *Front Sys Neurosci* 4.

Lythgoe N (1979). *The ecology of vision*. Oxford University Press.

Maisak MS, et al. (2013). A directional tuning map of Drosophila elementary motion detectors. *Nature* 500:212–216.

Martin GR, et al. (2007). Kiwi forego vision in the guidance of their nocturnal activities. *PLoS One* 2:e198.

McIntyre P, Caveney S (1985). Graded-index optics are matched to optical geometry in the superposition eyes of scarab beetles. *Philos Trans R Soc Lond B Biol Sci* 311:pp. 237–269.

Meinertzhagen I, Menzel R, Kahle G (1983). The identification of spectral receptor types in the retina and lamina of the dragonfly Sympetrum rubicundulum. *J Comp Physiol* 151:295– 310.

Menzi U (1987). Visual adaptation in nocturnal and diurnal ants. *J Comp Physiol A* 160:11–21.

Moeller J, Case J (1995). Temporal adaptations in visual systems of deep-sea crustaceans. *Mar Biol* 123:47–54.

Moser JC, et al. (2004). Eye size and behaviour of day- and night-flying leafcutting ant Alates. *J Zool* 264:69–75.

Mronz M, Lehmann FO (2008). The free-flight response of Drosophila to motion of the visual environment. *J Exp Biol* 211:2026–2045.

Narendra A, et al. (2011). Caste-specific visual adaptations to distinct daily activity schedules in Australian Myrmecia ants. *Proc Biol Sci* 278:1141–1149.

Nelson ME, MacIver MA (2006). Sensory acquisition in active sensing systems. *J Comp Physiol A* 192:573–586.

Nilsson DE (1989). *Facets of vision*. Springer. Springer, Berlin Heidelberg New York. Optics and evolution of the compound eye, pp. 30–73.

O'Carroll D, Warrant E (2011). Computational models for spatiotemporal filtering strategies in insect motion vision at low light levels. *in* 2011 Seventh International Conference on Intelligent Sensors, Sensor Networks and Information Processing (ISSNIP), pp. 119–124.

O'Carroll DC, Bidwell NJ, Laughlin SB, Warrant EJ (1996). Insect motion detectors matched to visual ecology. *Nature* 382:63–66.

O'Carroll DC, Laughlin SB, Bidwell NJ, Harris RA (1997). Spatio-temporal properties of motion detectors matched to low image velocities in hovering insects. *Vision Res* 37:3427–3439.

Payne RS (1971). Acoustic location of prey by barn owls (Tyto alba). *J Exp Biol* 54:535–573.

Perry M, et al. (2016). Molecular logic behind the three-way stochastic choices that expand butterfly colour vision. *Nature* 535:280–284.

Pick B, Buchner E (1979). Visual movement detection under light- and dark-adaptation in the fly, Musca domestica. *J Comp Physiol* 134:45–54.

Pirhofer-Walzl K, Warrant E, Barth FG (2007). Adaptations for vision in dim light: impulse responses and bumps in nocturnal spider photoreceptor cells (Cupiennius salei Keys). *J Comp Physiol A* 193:1081–1087.

Pittaway A (1993). *The Hawkmoths of the Western Palearctic*. Harley Books.

Reber T, et al. (2015). Effect of light intensity on flight control and temporal properties of photoreceptors in bumblebees. *J Exp Biol* 218:1339–1346.

Reichardt W (1961). *Sensory Communication*. MlT Press and Wiley. Autocorrelation, a principle for the evaluation of sensory information by the central nervous system, pp. 303–317.

Ribi WA (1975*a*). Golgi studies of the first optic ganglion of the ant, Cataglyphis bicolor. *Cell Tissue Res.* 160:207–217.

Ribi WA (1975*b*). The neurons of the first optic ganglion of the bee (Apis mellifera). *Adv Anat Embryol Cell Biol* 50:1–43.

Ribi WA (1977). Fine structure of the first optic ganglion (lamina) of the cockroach, Periplaneta americana. *Tissue Cell* 9:57–72.

Ribi WA (1978). Ultrastructure and migration of screening pigments in the retina of Pieris rapae L. (Lepidoptera, Pieridae). *Cell Tissue Res.* 191:57–73.

Ribi WA (1987). Anatomical identification of spectral receptor types in the retina and lamina of the Australian orchard butterfly, Papilio aegeus aegeus D. *Cell Tissue Res.* 247:393–407.

Rose A (1942). The relative sensitivities of television pickup tubes, photographic film, and the human eye. *Proc IRE* 30:293–300.

Roth E, Hall RW, Daniel TL, Sponberg S (2016). The integration of parallel mechanosensory and visual pathways resolved through sensory conflict. *Proc Natl Acad Sci USA* (in press).

Sane SP, Dieudonné A, Willis MA, Daniel TL (2007). Antennal mechanosensors mediate flight control in moths. *Science* 315:863–866.

Shaw SR, Moore D (1989). Evolutionary remodeling in a visual system through extensive changes in the synaptic connectivity of homologous neurons. *Vis Neurosci* 3:405–410.

Shimohigashi M, Tominaga Y (1999). Synaptic organization in the lamina of the superposition eye of a skipper butterfly, Parnara guttata. *J Comp Neurol* 408:107–124.

Simoncelli EP, Olshausen BA (2001). Natural image statistics and neural representation. *Annu Rev Neurosci* 24:1193–1216.

Single S, Borst A (1998). Dendritic integration and its role in computing image velocity. *Science* 281:1848–1850.

Snyder A (1979). *Handbook of Senory Physiology*. Springer. chapter The physics of compound eyes, pp. 225–213.

Snyder AW (1977). Acuity of compound eyes: Physical limitations and design. *J Comp Physiol A* 116:161–182.

Snyder AW, Stavenga DG, Laughlin SB (1977). Spatial information capacity of compound eyes. *J Comp Physiol* 116:183–207.

Srinivasan MV, Laughlin SB, Dubs A (1982). Predictive coding: a fresh view of inhibition in the retina. *Proc R Soc Lond B Biol Sci* 216:427–459.

Srinivasan MV, Lehrer M, Kirchner WH, Zhang SW (1991). Range perception through apparent image speed in freely flying honeybees. *Vis Neurosci* 6:519–535.

Srinivasan MV, Poteser M, Kral K (1999). Motion detection in insect orientation and navigation. *Vision Res* 39:2749–2766.

Srinivasan MV, Zhang S, Altwein M, Tautz J (2000). Honeybee navigation: nature and calibration of the "odometer". *Science* 287:851–853.

Srinivasan MV, Zhang SW (2000). Visual navigation in flying insects. *Int Rev Neurobiol* 44:67–92.

Srinivasan MV, Zhang SW, Chahl JS, Barth E, Venkatesh S (2000). How honeybees make grazing landings on flat surfaces. *Biol Cybern* 83:171–183.

Srinivasan, Zhang, Lehrer, Collett (1996). Honeybee navigation en route to the goal: visual flight control and odometry. *J Exp Biol* 199:237–244.

Stöckl A, et al. (2016). Differential investment in visual and olfactory brain areas reflects behavioural choices in hawk moths. *Sci Rep* 6:26041.

Strausfeld N (1976). *Atlas of an insect brain*. Springer.

Strausfeld NJ (1970). The optic lobes of Diptera. *Philos Trans R Soc Lond B Biol Sci* 258:135–223.

Strausfeld NJ (2012). *Arthropod Brains: Evolution, Functional Elegance, and Historical Significance*. Harvard Univ Pr.

Strausfeld NJ, Blest AD (1970). The optic lobes of Lepidoptera. *Philos Trans R Soc Lond B Biol Sci* 258:81–134.

Strausfeld NJ, Campos-Ortega JA (1973). The L4 monopolar neurone: a substrate for lateral interaction in the visual system of the fly Musca domestica (L.). *Brain Res* 59:97–117.

Straw AD, Rainsford T, O'Carroll DC (2008). Contrast sensitivity of insect motion detectors to natural images. *J Vis* 8:32.1–32.9.

Suver MP, Mamiya A, Dickinson MH (2012). Octopamine neurons mediate flight-induced modulation of visual processing in Drosophila. *Curr Biol* 22:2294–2302.

Takemura Sy, et al. (2013). A visual motion detection circuit suggested by Drosophila connectomics. *Nature* 500:175–181.

Tammero LF, Dickinson MH (2002). Collision-avoidance and landing responses are mediated by separate pathways in the fruit fly, Drosophila melanogaster. *J Exp Biol* 205:2785–2798.

Theobald JC, Coates MM, Wcislo WT, Warrant EJ (2007). Flight performance in night-flying sweat bees suffers at low light levels. *J Exp Biol* 210:4034–4042.

Theobald JC, Warrant EJ, O'Carroll DC (2010). Wide-field motion tuning in nocturnal hawkmoths. *Proc Biol Sci* 277:853–860.

Thomas J, Moss C, Vater M (2004). *Echolocation in bats and dolphins*. University of Chicago Press. Chicago.

Torralba A, Oliva A (2002). Depth estimation from image structure. *IEEE Trans Pattern Anal Mach Intell* 24:1226–1238.

Trujillo-Cenóz O, Melamed J (1966). Compound eye of dipterans: anatomical basis for integration–an electron microscope study. *J Ultrastruct Res* 16:395–398.

van Breugel F, Suver MP, Dickinson MH (2014). Octopaminergic modulation of the visual flight speed regulator of Drosophila. *J Exp Biol* 217:1737–1744.

van der Schaaf A, van Hateren JH (1996). Modelling the power spectra of natural images: statistics and information. *Vision Res* 36:2759–2770.

van Hateren J (1992*a*). Theoretical predictions of spatiotemporal receptive fields of fly LMCs, and experimental validation. *J Comp Physiol A* 171:157–170.

van Hateren J (1992*b*). A theory of maximizing sensory information. *Biol Cybern* 68:23–29.

van Hateren J (1993*a*). Spatiotemporal contrast sensitivity of early vision. *Vision Res* 33:257 – 267.

van Hateren JH (1992*c*). Real and optimal neural images in early vision. *Nature* 360:68–70.

van Hateren JH (1993*b*). Three modes of spatiotemporal preprocessing by eyes. *J Comp Physiol A* 172:583–591.

Walcott B (1975). *The compound eye and vision of insects*. Clarendon Press. Anatomical changes during light-adaptation in insect compound eyes, pp. 20–33.

Walls G (1942). *The Vertebrate Eye and its Adaptive Radiation*. The Cranbook Press.

Wardill TJ, et al. (2012). Multiple spectral inputs improve motion discrimination in the Drosophila visual system. *Science* 336:925–931.

Warrant E (2004). Vision in the dimmest habitats on earth. *J Comp Physiol A* 190:765–789.

Warrant E, Bartsch K, Günther C (1999). Physiological optics in the hummingbird hawkmoth: a compound eye without ommatidia. *J Exp Biol* 202 (Pt 5):497–511.

Warrant E, Oskarsson M, Malm H (2014). The remarkable visual abilities of nocturnal insects: Neural principles and bioinspired night-vision algorithms. *Proceedings of the IEEE* 102:1411–1426.

Warrant EJ (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Res* 39:1611–1630.

Warrant EJ (2008). Seeing in the dark: vision and visual behaviour in nocturnal bees and wasps. *J Exp Biol* 211:1737–1746.

Warrant EJ, et al. (2004). Nocturnal vision and landmark orientation in a tropical halictid bee. *Curr Biol* 14:1309–1318.

Warrant EJ, McIntyre PD (1990). Limitations to resolution in superposition eyes. *J Comp Physiol A* 167:785–803.

Warrant EJ, McIntyre PD (1991). Strategies for retinal design in arthropod eyes of low F-number. *J Comp Physiol A* 168:499–512.

Warrant EJ, McIntyre PD (1993). Arthropod eye design and the physical limits to spatial resolving power. *Prog Neurobiol* 40:413–461.

Warrant EJ, McIntyre PD (1996). The visual ecology of pupillary action in superposition eyes. *J Comp Physiol A* 178:75–90.

Warrant EJ, Nilsson DE (1998). Absorption of white light in photoreceptors. *Vision Res* 38:195–207.

Wcislo WTL, Arneson K, Roesch V, Gonzalez AS, Fernandez. H (2004). The evolution of nocturnal behaviour in sweat bees, Megalopta genalis and M. ecuadoria (Hymenoptera: Halictidae): an escape from competitors and enemies? *J Linn Soc* 83:377–387.

Weber F, Machens CK, Borst A (2010). Spatiotemporal response properties of optic-flow processing neurons. *Neuron* 67:629–642.

Weckström M, Jarvilehto M, Heimonen K (1993). Spike-like potentials in the axons of nonspiking photoreceptors. *J Neurophysio* 69:293–296.

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

Wicklein M, Varjúe D (1999). Visual system of the European hummingbird hawkmoth Macroglossum stellatarum (Sphingidae, Lepidoptera): motion-sensitive interneurons of the lobula plate. *J Comp Neurol* 408:272–282.

Wimmer EA (2003). Innovations: applications of insect transgenesis. *Nat Rev Genet* 4:225–232.

Wolburg-Buchholz K (1979). The organization of the lamina ganglionaris of the hemipteran insects, Notonecta glauca, Corixa punctata and Gerris lacustris. *Cell Tissue Res* 197:39–59.

Yeandle S (1958). Evidence of quantized slow potentials in the eye of Limulus. *Am J Ophthalmol* 46:82–87.

Yin C, et al. (2016). Insectbase: a resource for insect genomes and transcriptomes. *Nucleic Acids Res* 44:D801–D807.

Zuidema P, Verschuure H, Bouman MA, Koenderink JJ (1981). Spatial and temporal summation in the human dark-adapted retina *J Opt Soc Am* 71:1472–1480.

Printed by Media-Tryck, Lund University 2016
Nordic Ecolabel 3041 1003

I. Stöckl, A., O'Carroll D., Warrant E. (2016) Neural summation in the hawkmoth visual system boosts contrast sensitivity and information rate in dim light. Curr Biol. 26:821–826. doi:10.1016/j.cub.2016.01.030

II. Stöckl, A., O'Carroll D., Warrant E. (2016) Matching neural processing to visual ecology: spatial and temporal summation in motion vision of hawkmoths active at different light levels. (in preparation).

III. Stöckl, A., Ribi W., Warrant E. (2016) Adaptations for nocturnal and diurnal vision in the hawkmoth lamina. J Comp Neurol. 524:160–175. doi:10.1002/cne.23832

IV. Stöckl, A., Kihlström, K., Chandler, G.S., Sponberg, S. (2016) Behavioural adaptations for flower tracking in hawkmoths. Phil Trans B (in press). doi:10.1002/cne.23832

LUND UNIVERSITY

Department of Biology
ISBN 978-91-7753-064-0