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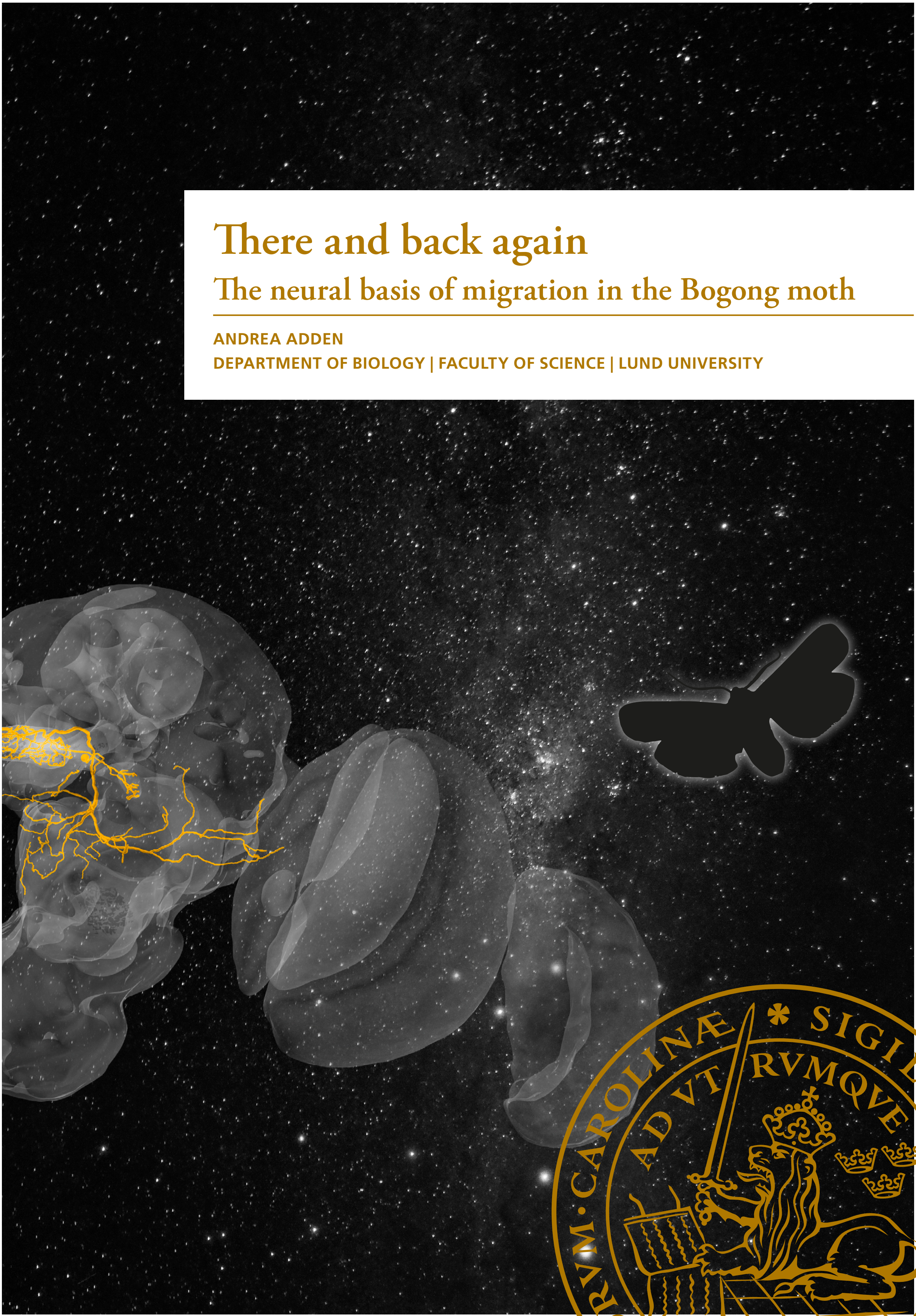

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The neural basis of migration in the Bogong moth

Andrea Adden

LUND
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DOCTORAL DISSERTATION

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Abstract

The Bogong moth (*Agrotis infusa*) is a small, night-active Australian moth that has a remarkable lifestyle. After hatching from its pupa in spring, it migrates over 1000 km to the Australian Alps, where it spends the summer in cool alpine caves. In the beginning of autumn, the moths emerge from the caves and fly back to their breeding grounds, where they mate, lay eggs, and die. The following year, a new generation of moths repeats the same journey to the mountains.

Migration is a difficult and dangerous task. If the moths get lost on the way, they will not arrive at the caves in time and will instead perish in the hot Australian summer. It is therefore crucial that they are efficient and reliable navigators. However, the brains of these moths are tiny – only 3 mm in diameter. How can such a small brain compute the trajectory of this extraordinary migration?

In this thesis, I investigated the neural basis of navigation and migration in the Bogong moth. I began by describing the Bogong moth brain in detail (Paper I). In insects, neurons in a brain region known as the central complex process spatial information and provide the spatial context for behavioural decisions. The central complex of the Bogong moth is well developed and can be expected to have the same function as in other insects. From previous studies, we know that brain regions that are of special importance for an animal tend to be bigger. I therefore compared the volume of several higher processing neuropils, including the central complex, across several moth species (Paper II), including both migrants and non-migrants. I found that that the relative volumes of the central complex across species were very similar. In fact, the central complex scaled hypo-isometrically, suggesting that the neural networks in this brain region are so fundamentally important that even the smallest moths cannot afford to reduce them further. Therefore, instead of being reflected in the overall volume of the central complex, migratory behaviour may be reflected in the response properties of individual neurons in this brain region. Knowing that the Bogong moth can chose a migratory heading based on the starry sky alone, I recorded from neurons in the central brain while presenting the moth with a rotating starry sky (Paper III). I found several neurons that consistently responded to this stimulus. Some of these neurons had branches in the optic lobes, the central complex or the lateral complex, which are all associated with visual compass processing. Thus, these neuropils provide a suitable substrate for processing compass cues during the moths' nocturnal migration. Finally, I investigated how a compass signal in the central complex is transmitted to downstream motor centres that coordinate wing and leg movement. To this end, I built a computational model of a proposed steering network (Paper IV). I showed that this network can theoretically steer based on input from olfaction as well as vision, providing a putative connection between the compass system in the central complex and thoracic motor centres. Taken together, these results have not only shed light on the neural basis of migration in the Bogong moth, but also on neural processing in the insect central complex and lateral accessory lobes in general. In the future, combining these results with insights from other insects may lead to a complete understanding of the neural basis of migration, from the sensory inputs to the behavioural output.

Key words navigation, central complex, lateral accessory lobe, insect brain, lepidoptera, noctuid, Milky Way

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There is a moth called a Bogong

With migrations both precise and long

How'd they know where to go?

Do they use optic flow?

They can't afford to get it wrong

(Benji Kessler)

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II. **Adden**, A., Kannan, K., Yuvaraj, J. K., Raif, T., Kelber, A., Heinze, S. Brain structure in lepidopteran insects – towards identifying anatomical correlates of migratory behaviour. *(Manuscript)*

III. **Adden***, A., Dreyer*, D., Frost, B., Mouritsen, H., Xu, J., Green, K., Whitehouse, M., Chahl, J., Wallace, J., Foster, J., Heinze, S., Warrant, E. The starry night sky provides true compass information for long-distance nocturnal navigation in the Australian Bogong moth. *(Manuscript)*

IV. **Adden**, A., Stewart, T.C., Webb, B., Heinze, S. A neural model for insect steering applied to olfaction and path integration. *(Manuscript)*

Author contributions

I. S.H. and E.W. designed the study. A.A., S.H. and S.W. collected the data. K.P. generated the average shape brain. A.A. and S.H. analysed and interpreted the data. A.A. wrote the first draft and compiled the figures. All authors contributed to the final version of the manuscript.

II. A.A., S.H. and A.K. designed the study. A.A., J.K.Y., K.K. and T.R. collected the data. A.A., K.K., S.H. and A.K. analysed and interpreted the data. A.A. wrote a first draft of the manuscript and compiled the figures. A.A. and S.H. prepared the final manuscript with contributions from all other authors.

III. A.A. and S.H. designed the electrophysiological experiments. D.D. and E.W. designed the behavioural experiments and the visual and magnetic stimuli. A.A. analysed, interpreted and visualised the electrophysiological and neuroanatomical data with input from S.H.. D.D. analysed, interpreted and visualised the behavioural data. A.A. prepared a first draft of the physiological methods and results and compiled the corresponding figures. E.W. wrote the final manuscript with input from all authors.

IV. A.A., S.H. and B.W. designed the study. A.A. and T.S. prepared the computational models and collected the data. A.A., T.S. and B.W. analysed, interpreted and visualised the data. A.A. and T.S. wrote a first draft. All authors contributed to the final version of the manuscript.

Articles not contained in this thesis

Warrant, E. J., Frost, B., Green, K., Mouritsen, H., Dreyer, D., **Adden**, A., Brauburger, K., Heinze, S. (2016) The Australian Bogong moth *Agrotis infusa*: A long-distance nocturnal navigator. *Front Behav Neurosci 10*.
doi: 10.3389/fnbeh.2016.00077

de Vries, L., Pfeiffer, K., Trebels, B., **Adden**, A. K., Green, K., Warrant, E., Heinze, S. (2017) Comparison of navigation-related brain regions in migratory versus non-migratory noctuid moths. *Front Behav Neurosci 11*.
doi: 10.3389/fnbeh.2017.00158

Stone, T., Webb, B., **Adden**, A, Weddig, N. B., Honkanen, A, Templin, R., Wcislo, W., Scimeca, L., Warrant, E., Heinze, S. (2017) An Anatomically Constrained Model for Path Integration in the Bee Brain. *Curr Biol 27*. 20: 3069-3085.e11.
doi: 10.1016/j.cub.2017.08.052

Honkanen, A., **Adden**, A., da Silva Freitas, J., Heinze, S. (2019) The insect central complex and the neural basis of navigational strategies. *J Exp Biol 222*.
doi: 10.1242/jeb.188854

Steinbeck, F., **Adden**, A., Graham, P. (2019) Connecting brain to behaviour: general purpose steering circuits in insect orientation? *J Exp Biol (under review)*

Popular summary

The Bogong moth (*Agrotis infusa*) is a small, night-active Australian moth that has a remarkable lifestyle. After hatching from its pupa in spring, it migrates over 1000 km to the Australian Alps, where it spends the summer in cool alpine caves. In the beginning of autumn, the moths emerge from the caves and fly back to their breeding grounds, where they mate, lay eggs, and die. The following year, a new generation of moths repeats the same journey to the mountains.

Migration is a difficult and dangerous task. If the moths get lost on the way, they will not arrive at the caves in time and will instead perish in the hot Australian summer. It is therefore crucial that they are efficient and reliable navigators. However, the brains of these moths are tiny – only 3 mm in diameter. How can such a small brain compute the trajectory of this extraordinary migration?

In this thesis, I investigated the neural basis of navigation and migration in the Bogong moth. I began by describing the Bogong moth brain in detail (**Paper I**). In insects, neurons in a brain region known as the central complex process spatial information and provide the spatial context for behavioural decisions. The central complex of the Bogong moth is well developed and can be expected to have the same function as in other insects. From previous studies, we know that brain regions that are of special importance for an animal tend to be bigger. I therefore compared the volumes of higher processing neuropils, including the central complex, across several migratory as well as non-migratory moth species (**Paper II**). I found that that the relative volumes of the central complex across species were very similar. In fact, the central complex scaled hypo-isometrically, suggesting that the neural networks in this brain region are so fundamentally important that even the smallest moths cannot afford to reduce them further. Therefore, instead of being reflected in the overall volume of the central complex, migratory behaviour may be reflected in the response properties of individual neurons in this brain region. Knowing that the Bogong moth can chose a migratory heading based on the starry sky alone, I recorded from neurons in the central brain while presenting the moth with a rotating starry sky (**Paper III**). I found several neurons that consistently responded to this stimulus. Some of these neurons had branches in the optic lobes, the central complex or the lateral complex, which are all associated with visual compass processing. Thus, these neuropils provide a suitable substrate for processing compass cues during the moths' nocturnal migration. Finally, I investigated how a compass signal in the central complex is transmitted to downstream motor centres that coordinate wing and leg movement. To this end, I built a computational model of a proposed steering network (**Paper IV**). I showed that this network can theoretically steer based on input from olfaction as well as vision, providing a putative connection between the compass system in the central complex and thoracic motor centres.

Taken together, these results have not only shed light on the neural basis of migration in the Bogong moth, but also on neural processing in the insect central complex and lateral accessory lobes in general. In the future, combining these results with insights from other insects may lead to a complete understanding of the neural basis of migration, from the sensory inputs to the behavioural output.

Zusammenfassung

Die Bogongmotte (*Agrotis infusa*) ist ein kleiner, nachtaktiver australischer Eulenfalter mit einer bemerkenswerten Lebensweise. Im Frühjahr schlüpfen Millionen Falter aus ihren Puppen und wandern über 1000 km weit in die australischen Alpen, wo sie den Sommer in kühlen Alpenhöhlen verbringen. Im Frühherbst verlassen die Nachtfalter die Höhlen und fliegen zurück zu ihren Brutgebieten, wo sie sich paaren, Eier legen und sterben. Im folgenden Jahr wiederholt eine neue Generation von Bogongmotten die gleiche Reise in die Berge.

Migration ist ein schwieriges und gefährliches Unterfangen. Wenn die Wanderfalter sich verfliegen, erreichen sie die Höhlen nicht rechtzeitig und kommen im heißen australischen Sommer um. Es ist daher entscheidend, dass sie effizient und zuverlässig navigieren können. Das Gehirn dieser Nachtfalter ist jedoch winzig, mit einem Durchmesser von nur 3 mm. Wie kann ein so kleines Gehirn diese außergewöhnliche Wanderung steuern?

In der vorliegenden Arbeit untersuchte ich die neuronalen Grundlagen von Navigation und Migration der Bogongmotte. Zunächst beschrieb ich das Gehirn der Bogongmotten im Detail (**Manuskript I**). Bei Insekten verarbeiten Nervenzellen in einer Gehirnregion, die als Zentralkomplex bekannt ist, räumliche Informationen vom Sehsystem und anderen Sinnesorganen und liefern den räumlichen Kontext für Verhaltensentscheidungen. Der Zentralkomplex der Bogongmotte ist gut entwickelt und hat vermutlich dieselbe Funktion wie bei anderen Insekten. Aus früheren Studien wissen wir, dass Gehirnregionen, die für ein Tier von besonderer Bedeutung sind, tendenziell größer sind. Ich verglich daher die Volumina aller höheren Gehirnregionen, einschließlich des Zentralkomplexes, von verschiedenen Falterarten (**Manuskript II**). Einige dieser Arten machen eine ähnliche Langstreckenwanderung wie die Bogongmotte. Ich konnte zeigen, dass die relativen Volumina des Zentralkomplexes zwischen den Arten sehr ähnlich waren. Tatsächlich skaliert der Zentralkomplex hypoisometrisch, was darauf hindeutet, dass die neuronalen Netzwerke in dieser Gehirnregion von so grundlegender Bedeutung sind, dass es sich selbst die kleinsten Motten nicht leisten können, sie weiter zu reduzieren. Da sich das Migrationsverhalten nicht im Gesamtvolumen des Zentralkomplexes widerspiegelte, untersuchte ich im Folgenden die Nervenzellen in dieser Gehirnregion. Aus vorherigen Verhaltensxperimenten war bekannt, dass die Bogongmotte allein mit Hilfe des Sternenhimmels navigieren kann. Daher maß ich die Aktivität von Nervenzellen im Gehirn, während ich der Motte einen sich drehenden Sternenhimmel präsentierte (**Manuskript III**). Ich fand mehrere Zellen, die zuverlässig auf diesen Stimulus reagierten. Einige dieser Neurone hatten Verzweigungen in den optischen Loben, dem Zentralkomplex oder dem Lateralkomplex, die alle mit der visuellen Kompassverarbeitung verbunden sind. Das bestätigt, dass all diese Gehirnregionen an

der Verarbeitung von Kompassinformationen während der nächtlichen Wanderung der Falter beteiligt sind. Schließlich untersuchte ich, wie ein Kompasssignal vom Zentralkomplex an nachgeschaltete motorische Zentren übertragen wird, die die Bewegung von Flügeln und Beinen koordinieren. Zu diesem Zweck erstellte ich ein neuronales Modell eines bereits beschriebenen Netzwerks (**Manuskript IV**). Ich konnte zeigen, dass dieses Netzwerk das Tier theoretisch mit Hilfe von sowohl olfaktorischen als auch visuellen Sinneseindrücken lenken kann, also eine mögliche Verbindung zwischen dem Kompasssystem im Zentralkomplex und den motorischen Zentren im Thorax herstellt.

Zusammengenommen geben diese Ergebnisse Aufschluss über die neuronale Grundlage der Migration der Bogongmotte, aber auch über die neuronale Verarbeitung im Insektengehirn im Allgemeinen. In Zukunft können diese Ergebnisse mit Erkenntnissen über andere Insekten kombiniert werden, um zu einem vollständigen Verständnis der neuronalen Mechanismen zu führen, die dem Wanderverhalten von Tieren zu Grunde liegen – von den Sinneseindrücken bis zum Verhalten.

Sammanfattning

Den australiska Bogong-nattfjärilen (*Agrotis infusa*) är en liten, nattaktiv insekt med en väldigt anmärkningsvärd livscykel. Efter att ha kläckts ur sin puppa, under den australiska våren, flyger den över 1000 km till de australiska Alperna. Där tillbringar den sommaren i svala grottor i ett dvalliknande tillstånd för att sedan, i början av hösten flyga samma väg tillbaka till sina fortplantningsområden, där den parar sig, lägger ägg och dör. Nästa generation nattfjärilar kläcks långt efter att föräldrarna har dött men flyger, trots detta, samma långa väg till bergen.

Att migrera så långa sträckor är både farligt och krävande för nattfjärilarna. Om de flyger vilse riskerar de att inte nå grottornas svalka i tid, utan dör istället under den heta australiska högsommarsolen. Det är därför viktigt att de är effektiva och pålitliga navigatörer. Men hjärnorna hos dessa insekter är väldigt små – bara 3 mm i diameter. Hur kan en så liten hjärna beräkna och navigera denna extraordinära långflygning?

I den här avhandlingen undersökte jag den neurala grunden för navigering och migration hos Bogong-nattfjärilen. Jag började med att beskriva nattfjärilens hjärna i detalj (**manus I**). Hos insekter finns en särskild hjärnregion som kallas centralkomplexet med neuroner som behandlar rumslig information och ger ett rumsligt sammanhang för beteendebeslut. Nattfjärilens centralkomplex är välutvecklat och kan förväntas ha samma funktion. Från tidigare studier vet vi att regioner i hjärnan som är särskilt viktiga för ett djur har en tendens att vara större. Jag jämförde därför volymen hos flera högre hjärnregioner, inklusive centralkomplexet, mellan ett flertal nattfjärilsarter (**manus II**). Vissa arter var migrerande, andra inte. Jag fann att centralkomplexens relativa volym i själva verket var väldigt lika mellan de olika arterna. Det antyder att de neurala nätverken i denna hjärnregion fyller en grundläggande och viktig funktion så att även de minsta nattfjärilarna inte har råd att minska dem ytterligare. Eftersom migrationsbeteende inte återspeglas i centralkomplexets totala volym, undersökte jag istället responsegenskaperna hos enskilda neuroner i centralkomplexet. Med tanke på att Bogong-nattfjärilen kan välja flygriktning enbart baserad på stjärnhimlen, mätte jag aktiviteten hos nervceller i den centrala delen av hjärnan samtidigt som nattfjärilen placerades framför en roterande stjärnhimmel (**manus III**). Här upptäckte jag flera neuroner som konsekvent svarade på detta stimulus. Vissa av neuronerna förgrenade sig antingen i de optiska loberna, centralkomplexet eller det laterala komplexet, som alla är förknippade med bearbetning av visuella kompassignaler. Således tillhandahåller dessa regioner i hjärnan ett lämpligt substrat för bearbetning av kompassignaler under Bogongens nattliga migration. Slutligen undersökte jag hur en kompassignal i centralkomplexet överförs till motorcentrum som koordinerar ving- och benrörelser. För detta ändamål skapade jag en beräkningsmodell av ett föreslaget styrnätverk (**manus IV**). Jag visade att detta nätverk teoretiskt sett kan fungera som ett styrsystem, baserat på information från lukt- såväl som visuella signaler, vilket tyder på en koppling mellan kompassystemet i centralkomplexet och motorcentralen i thorax.

Sammantaget belyser dessa resultat både den neurala grunden för migration hos Bogong-nattfjärilen, samt neuroners bearbetning av information generellt, i insektens centralkomplex och laterala komplexets lober. I framtiden kan dessa resultat från Bogong-nattfjärilen kombineras med kunskap från andra insekter för att ge en fullständig förståelse för den neurala grunden för migration – från sensorisk information till beteende.

1 The scope of this thesis

Light provides information about the structure and layout of an animal's environment, and it is an important cue to guide the animal through its daily life. Therefore, many animals rely heavily on vision and have evolved brains that are well adapted to using visual information in order to solve complex tasks. These tasks range from locating a food source, to finding a mate, to navigating home. As diverse as these daily challenges seem to be, they have one thing in common: they all require the ability to navigate. External cues provide the most effective basis for navigation, and they can be subdivided into two types. The first are local cues, such as landmarks, which provide the animal with information about where it is in its local environment. The second type are compass cues, which give the animal a global reference frame. The Sun and the geomagnetic field are well-known compass cues, and they are especially important for animals that navigate over long distances (Heinze, 2017).

A famous example for a long-distance migrating insect is the Monarch butterfly. Every year, these colourful butterflies migrate over 4000 km from their breeding grounds in the northern USA and southern Canada to their overwintering sites in central Mexico. It is now known that they use a time-compensated Sun compass in order to keep a straight heading over many weeks (Mouritsen and Frost, 2002; Reppert, 2006). While the Monarch butterfly has been a useful model system to study long-distance navigation during the day, nocturnal long-distance migrating insects have received less attention. Little is known about how sensory information is used for long-distance navigation in dim light, and how it is processed in the brain (Warrant and Dacke, 2016).

To shed light on the neural mechanisms underlying nocturnal migration, I studied the Australian Bogong moth *Agrotis infusa*, which is a recently established insect model for nocturnal long-distance migration. Every year, millions of Bogong moths travel over 1000 km from their breeding grounds to their aestivation sites in the Snowy Mountains (Warrant et al., 2016). Recent behavioural experiments showed that these moths can use the geomagnetic field in combination with visual landmarks to select a flight direction (Dreyer et al., 2018). However, the nature of the visual landmarks remains unclear, as well as the possible use of other compass cues. Moreover, we do not yet understand how compass cues are processed and integrated in the Bogong moth brain to provide an unambiguous navigational heading.

In this thesis, I combined anatomical, functional and computational approaches to investigate the neural basis of the Bogong moth's migration (Figure 1). I began by describing the Bogong moth brain in detail (**Paper I**), in order to provide a framework in which functional studies can be embedded. Expanding this approach to other lepidopteran species allowed me to compare the brains of migratory and non-migratory moths and butterflies, and revealed that a migratory lifestyle correlates with small but consistent volumetric changes in higher processing areas (**Paper II**). Using intracellular electrophysiology combined with behavioural experiments, my colleagues and I were able to demonstrate that Bogong moths can use the starry sky as a navigational cue, and that neurons in the compass centre of the moth's brain are sensitive to this type of celestial information (**Paper III**). Finally, following such a compass cue requires precise steering. Using a computational model, I showed that a simple neural circuit in the brain's lateral accessory lobes is suited to compute steering independent of stimulus modality (**Paper IV**).

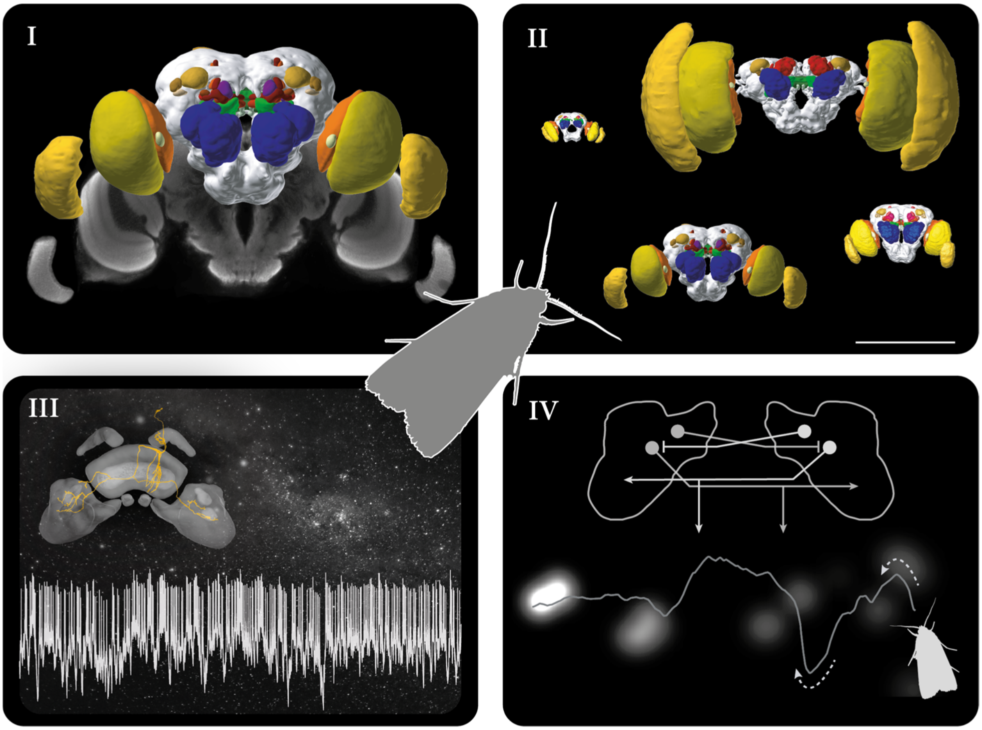

Figure 1: The scope of this thesis.
This thesis examines the neural basis of migration. Papers I and II focus on the anatomical layout of the Bogong moth brain, as well as the brains of other migratory and non-migratory moths and butterflies. Paper III describes neurons in the compass centre of the Bogong moth brain that respond to the starry sky, which can be linked back to the behaviour of the moth. Paper IV explores a simple neural circuit that is sufficient to underlie steering during general orientation behaviours and likely also during long-distance migration.

2 The migration of the Bogong moth

The ecology of Bogong moth migration

The Bogong moth *Agrotis infusa* (Boisduval, 1832) is a noctuid moth that is native to the temperate regions of Queensland, New South Wales and Victoria in Australia. It is a multivoltine species, meaning that if conditions are favourable, the Bogong moth can produce up to four overlapping generations per year (Figure 2A). The larvae are known as cutworms and are considered a pest (Common, 1957). However, in most known breeding grounds larval food plants become sparse during the hot summers. It is therefore necessary to delay the reproductive period in order to promote survival of the following generation. This need to delay mating has given rise to an extraordinary migration event that takes place each year. In spring, as temperatures in the breeding grounds rise, newly eclosed Bogong moth adults begin their migration to the Snowy Mountains in Southern New South Wales (Figure 2B). The moths have been reported to fly during the night and feed during the late afternoon, resting in trees throughout the day. Upon arrival in the mountains, they settle in a number of alpine caves, located at altitudes above 1800 meters, where they enter a state called aestivation. Aestivation closely resembles hibernation in that the metabolic rate of the moth is decreased and they are largely inactive, only rarely flying at dusk and dawn (Common, 1952, 1954). The reproductive organs of the aestivating moths are immature and only become mature after ingestion of food during the return migration at the end of the summer (Common, 1954). Once the moths arrive at their breeding grounds, they mate, lay eggs and die. The larvae hatch in winter and pupate in early spring, and the newly eclosed adults repeat the same journey to the Snowy Mountains (reviewed in Warrant et al. 2016).

During the summer, the aestivating moths form an important part of the food chain in the Snowy Mountains, and are predated upon by ravens, bush rats and foxes (Green, 2011). The moths are also of great cultural importance in Australia, as they were an abundant food source for local Aboriginal tribes during the summer (Flood, 1980). However, Bogong moth numbers have been declining over the past century, and in recent years we observed another sharp drop. Many caves that would usually be occupied by moths now remain empty throughout the summer, and our light-trapping records show that mass migration events happen on far fewer nights than in previous years (unpublished observations). The slow decline of the Bogong moth has been attributed to changing agricultural practices and increased use of pesticides in their breeding grounds (Green et al., *in preparation*). In contrast, the recent sudden collapse of the population appears to be linked to the ongoing drought that affects most of South-Eastern Australia.

Several details of the Bogong migration are noteworthy. First of all, the distance over which these moths migrate typically exceeds 1000 km, which is an astonishing distance for a moth that is only 3 cm long. Secondly, Bogong moths are nocturnal and have only been observed flying at night, although they feed and make orientation flights at dusk and dawn (Common, 1954). The challenges and strategies involved in navigating over such enormous distances in dim light will be discussed in detail in this thesis. Last but not least, the moths occupy the same caves every year, but as yet no commonality between those caves has been found (Warrant et al., 2016). It has however been suggested that the moths use olfactory cues to find the caves: Excrements on the cave walls left by previous generations of moths, as well as moth debris on the cave floor, emit a strong odour that even humans can perceive (personal observation) and that may be sufficient to guide the moths to the caves over short distances.

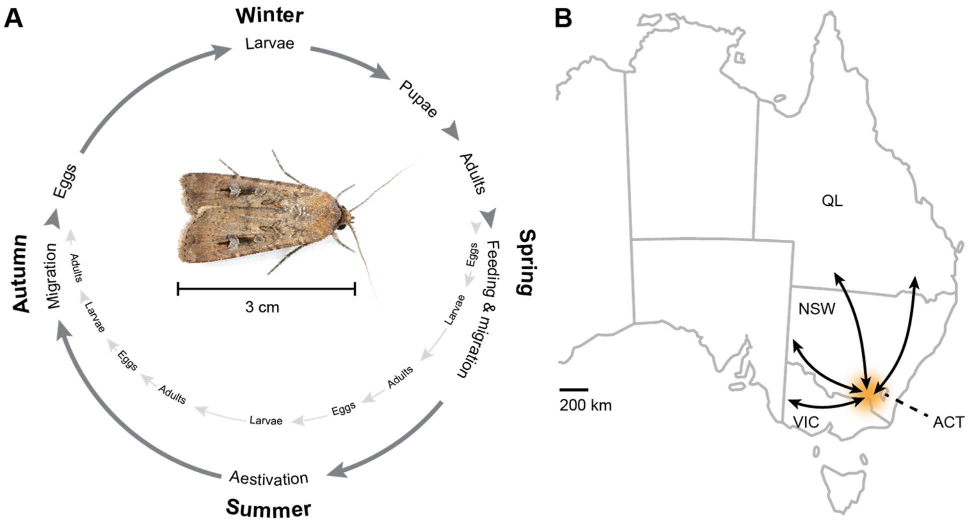

Figure 2: Life cycle and migration of the Bogong moth.
A: Adult moths emerge in spring and embark on their migration to the Snowy Mountains, where they aestivate. They migrate back to their breeding grounds at the end of the summer. The moths then mate, lay eggs and die. Their larvae feed during the winter and pupate in early spring. Under favourable conditions, the Bogong moth can be multivoltine (light grey arrows) and can have up to four generations per year, avoiding the need for migration and aestivation. Life cycle adapted from Common (1954). Photo of the Bogong moth courtesy of Ajay Narendra, Macquarie University, Australia. B: Bogong moths migrate to the Snowy Mountains (orange) from their breeding grounds in southern Queensland, western New South Wales and western Victoria.

How does the Bogong moth navigate?

A long-distance migration such as the Bogong moth's requires extremely precise navigation. Any deviation from the migratory route would result in the moth missing its target, and would therefore be fatal. So far, we know little about how Bogong moths can navigate so precisely, but a recent behavioural study showed that the moths are able to perceive the geomagnetic field and combine it with visual landmarks to select a stable flight direction (Dreyer et al., 2018). This agrees well with the observation by Common (1954) that Bogong moths continue their migration under heavily overcast skies, when no celestial compass cues are available (Common, 1954; Warrant et al., 2016). Interestingly, in their 2018 study, Dreyer and colleagues were only able to show that the moths use the geomagnetic field in a cue conflict experiment: When the magnetic field vector and the visual landmark were moved together, but their relative position was unchanged, the moths remained oriented. However, when the relative position of the magnetic field vector and the visual landmark changed, the moths lost their flight direction and started circling. Furthermore, the delay of the cue conflict response was relatively long at approximately 1.5-2 minutes (Dreyer et al., 2018). The authors of the study suggest that the moths may employ a multisensory 'snapshot' strategy, in which the moths periodically take a snapshot of all available cues and their current relative orientations – including the geomagnetic field, but also visual landmarks – and align it to an internal reference. As the moth's position relative to landmarks changes over time, landmarks alone would be an unreliable cue, but with every new snapshot, the moth can adjust its heading with respect to the geomagnetic field (Dreyer et al., 2018). Such a strategy has the advantage that it does not need to be time-compensated. However, it appears that uncontrolled visual landmarks override the magnetic sense, and moths will select a flight direction more readily relative to a visual cue than to the magnetic field vector (D. Dreyer, personal communication). This observation suggests that the magnetic sense acts as a 'backup sense' that can be used when no other reliable cues are available, for example under thick cloud cover. Under a clear night sky, the moth has additional options: the Moon and the stars are salient cues that can guide navigation. However, skylight cues come with challenges, which I will discuss in chapter 3. In **Paper III**, I present the first evidence that Bogong moths can use the starry sky to navigate in their migratory direction.

3 Compass cues

It is common knowledge that moths are attracted to light – they come to illuminated windows or crowd around lightbulbs. Why? One hypothesis is that they mistakenly use the light as a compass cue, an external reference that informs the moth's internal compass and allows it to move in a straight line (Cheung et al., 2007). However, the distance between the moth and this compass cue matters: If the cue is nearby, its position relative to the moth changes rapidly as the moth moves. The further away a cue is, the less its position changes relative to the moth. Thus, a cue that is infinitely far away from the moth is the most stable reference for orientation. This means that celestial bodies such as the Moon are ideal compass cues, while landmarks such as trees are relatively bad compass cues. In order to fly in a straight line, a moth may fly at a constant angle relative to a celestial light source. However, if it mistakenly identifies a street lamp as a celestial cue, flying at a constant angle will lead the moth to spiral around the street lamp. Thus, artificial lights may severely disrupt the navigational capabilities of moths.

Nocturnal celestial cues: the Moon and the Milky Way

The brightest and therefore most salient celestial body in the night sky is the Moon. The Moon is Earth's natural satellite and completes one orbit around the Earth in approximately 27.3 days (Williams, 2019). During this orbit, the Moon varies from fully reflecting sunlight (full moon) to being located between the Earth and the Sun, in which case it does not reflect any sunlight back to the Earth (new moon). Therefore, both the brightness and the apparent size of the Moon change over the course of one orbit. While the Moon orbits the Earth, the Earth also completes a full rotation once per approximately 24 hours. Due to this rotation, the Moon appears to move across the sky over the course of one night. When viewed from Earth, the Moon has an angular diameter of approximately 0.52°, which is only marginally smaller than the angular diameter of the Sun (≈ 0.53°; Stellarium Developers, www.stellarium.org).

The Moon also has an associated pattern of polarised light. Unpolarised light from the Sun and the Moon becomes polarised when it passes through the atmosphere, through a process called Rayleigh scattering (Wehner, 2001; Johnsen, 2012). A light beam is said to be linearly polarised if the electric field vectors (e-vectors) of its component waves oscillate in the same plane perpendicular to the direction of propagation of the beam. Unpolarised light coming from the Sun or Moon is scattered by atmospheric particles (mostly O_2 and N_2 molecules), with the maximum degree of polarisation at scattering angles of 90°. Therefore, sunlight is maximally polarised in a band 90° away from the Sun, and the angle of polarisation forms concentric circles around the Sun. Sunlight and Moonlight can reach a maximum degree of polarisation of over 60% under clear skies (Horváth et al., 2014; Foster et al., 2019), although the polarisation pattern around the Moon is about 6 orders of magnitude dimmer than that around the Sun (Gál et al., 2001; Smolka et al., 2016). Polarised light is an axially symmetric cue, and therefore inherently ambiguous. However, it is theoretically possible to infer the Sun's position from the polarisation pattern alone, unless the Sun is at the azimuth or very close to the horizon (Bech et al., 2014; Gkanias et al., 2019). Two other properties of skylight can be used to increase the reliability of this compass system: the spectral and intensity gradient of the sky. The solar hemisphere contains relatively more long-wavelength light and is brighter than the anti-solar hemisphere (el Jundi et al., 2014). The same spectral and intensity gradient distinguishes the lunar from the anti-lunar hemisphere, although the nocturnal spectral gradient is dimmer and contains approximately 14% less UV light (Foster et al., 2019). Therefore, by combining skylight polarisation and the spectral or intensity gradient, one can unambiguously infer the Sun's or Moon's azimuth.

Another celestial structure that could be used as a compass cue is the Milky Way. The Milky Way is the Earth's home galaxy and can be viewed from Earth as a bright band that spans the night sky. Its angular width is approximately 30°, but it is far dimmer than the moon (Foster et al., 2017). It is therefore often obscured by light pollution in urban areas (Foster et al., 2018). Rather than being a band of uniform brightness, the Milky Way has a distinct gradient from the bright galactic centre to the dimmer outer regions (Foster et al., 2017). Furthermore, the Milky Way is tilted by approximately 60° with respect to the Earth's ecliptic plane. This tilt, together with the Earth's rotation, result in a noticeable change of the Milky Way's position over the course of a year: While it is highly visible and located approximately at the zenith in March, it is located near the horizon throughout almost the entire night in October (at the field site near Adaminaby, NSW; Figure 3).

Finally, the celestial centre of rotation can be a useful reference for navigation. As the Earth rotates, the sky also appears to rotate around the Earth's axis of rotation, which is the North-South axis. In the Northern hemisphere, the centre of rotation is Polaris, the North Star, which marks geographical North (Stellarium Developers, www.stellarium.org). In the Southern hemisphere, the centre of rotation is not a single star, but an area of sky near the star Sigma Octantis. However, even if the celestial pole is unknown, it can be inferred from the apparent movement of the stars and constellations around it. In contrast to the Moon and the Milky Way, which appear to move across the night sky, the celestial centres of rotation provide a fixed reference to North and South.

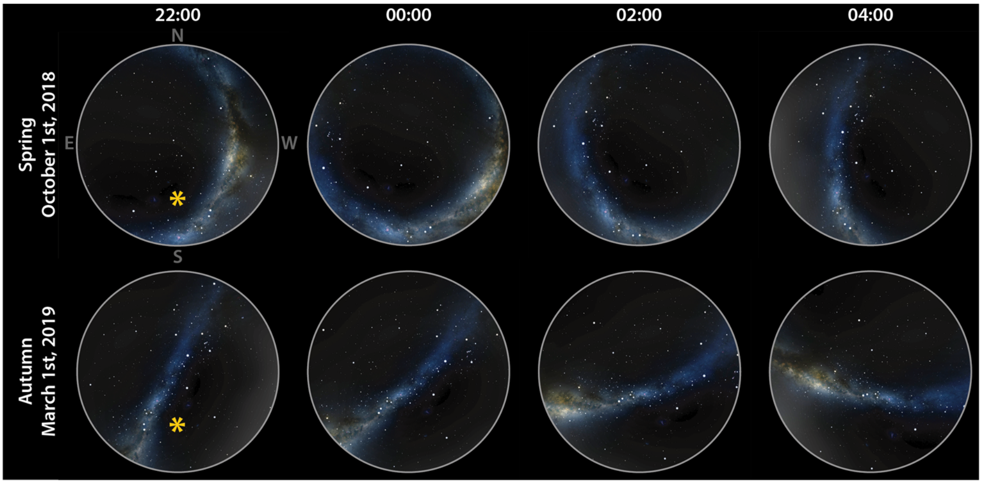

Figure 3: The orientation of the Milky Way changes over the course of one night and between seasons. To an observer located in Canberra, Australia, the Milky Way is located near the horizon in spring, while it rises to near the zenith in autumn. The orange asterisk marks the Southern centre of celestial rotation. All images are stereographic projections generated in Stellarium 0.19.1 (Stellarium Developers, www.stellarium.org). Brightness was increased for better visibility.

Time compensation

Broadly speaking, we can distinguish between two types of compass cues: constant cues that provide a fixed reference frame, and variable cues. The celestial centre of rotation is a constant cue, as it always denotes South (or North in the Northern hemisphere). However, the Moon and the Milky Way are variable cues, as their position changes over time as the night progresses. While this is not a problem for animals that only move over short distances, long-distance migrators like the Bogong moth would divert from their straight course if they kept a constant angle to these cues over the course of a night (Figure 4). In order to keep a constant bearing, animals need to find a way to compensate for the apparent movement of the sky, and adjust their heading accordingly. This can be done by integrating the compass system with the circadian clock. Monarch butterflies have been shown to possess a time-compensated Sun compass that allows them to navigate precisely over 4000 km, with clock-shifted Monarch butterflies predictably changing their direction depending on the azimuth of the Sun (Mouritsen and Frost, 2002). The clock mechanism has been shown to reside in the antennae (Merlin et al., 2009). However, where compass cues are integrated with timing information, and the exact mechanism for azimuth compensation, is as yet unclear (see next chapter; Heinze and Reppert 2011; Heinze and Reppert 2012).

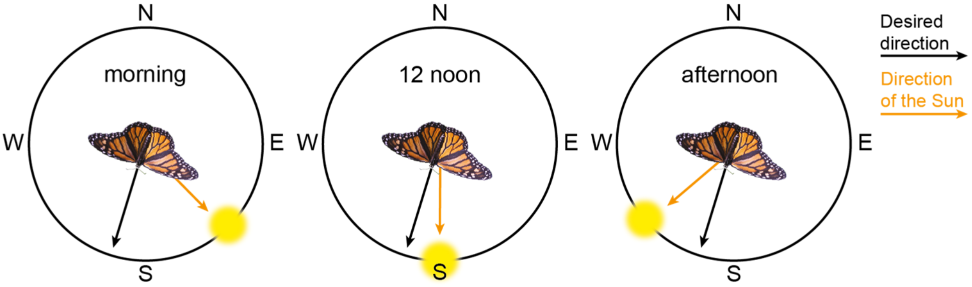

Figure 4: Time compensation is essential when following compass cues during long-distance migration. Over the course of a day, the Sun and other celestial bodies move across the sky in a predictable manner. Long-distance migrators such as the Monarch butterfly need to integrate the time of day with the position of the Sun in order to compensate for this change and to keep their migratory heading. Figure adapted from Honkanen et al. (2019).

Landmarks and the 'snapshot strategy'

Landmarks are used for orientation and navigation in short-range homing foragers such as bees and ants (Collett, 1996), but in long-distance migrators, evidence for landmark use is so far lacking. However, it has been suggested that Monarch butterflies may be guided to their overwintering grounds in part by following mountain ranges and coastlines (Mouritsen et al., 2013). These geographical features can be prominent visual cues, even in dim light. However, landmarks provide reliable information about the environment to an animal only as long as they are in sight, and thus are of limited use to a moving animal. Therefore, landmarks can only guide migration when used in concert with compass cues. If a flying animal's migratory direction aligns with a geographic landmark (e.g. a coastline or a river), it is conceivably easier to follow that visible geological feature while only occasionally updating the compass direction. Alternatively, the feature might act like a barrier that cannot be crossed, therefore guiding the animal in a desirable direction (Mouritsen et al., 2013). Similarly, if a high mountain lies in an animals flight path, it seems straightforward to fly towards the mountain and only periodically check the compass direction if the mountain is either no longer visible or if the animal has passed it. Anything sufficiently constant and salient can be a landmark, and even the (non-time-compensated) Milky Way may be used. An appropriate strategy for navigation could then be a 'snapshot' strategy, in which the animal takes a panoramic image of all available cues and aligns itself with that snapshot. Alternatively, the animal might periodically check its heading against a suitable compass cue and then align with a landmark that guides the animal's flight up to the next checkpoint.

Using nocturnal compass cues

Several nocturnal invertebrates are known to use the Moon or the Milky Way for navigation. One example is the sandhopper (*Talitrus saltator*), which navigates back and forth between the shoreline and its burrowing zone on the beach. Sandhoppers use the Moon as a reference for directed movement, with movement becoming confused or ceasing under an overcast sky. Furthermore, sandhoppers can use the Sun for the same purpose during the day, but have two different chronometric systems to compensate for the apparent movement of the Sun and the Moon (Ugolini et al., 1999). An example for an insect that uses the Milky Way to orient is the dung beetle (*Scarabaeus satyrus*). When rolling its dung ball, this beetle uses the Milky Way to maintain a straight heading (Dacke et al., 2013a; Smolka et al., 2016; Foster et al., 2017). However, both sandhoppers and dung beetles only navigate over short distances. When it comes to long-distance navigation, it is well-known that other animals, such as birds, use the fixed rotation axis around the northern celestial pole (Emlen, 1970; Keeton, 1979). An early study in the heart-and-dart moth (*Agrotis exclamationis*) supports the notion that these moths can use magnetic fields to calibrate a Moon compass (Baker and Mather, 1982; Baker, 1987). However, to my knowledge, unambiguous evidence for a Moon or Milky Way compass in long-distance migrating insects is so far lacking (but see Sotthibandhu and Baker, 1979).

The Bogong moth has been shown to use to geomagnetic field in combination with visual landmarks to navigate (Dreyer et al., 2018). As the response to a change in the geomagnetic field was on the order of 1.5 minutes and thus relatively slow, the authors of the study suggested that the moths employ a 'snapshot strategy'. This would allow the moth to periodically take a snapshot of all available cues, including the geomagnetic field and visual landmarks, and use this snapshot to align itself to an internal directional reference (most likely the geomagnetic field). The visual aspect of the snapshot is then used to ensure stable flight in the correct direction for the next 1-2 minutes, until the alignment with the internal reference is checked once more. This strategy is robust to drift, as well as to changes in the environment as the moth moves, because the regular compass updates allow the moth to compensate for deviations from its migratory direction and re-adjust its heading. Any salient cue can be used as a visual landmark in the snapshot, including the silhouetted horizon, the Moon and the Milky Way. Used in this way, celestial cues would not need to be time-compensated. However, if the Bogong moth can compensate for the movement of the Moon or the Milky Way, these celestial cues can be used as true compass cues, potentially providing a parallel compass system to the geomagnetic compass.

Aside from the celestial cues discussed above, other cues are likely to play a role during the migration of the Bogong moth. Whether the superposition eye of the Bogong moth is sensitive enough to detect and resolve single stars is not yet known, making it difficult to speculate whether they can potentially use the rotation axis around the celestial pole. However, terrestrial features will likely help guide the moths to their goal. Bogong moths that migrate from southern Queensland to the mountains fly in parallel to the eastern coastline of Australia, as well as to the Great Dividing Range, the mountain range that separates the coastal areas of eastern Australia from the arid regions further inland. These geographic features may act as barriers that cannot be crossed, effectively channelling the moths to their destination in the Snowy Mountains. Finally, after the moths arrive in the mountains, they have to find their aestivation caves. It has been suggested that this search is based on odour cues from the caves (Warrant et al., 2016). Indeed, when given the choice between the smell of cave soil and the smell of control soil in a y-maze, preliminary experiments showed that the moths were strongly attracted to cave odour but not control odour (D. Dreyer, personal communication). The journey from the breeding grounds to the caves can thus be subdivided into two parts: (1) long-distance migration, which relies on the magnetic sense and visual compass cues, and (2) short-distance search for the caves, which appears to be based on olfactory cues. When the moths switch from one to the other, and what triggers the switch, are intriguing questions for the future.

4 The insect brain as the interface between senses and behaviour

The compass cues that guide Bogong moths on their journey are perceived by the eyes and subsequently analysed by specific neural networks in the brain. The neuropils that house these networks include the optic lobes, the anterior optic tubercle, the central complex and the lateral complex. In this chapter, I will introduce these brain regions and give an overview of the general layout of insect brains, in order to provide the anatomical framework in which specific compass-related neural networks can be embedded. Note that in this thesis, I am using the nomenclature as proposed by the Insect Brain Name Working Group (Ito et al., 2014). For neuron names, the system I use corresponds to that established in the locust.

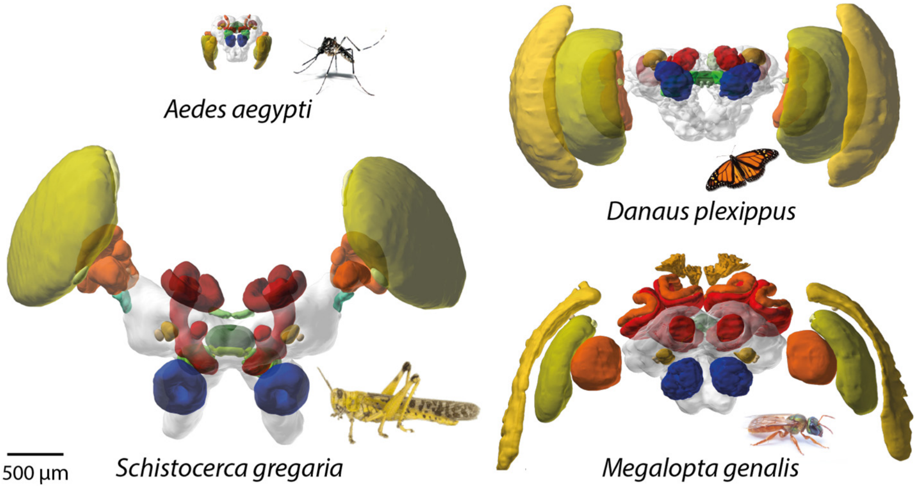

Figure 5: All insect brains follow the same basic anatomical layout.
Despite their differences in size and shape, the brains of the yellow fever mosquito (*Aedes aegypti*, Diptera), the Monarch butterfly (*Danaus plexippus*, Lepidoptera), the desert locust (*Schistocerca gregaria*, Orthoptera) and the sweat bee (*Megalopta genalis*, Hymenoptera) are remarkably similar. The major neuropils include the optic lobes (yellow), antennal lobes (blue), anterior optic tubercles (brown), ocellar neuropils (light brown), mushroom bodies (red) and central and lateral complexes (green), as well as the unstructured protocerebrum (grey). Insect pictures not to scale. All 3D brains were retrieved from the insect brain database, species handles https://hdl.handle.net/20.500.12158/SIN-0000024.2 (*A. aegypti*), https://hdl.handle.net/20.500.12158/SIN-0000005.1 (*D. plexippus*), https://hdl.handle.net/20.500.12158/SIN-0000009.1 (*S. gregaria*) and https://hdl.handle.net/20.500.12158/SIN-0000003.1 (*M. genalis*).

The anatomical layout of insect brains

The basic layout of the brain is very similar across insects, although the brains of different superfamilies, families and species differ in shape and size (Figure 5). The well-defined primary sensory areas – the optic lobes, ocellar neuropils and antennal lobes – are situated at the periphery of the central brain and receive direct input from the eyes, the ocelli and the antennae, respectively (Figure 6A, B). The optic lobes lie laterally to the central brain underneath the retinae. They generally consist of four retinotopically-organised neuropils: lamina, medulla and lobula complex (lobula and lobula plate), as well as the accessory medulla (Strausfeld, 2005). The retinotopic neuropils are the first processing stages for visual information coming from the eyes, whereas the accessory medulla plays a vital role for circadian rhythm (Homberg et al., 2003b). Visual information perceived by the ocelli is processed in the much smaller ocellar neuropils, which are located at the dorsal surface of the brain, in close proximity to the skyward-facing ocelli (Mizunami, 1995). Olfactory information is sensed by the antennae and transferred to distinct processing units termed glomeruli in the antennal lobe, which are situated on the anterior surface of the brain (Strausfeld and Reisenman, 2009).

Sensory information is then transferred from the primary sensory areas to central brain neuropils, including the anterior optic tubercle, lateral complex, central complex and mushroom bodies (Figure 6A-D). The anterior optic tubercle is a secondary sensory area for visual information (Homberg et al., 2003a). It consists of the upper unit and the lower unit complex, which is comprised of the lower and nodular units in lepidopteran insects. This neuropil processes compass cues such as polarised light (Pfeiffer et al., 2005), but also colour information (Mota et al., 2011). Projection neurons connect the anterior optic tubercle to the bulbs of the lateral complex. The bulbs are small neuropils that have a glomerular structure (Träger et al., 2008; Heinze and Reppert, 2011; Held et al., 2016; Omoto et al., 2017) and are located next to the gall, on the dorsal surface of the lateral accessory lobes, the largest neuropil of the lateral complex (Namiki and Kanzaki, 2016). This region has been described as a pre-motor region, as several descending neurons that directly steer behaviour originate here (Namiki et al., 2014). The lateral complex is closely associated with the central complex, and is located anterio-ventrally to either side of it. The central complex comprises four neuropils: the fan-shaped body, ellipsoid body, protocerebral bridge and noduli (Figure 6D; Honkanen et al., 2019). The fan-shaped body is the largest subdivision of the central complex and forms the upper part of the central body. The lower part of the central body is formed by the ellipsoid body. Together, these two neuropils are unique in the insect brain in that they span the midline. The protocerebral bridge, located posterior to the central body, is also connected across the midline in many insect groups. It is associated with the posterior optic tubercles, small neuropils that lie to either side of the protocerebral bridge on the posterior optic tract. The last component of the central complex are the paired noduli, which are situated ventrally relative to the central body. The central complex is the compass centre of the insect brain, and computes for example the insect's heading direction and spatial memory (Honkanen et al., 2019). The brain region predominantly associated with learning and memory is the mushroom body. It is a complex structure consisting of the calyx, peduncle, and at least two lobe systems: the vertical lobe, comprised of the alpha, alpha' and vertical gamma lobes, and the medial lobe, which consists of the beta, beta' and medial gamma lobes as well as the associated spur (Figure 6D; Strausfeld et al., 1998, 2009). In butterflies and moths, a third lobe, the Y-lobe, has been described (Homberg et al., 1988; Sjöholm et al., 2005; Heinze and Reppert, 2012). The mushroom body calyx processes visual and olfactory information, and the peduncle and lobes are sites for visual and olfactory learning and memory (Menzel, 2014). Aside from these well-defined neuropils, over 50% of the central brain is unstructured protocerebrum (Ito et al., 2014), which includes important primary (antennal mechanosensory and motor centre, AMMC; Homberg et al., 1988, 1989; Stocker et al., 1990) and secondary sensory areas (lateral horn; Paulk and Gronenberg, 2008; Schultzhaus et al., 2017). However, the neuropil boundaries of these brain regions cannot be easily defined.

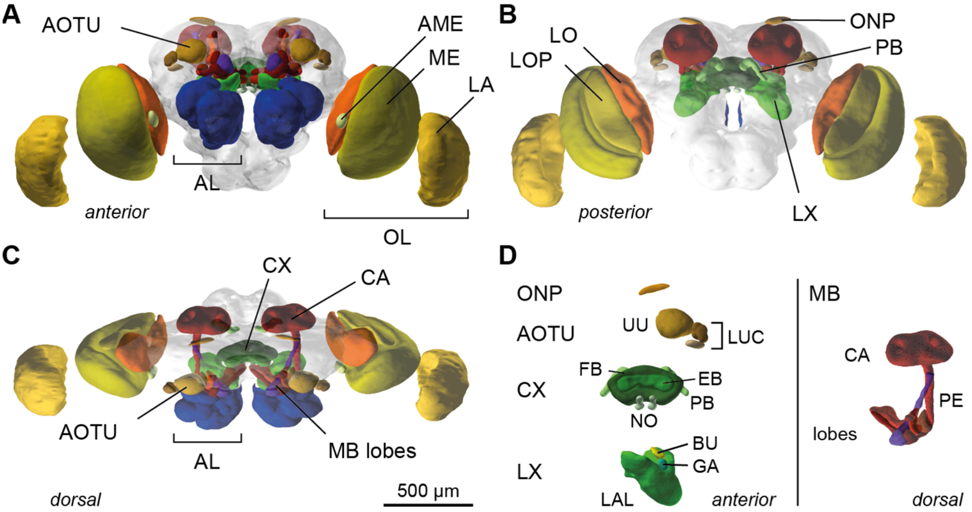

Figure 6: Overview over the defined brain areas of the insect brain.
A: Anterior view, showing the anterior optic tubercles (AOTU), antennal lobes (AL) and optic lobes (OL), which include the accessory medulla (AME), medulla (ME) and lamina (LA). B: Posterior view, showing the lobula (LO) and lobula plate (LOP) of the OL, as well as the ocellar neuropil (ONP), the protocerebral bridge of the central complex (PB), and the lateral complex (LX). C: Dorsal view, showing the central complex (CX), AOTU, AL and the calyx (CA) and lobes of the mushroom body (MB). D: Central neuropils and their sub-structures. The AOTU consists of the upper unit (UU) and the lower unit complex (LUC). The CX contains the fan-shaped body (FB), ellipsoid body (EB), noduli (NO) and PB. The LX comprises the lateral accessory lobe (LAL), bulb (BU) and gall (GA). The sub-neuropils of the MB are the CA, peduncle (PE) and lobes. Brain shown is a Bogong moth brain, retrieved from the insect brain database, species handle https://hdl.handle.net/20.500.12158/SIN-0000002.1.

Why is it important to understand the anatomical layout of the insect brain? One answer to this question is that it adds new access points we have to an insect. For example, using electrophysiological methods to record from a specific brain area is difficult without a detailed anatomical map of the brain. The map reveals how big individual neuropils are and where they are located relative to each other, allowing us to target individual neuropils specifically. A neuroanatomical description of the brain is therefore a valuable tool on which future research can be based. To this end, I present a detailed atlas of the Bogong moth brain in **Paper I**.

Structure reflects function

Another answer to the question why neuroanatomy is important is that it can give us insights into the neural basis of the insect's behaviour. Neural tissue is energetically expensive, as it consumes energy even at rest. Since every living organism has a limited energy budget, there is also a limit to the size of brain areas that can be afforded (Niven and Laughlin, 2008). This means that it is advantageous to invest in neuropils that are particularly important to the survival and fitness of the animal, at the expense of neuropils that are of secondary importance. This effect can be seen when comparing closely related species that have a different ecology, for example a different activity period. The day-active hawkmoth *Macroglossum stellatarum* has relatively larger optic lobes than its night-active relative, *Deilephila elpenor*. On the other hand, *D. elpenor* has relatively larger antennal lobes. This finding can be linked to ecological differences between the two species: *M. stellatarum* uses predominantly colour vision to find flowers to feed from, while the nocturnal *D. elpenor* prefers to use olfaction to solve the same task (Stöckl et al., 2016). Thus, the difference in neuropil size reflects the behavioural difference between the two species.

Similar analyses have been conducted for the mushroom bodies, the insect brain's centres for visual and olfactory learning and memory. Hymenopterans have extremely well-developed mushroom bodies, as well as a sophisticated social structure within their colonies, and the tasks that are carried out by each individual depend on that individual's caste. Mushroom body volume, particularly that of the calyx, was positively correlated with task complexity in ants (Muscedere and Traniello, 2012; Amador-Vargas et al., 2015; Ilieş et al., 2015) and with social dominance in paper wasps (O'Donnell et al., 2006; Molina and O'Donnell, 2008). However, analogous studies across many species have not been done with respect to the neuropils that underlie orientation, navigation and spatial memory. Do the central complex and lateral complex scale differently in species that have especially sophisticated navigation behaviours, for example long-distance migrating moths? I address this question in **Paper II**.

5 The neural circuitry underlying orientation and navigation

In order to probe how navigational cues are processed in the Bogong moth brain (Figure 7), it is helpful to compare it with other insects in which these pathways have already been investigated. The neural architecture of the central processing sites, most notably the central complex, turns out to be remarkably conserved between insect species, making it likely that the processing pathways in the Bogong brain are at least similar to the ones described in desert locusts (*Schistocerca gregaria*), Monarch butterflies (*Danaus plexippus*), fruit flies (*Drosophila melanogaster*) and dung beetles (*Scarabaeus sp.*), among others. A large part of our knowledge of compass processing was investigated in diurnal insects such as the desert locust and the Monarch butterfly, and the neural networks were therefore described in the context of Sun compass navigation and the Sun-associated pattern of polarised light. However, it is likely that nocturnal compass cues are processed by the same neural networks, as the characteristics and challenges associated with compass cues are the same independent of the time of day or the brightness of the cue.

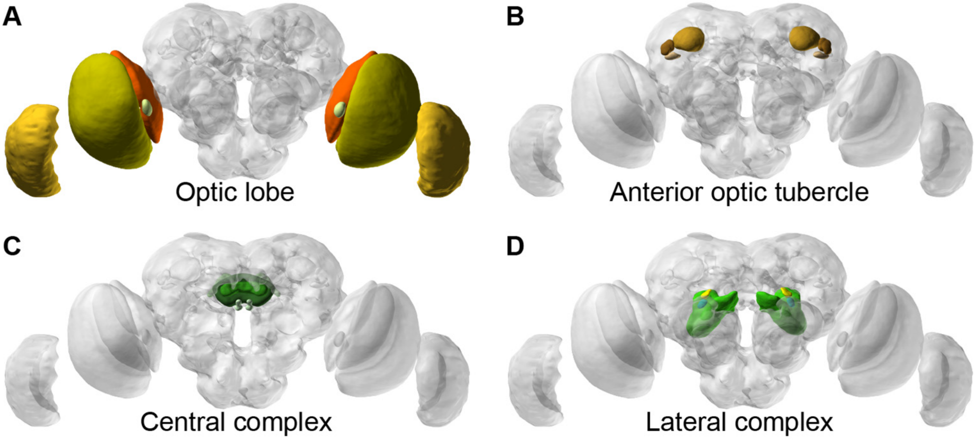

Figure 7: The processing stations of visual compass input.
(A) Optic lobes. (B) Anterior optic tubercles. (C) Central complex. (D) Lateral complex.

Input pathways

Compass cues

The eye and optic lobe

Bogong moths have refracting superposition eyes which allow them to use visual information to guide behaviour even at extremely dim light levels (Warrant et al., 2003; Cronin et al., 2014). Refracting superposition eyes are compound eyes that have two specialisations: refracting crystalline cones and a clear zone. Unlike in apposition eyes, in which light originating from one point in space exclusively enters the ommatidium directed at that point in space, in refracting superposition eyes this light is captured by many ommatidia. It is then refracted in the corneal lens and the crystalline cone, with the angle of refraction depending on the incidence angle of the light due to refractive index gradients. The refracted light beam crosses the clear zone and is absorbed by a single rhabdom in the retina. Thus, while each point in space is still viewed by a single photoreceptor, this photoreceptor receives light from many lenses at the same time, resulting in a higher sensitivity at the level of the photoreceptor (Kunze, 1969; Kunze and Hausen, 1971).

Moths have microvillar photoreceptors, containing visual pigments housed within the membranes of the microvilli. The arrangement of the visual pigments is crucial for the functioning of the photoreceptor: if the photoreceptor is predisposed to detect polarised light, the visual pigments need to be aligned with the e-vector angle in order to maximally absorb the light (Johnsen, 2012; Cronin et al., 2014). Therefore, an ordered alignment within one photoreceptor is ideal for detecting a specific e-vector angle, and to cover the whole 180° range of e-vectors, different photoreceptors should have differently oriented pigment alignments. Photoreceptors that are not specialised for detecting polarised light tend to have non-aligned visual pigments via a twisted rhabdom (Wehner and Bernard, 1993). Since polarisation vision interferes with colour vision, insects tend to spatially separate the two types of photoreceptors: in moths and other insects that need to detect skylight polarisation, polarisation-sensitive photoreceptors are located in the dorsal rim area (DRA) of the eye. This topology gives rise to the different processing pathways for polarised light and other visual information, such as visual landmarks and optic flow (Figure 8).

DRA photoreceptors detect the e-vector angle of polarised light and project to the dorsal rim area of the medulla, and in locusts also to the dorsal rim of the lamina, in the optic lobe. In particular, layer 4 of the medulla has been shown to process polarised light information (el Jundi et al., 2011), and several polarised light sensitive neurons were described to be associated with this layer. Among these, POL1 interneurons (first characterised in the field cricket and morphologically similar to locust MeMe neurons

(Labhart, 1988; el Jundi et al., 2011) are known to integrate e-vector responses from about one third of the entire DRA of the eye, which result in an overall response to the mean e-vector angle across the receptive field. In locusts, these neurons also respond to green and UV light spots, indicating that the e-vector is already integrated with the Sun position at this level (el Jundi et al., 2011). Transmedulla neurons then transmit this information to the next processing centre, the anterior optic tubercle (AOTU) (Homberg et al., 2003a; el Jundi et al., 2011; Zeller et al., 2015)

The anterior optic tubercle

In Bogong moths, the AOTU consists of three subunits: the upper, lower and nodular unit, of which the lower and nodular units are combined into the lower unit complex. While the lower unit was shown to be a part of the primary compass pathway in a variety of species, the upper unit of the AOTU has been functionally examined only in bees, where it is associated with colour vision (Mota et al., 2011). Functionally, little is known about the nodular unit, but it also has connections to the lateral complex (Heinze et al., 2013), thus likely constituting a parallel input pathway to the compass system.

Most neurons that process polarised light information show polarisation opponency, meaning that the neurons are maximally excited by one e-vector angle ϕ_{max} and maximally inhibited by the e-vector angle perpendicular to it, ϕ_{min}. This response pattern is seen already in POL1 neurons and indicates that the polarisation vision system may receive opposing input in early processing stages, e.g. from photoreceptors that respond to perpendicular e-vector orientations, in order to sharpen contrast and extract the predominant e-vector. Like MeMe neurons, cells in the AOTU also respond to green and UV light spots, meaning that the neurons encode both the solar azimuth and its associated e-vector angle (Pfeiffer and Homberg, 2007; el Jundi et al., 2011; Heinze and Reppert, 2011). Several AOTU neuron types have also been shown to be inhibited by weakly polarised light that would typically occur close to the Sun itself, thereby filtering out irrelevant e-vectors that might confound the compass (Pfeiffer et al., 2011).

Information from the AOTU is relayed to the lateral complex via TuLAL neurons. In the locust, two distinct cell types – TuLAL1a and TuLAL1b – receive input in the lower and nodular units of the AOTU (el Jundi and Homberg, 2012) and project onto TL2 and TL3 interneurons (termed ring neurons in *Drosophila* nomenclature) in the bulbs of the lateral complex, where their synapses form large microglomerular complexes (Pfeiffer et al., 2005; Träger et al., 2008; Held et al., 2016). In locusts, TuLAL neurons respond to the e-vector angle of polarised light, and there is evidence for additional responses to unpolarised light spots from Monarch butterflies (Heinze and Reppert, 2011; el Jundi and Homberg, 2012). TuLAL1a and TuLAL1b constitute two parallel streams of information. TuLAL1a neurons project onto TL2 neurons in the lateral bulb, while TuLAL1b neurons synapse onto TL3 neurons in the medial bulb

(Träger et al., 2008). These two pathways are functionally different in that the TuLAL1b—TL3 pathway transmits monocular information from the ipsilateral field of view, while the TuLAL1a—TL2 pathway integrates binocular information (Vitzthum et al., 2002; Heinze et al., 2009). In *Drosophila*, a homologous system of parallel neurons processes small and wide field visual information rather than polarised light and compass cues (Omoto et al., 2017). However, similar to in the locust, there are different types of TuLAL cells (called TuBu in *Drosophila*) that are functionally different: The receptive fields of TuBu$_s$ neurons are restricted to the ipsilateral hemifield, whereas TuBu$_i$ neurons have a receptive field that covers both hemispheres (Omoto et al., 2017). This likely corresponds to the monocular versus binocular pathways in locusts, but the exact homology is still unclear.

Aside from the primary input pathway for compass information, a second pathway for polarised light has been proposed in locusts. It also originates from the DRA of the medulla but then passes information via the accessory medulla to the posterior optic tubercle (POTU), and potentially to the central complex (el Jundi and Homberg, 2010). This pathway has been suggested to be more directly involved in time-compensating compass cues, as the accessory medulla is known to be the seat of the internal circadian clock in the cockroach (el Jundi and Homberg, 2010). However, whether this pathway actually processes polarised light is unknown, as it was described purely based on anatomical connections between neurons. Furthermore, whether and how this pathway is functionally connected to the central complex compass network is not clear.

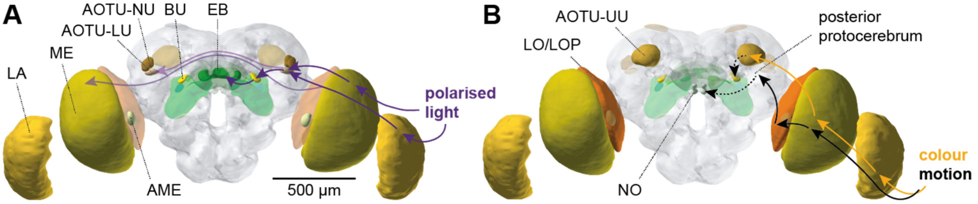

Figure 8: Input pathways for polarised light, colour and motion cues.
(A) Polarised light is processed in dorsal rim area of the lamina (LA) and medulla (ME) of the optic lobes and transferred to the lower and nodular units of the anterior optic tubercle (AOTU-LU and AOTU-NU, respectively). The signal is then fed into the ellipsoid body of the central complex (EB) via the bulbs (BU). A secondary pathways via the accessory medulla (AME) and the posterior optic tubercle (POTU) is not shown. **(B)** Motion information (black arrows) bypasses the LA and is first processed in the ME and subsequently in the lobula and lobula plate (LO and LOP, respectively). From there, the information is transferred to the posterior protocerebrum. The link between this area and the noduli of the central complex (NO) is unclear. Colour information (orange arrows) is processed in the lamina, medulla and then in the upper unit of the anterior optic tubercle (AOTU-UU). From there, colour and object information may be transferred to the BU via TuLAL neurons.

Visual landmarks and optic flow

Aside from compass cues, visual information that can be used for orientation and navigation includes visual landmarks and optic flow. Optic flow is the displacement of the visual image across the retina as an animal moves through the world (Koenderink, 1986), and its neural processing stages have been investigated in detail in flies (e.g. Krapp and Hengstenberg, 1996; Krapp et al., 2001; Geurten et al., 2012). How these cues are processed in the central brain has been studied predominantly in the fruit fly (*Drosophila melanogaster*; Weir and Dickinson, 2015) and the cockroach (*Blaberus* discoidalis; Kathman et al., 2014), but also in the locust (Rosner and Homberg, 2013) and bees (Paulk et al., 2009; Stone et al., 2017). Single-neuron physiologies and anatomies are available from intracellular recordings in the locust, and from two-photon calcium imaging experiments of single cells in the fruit fly. Results from the cockroach were obtained through extracellular multi-unit recordings and cannot be assigned to specific neurons.

Visual information other than polarised light is detected by the photoreceptors in the eye (outside the DRA) and transmitted to the visual neuropils in the optic lobe (Figure 8B), where wide-field motion cues are extracted and disentangled from other visual information. In particular, motion cues are extracted from visual information in the lamina and further processed in the lobula complex. There, lobula plate tangential cells show strong direction-dependent responses to either horizontal or vertical optic flow (Krapp and Hengstenberg, 1996; Krapp et al., 2001), and small target motion detector neurons extract information about small moving objects (O'Carroll, 1993; Nordström et al., 2006). Motion-sensitive neurons from the lobula complex project predominantly to optic glomeruli in the posterior protocerebrum, which are especially distinct in flies (Strausfeld and Okamura, 2007). Each of these optic glomeruli give rise to one or several descending neurons that provide a direct connection to thoracic motor centres, such that artificial activation of a glomerulus leads to a specific and stereotyped behavioural response (Wu et al., 2016). In bees, neurons processing colour information project to the anterior protocerebrum and the upper unit of the AOTU (Paulk et al., 2009). It is unclear how visual information is then passed on to the central complex. As discussed above, however, the likely *Drosophila* homologues of TuLAL1a and 1b neurons transmit information about small field targets rather than polarised light (Omoto et al., 2017). This finding indicates that the same neural substrate that underlies the compass in locusts and the Monarch butterfly may also be used for processing other visual information, depending on the visual ecology of the insect. In the nocturnal sweat bee *Megalopta genalis*, optic flow information was shown to be processed by neurons projecting from the lateral accessory lobes and the ventrolateral protocerebrum to the noduli of the central complex (Stone et al., 2017). Thus, the input to the central complex is already highly processed and separated into speed and direction components of large and small field motion cues.

Time compensation

Certain compass cues, such as the position of the Sun or the Milky Way, appear to move across the sky over the course of a day or night due to the Earth's rotation. Therefore, these cues need to be time-compensated in order to provide an external reference frame that remains consistent. It is well known that animals have internal clocks that regulate circadian activity patterns and are therefore suited to time-compensate the compass system. However, it is still unclear how the clocks are functionally connected to the compass and where in the system the time compensation occurs. In the following, I will briefly discuss two endogenous clocks that are potentially involved in time compensation.

One well-described clock resides in the accessory medulla of the optic lobes and was originally described in the cockroach (*Rhyparobia maderae*) (Homberg et al., 2003b). This clock has an endogenous rhythmicity that persists in darkness, and it is entrained by light input from the eye. One major characteristic of this clock is the circadian expression pattern of pigment-dispersing factor (PDF), a neuropeptide whose expression oscillates with a 24-hour periodicity (Homberg et al., 2003b). Neurons expressing PDF project from the anterior medulla and branch widely in the midbrain, but only a few neurons projecting to the superior median and lateral protocerebrum are thought to be predominantly involved in motor control (Reischig and Stengl, 2003). How these neurons interact with the compass system is currently unclear. However, one type of neuron, which connects the accessory medulla with the contralateral medulla, was shown to be polarisation sensitive and may therefore be a potential candidate for a clock-compass interface (Homberg and Würden, 1997; Loesel and Homberg, 2001). Furthermore, there is evidence for connections from the accessory medulla to the posterior optic tubercles (Petri et al., 1995; el Jundi and Homberg, 2010) and to layer 4 of the medulla, which specifically processes polarised light (Zeller et al., 2015). Thus, while the interface between the clock and the compass remain unknown, there are several possibilities where the two might be integrated.

A second clock exists in the antennae of the Monarch butterfly (Merlin et al., 2009). Like the brain clock, it has an endogenous rhythmicity that persists in darkness, and it is entrained by light. However, this clock likely gets light input not from the eyes, but from the light-sensitive cryptochrome CRY1, which is expressed in the antennae. Intriguingly, if the two antennal clocks are out of sync or ablated, Monarch butterflies can still keep a constant heading, but they do not follow their migratory direction anymore (Merlin et al., 2009). How information from the antennae is relayed to the compass system, however, remains unknown.

At this stage it is unclear whether the Bogong moth uses time-compensated compass cues, and if so, how this time compensation works in the moth. Assuming that celestial cues are time-compensated throughout the night, this leads to an interesting hypothesis. It is now known that the Bogong moth can use the geomagnetic field as a compass cue. As the geomagnetic field does not change substantially over the course of a day, this cue does not need to be time-compensated. However, one would still expect it to be processed in the heading direction network of the central complex. Thus, time-compensating the heading direction signal or central complex output seems unlikely, as this would introduce an error into any heading direction that is based on the geomagnetic field. This means that any compass cue that requires time compensation needs to be integrated with a clock signal upstream of the central complex. It therefore seems reasonable to assume that the clock signal is integrated either in the optic lobes or in the anterior optic tubercle. If and where this occurs remains to be investigated.

Compass networks

We have now seen how visual information is extracted from visual input to the eye, and how it is transferred to the central brain. There, one crucial brain area is the central complex, which has been shown to be critically involved in processing spatial information for orientation and navigation purposes. In the following, I will discuss what is known about the central complex networks that process visual compass cues, as well as optic flow and visual landmarks.

The central complex

The central complex consists of four subunits: the fan-shaped body (FB, or CBU), the ellipsoid body (EB, or CBL), the protocerebral bridge (PB) and the paired noduli (Figure 9A; Heinze and Homberg, 2008; but see Wolff and Rubin, 2018). Important input neurons to the central complex are TL2 and TL3 neurons (*Drosophila*: ring neurons), which transmit compass information such as polarised light and the solar azimuth, but likely also other visual cues such as landmarks, into the compass system (Heinze and Reppert, 2011; el Jundi and Homberg, 2012; Seelig and Jayaraman, 2013; Omoto et al., 2017; Fisher et al., 2019; Kim et al., 2019). The precise nature of the input is expected to depend on the environment and the lifestyle of the insect.

Processing within the central complex is intricate. However, in recent years enormous progress has been made in characterising specific circuits within this brain region, which is now understood to be the neural basis for the heading direction system, path integration, spatial memory and sleep, among other functions. In the context of this thesis, I want to focus on two circuits in particular: (1) the heading direction network, and (2) the path integration network. The two networks have been identified separately but likely work together to represent the insect's heading, movement direction and forward velocity, thus allowing the insect to remain oriented in space as it moves through its environment. Connections between neurons that give rise to these networks were inferred from their morphologies, such that a connection was assumed if the presynaptic branches of one neuron overlap with the dendrites of a downstream neuron. In some cases, these connections were later confirmed based on electron micrographs (Turner-Evans et al., 2019). It is important to note that these networks do not exist in isolation but are part of a complex array of neurons, most of which are not well characterised. These networks are therefore simplifications and should be taken as such.

The heading direction network

In order to understand its own position within the environment, any animal needs to know its current heading, and how this heading changes as the animal rotates to the left or right. Both idiothetic (internal) and allothetic (external) cues can be used to track one's heading. Idiothetic cues comprise mechanosensory and proprioceptive input from the insect's own body, providing information about e.g. its angular velocity. Indeed, central complex neurons respond to mechanosensory information from the antennae (locust: Homberg, 1994; cockroach: Ritzmann et al., 2008) as well as proprioceptive input from the legs (fruit fly: Green et al., 2017; Turner-Evans et al., 2017), which provide the animal with information about its own angular velocity even in the absence of visual cues. However, relying exclusively on idiothetic cues to estimate one's heading is prone to accumulating error and would therefore not provide a sustainable strategy to keep track of the heading direction over longer time spans (Cheung et al., 2007). Therefore, it is essential to integrate idiothetic angular velocity information with an external reference. Depending on the insect's environment and lifestyle, the external cues used can vary: *Drosophila* appears to largely rely on landmarks in its immediate environment (Green et al., 2017; Turner-Evans et al., 2017) but can also use celestial cues (Giraldo et al., 2018), while locusts, dung beetles and Monarch butterflies prefer to use celestial compass cues such as the Sun position and the polarisation pattern (Heinze and Reppert, 2011; Dacke et al., 2013b; el Jundi et al., 2014). In general, it can be expected that all available allothetic and idiothetic cues are integrated to provide an optimal heading estimate.

The idiothetic and allothetic cues described above feed into a simple neural circuit that computes the insect's heading. This circuit has the properties of a ring attractor network (Seelig and Jayaraman, 2015). A ring attractor can be theoretically modelled as a network of connected nodes that form a topological ring. Short-range excitation combined with long-range inhibition leads to the formation of a single "bump" of neural activity on this ring, the position of which represents an angular variable such as head direction. The activity bump is moved around the ring by asymmetric incoming activity (Kakaria and de Bivort, 2017). Neural correlates of ring attractor nodes can be found in the ellipsoid body. CL1 and CL2 cells (wedge/E-PG neurons and tile/P-EN neurons respectively in *Drosophila* nomenclature), which project on to each other in a loop, connect ellipsoid body slices to the corresponding protocerebral bridge slices.

Crucially, however, a CL2 cell which gets input from a CL1 cell in slice X of the protocerebral bridge does not connect to the same CL1 cell in the ellipsoid body, but to a neighbouring CL1 cell that projects to protocerebral bridge slice X+1 (Figure 9C; Green et al., 2017; Turner-Evans et al., 2017). This connectivity allows activity to be pushed around the ring depending on the fly's angular velocity. As both CL1 and CL2 cells give excitatory output onto each other, a source of inhibition has to be built into the system in order to avoid spreading of activity across slices, which would result in loss of the activity bump (Kim et al., 2017). The inhibition is likely realised through TL cells in the ellipsoid body and TB1 cells in the protocerebral bridge (Kakaria and de Bivort, 2017; Turner-Evans et al., 2017; Green and Maimon, 2018). Therefore, this network also explains the properties of TB1 neurons of the locust's protocerebral bridge. Here, each slice encodes an e-vector angle such that an ordered array of e-vector angles emerges (Figure 9B), across which the activity bump travels (Heinze and Homberg, 2007).

The circuit encodes heading based on the angular velocity of the fly's turns, a representation that can rely exclusively on internal input such as proprioceptive information from the legs, or self-generated cues such as optic flow (Green et al., 2017; Turner-Evans et al., 2017). Thus, the heading estimate can be maintained purely based on idiothetic information. However, it is calibrated by adding allothetic information, such as external landmarks in flies, and compass cues in locusts and bees (Homberg, 1994; Heinze et al., 2009; Rosner and Homberg, 2013; Seelig and Jayaraman, 2013, 2015; Stone et al., 2017; Giraldo et al., 2018). In the context of migration, it is crucial to have an accurate heading direction estimate and compare it to the intended migratory heading, to be able to compensate for discrepancies as soon as they occur. Considering that the network appears to be morphologically and functionally similar in fruit flies, locusts and butterflies, it is likely that this is a basic neural circuit that encodes heading direction in all insects, including the Bogong moth. In **Paper III**, I investigated whether this circuit also processes nocturnal compass cues.

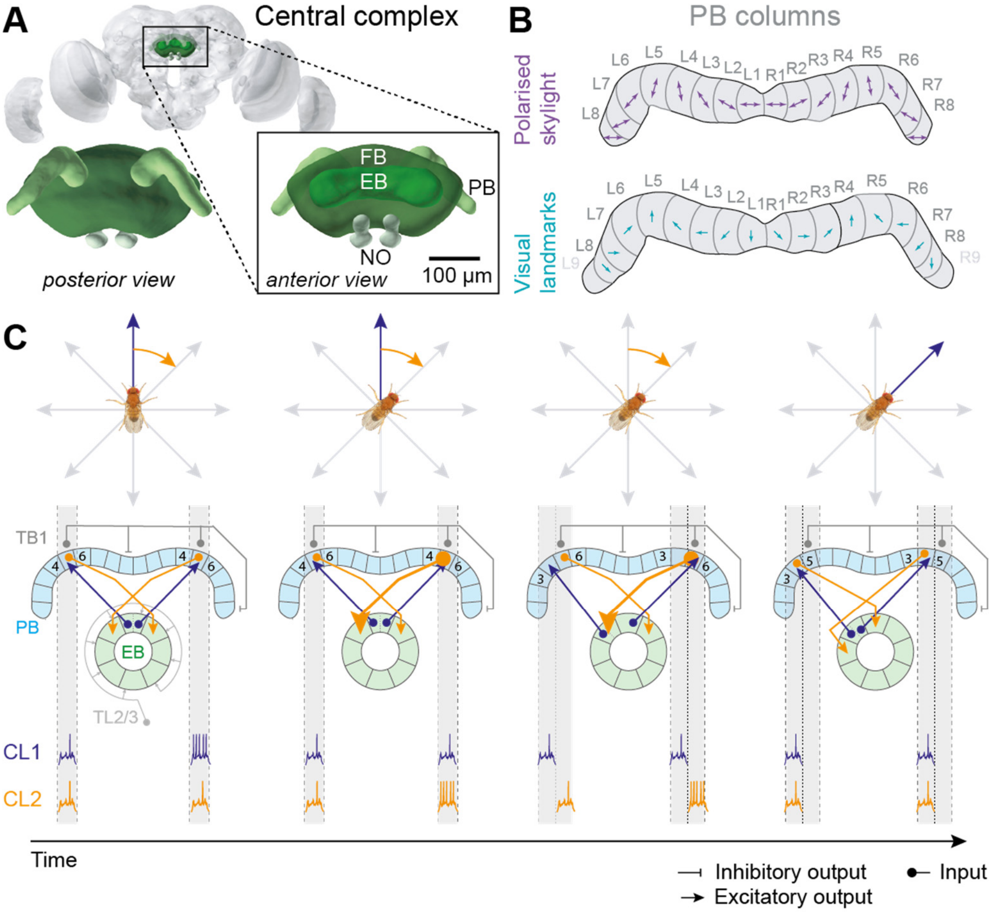

Figure 9: The heading direction network of the central complex.
(A) The central complex (CX) of the Bogong moth is located in the centre of the brain. It consists of the fan-shaped body (FB), the ellipsoid body (EB), the protocerebral bridge (PB) and the noduli (NO). Note that the PB is not fused in lepidoptera, unlike e.g. in locusts and flies. (B) Ordered representation of polarised skylight e-vectors (upper panel, as in desert locust) and visual landmarks (lower panel, as in the fruit fly) across the slices of the PB. (C) The heading direction network. CL1 neurons (dark blue) get input in the ellipsoid body (EB, green) and project to the protocerebral bridge (PB, blue), where they provide input to CL2 neurons (orange). CL2 neurons project from the PB to neighbouring CL1 neurons in the EB. When the insect rotates to the right, the right CL1 neuron gets relatively more input than the left CL1 neuron. It activates its postsynaptic CL2 cell in the PB, which pushes the activity bump in the EB to the neighbouring slice. As each slice covers 45° of the space around the animal, a 45° right turn results in a shift to the left by one EB slice. The activity bump in the EB and in both hemispheres of the PB is stabilised by TB1 neurons (dark grey), which get input from CL1 cells and inhibit PB slices that encode the opposite headings. While this circuit works with purely idiothetic information, TL2 and TL3 neurons (light grey) provide additional allothetic information, such as compass cues. Figure modified from Green et al., 2017.

The path integration network

Rather than rotating on the spot, insects move through their environment. Therefore, updating their heading direction is not sufficient, as the direction and velocity of their movement need to be taken into account when estimating their current position. A recent model of the central complex demonstrates how this can be accomplished (Stone et al., 2017). The model uses output from the heading direction network described above and combines it with velocity information obtained from optic flow. Taken together, the two types of information result in a vector that points to the origin of the outbound path. Like the heading direction network, this model is anatomically constrained, and it combines data from the locust and the nocturnal sweat bee (*Megalopta genalis*). The model was originally developed to explain path integration, a navigation strategy that allows ants and bees to return to their nest in a straight line after foraging (Figure 10A; Srinivasan, 2015). However, in theory it can be easily adapted to function during migration (Honkanen et al., 2019).

The path integration network comprises three main processing units that perform distinct tasks: (1) the heading direction network, which is located in the protocerebral bridge, (2) the speed sensor (odometer), which is located in the noduli, and (3) the memory, which combines the heading estimate with the speed estimate and is located in the fan-shaped body (Figure 10B). The heading direction network has been discussed in detail and for the purpose of this model, only the TB1 neurons are relevant. As mentioned above, the tuning of TB1 neurons to external cues results in an ordered array of heading direction cells, which span the width of the protocerebral bridge (Figure 9B; Heinze and Homberg, 2007). TB1 cells are assumed to form inhibitory synaptic connections with each other, such that the neuron with the highest activity inhibits TB1 neurons in slices furthest away from it, which stabilises and sharpens the activity bump. Thus, the TB1 neurons in the protocerebral bridge represent the insect's current heading. In the noduli, another set of cells, called TN, processes translational optic flow (Stone et al., 2017). Optic flow is the displacement of the visual image across the retina as an animal moves, and the animal can infer its own movement velocity from the velocity of the optic flow. Thus, TN neurons are the speed sensors of the path integrator Finally, CPU4 cells of the fan-shaped body complete the network. They integrate and store the current information from both the heading direction network and the odometer by forming a recurrent loop between the noduli and the protocerebral bridge. As each slice in the protocerebral bridge is tuned to one particular heading, all CPU4 neurons in that slice can also be associated with that heading. Since TB1 neurons are inhibitory, the location of the activity bump in the protocerebral bridge is also the origin of highest postsynaptic inhibition. Thus, the CPU4 neurons that are associated with the current heading are maximally inhibited, while those associated with the opposite heading are least inhibited. Simultaneously, all CPU4 neurons get speed input from TN cells in the noduli, but only the CPU4 neurons that are least inhibited can then accumulate speed information. Thus, the memory accumulates in the opposite direction to the current heading of the animal, resulting in a home vector that encodes both the distance and direction to the origin of the path (Figure 10B, C; Stone et al., 2017). Note that the home vector is encoded by the population of CPU4 neurons across slices in this model. The home vector can be generalised to be a desired heading, and during navigation it can be compared to the current heading (represented in the heading direction network) to produce compensatory steering.

How is CPU4 activity translated into a steering signal? Stone et al. (2017) propose that CPU1 neurons integrate the CPU4 memory signal. CPU1 neurons have input branches in the protocerebral bridge and the fan-shaped body. Importantly, CPU4 output branches in the fan-shaped body are offset by one slice as compared to the protocerebral bridge. Thus, each CPU1 neuron integrates the current heading from protocerebral bridge slice X with the activity of CPU4 cells in slice X+1 of the fan-shaped body. This offset produces a shift to the left in right hemisphere CPU1 neurons, and a shift to the right in left hemisphere CPU1 neurons, essentially assessing whether a left turn or a right turn is necessary to compensate for the difference between current heading and intended heading. Across the population of CPU1 neurons, this results in an imbalance between activity in the right versus the left hemisphere. If the activity in the right hemisphere is higher than in the left, the animal needs to turn right to compensate for the heading mismatch (Stone et al., 2017). CPU1 neurons project to the lateral accessory lobes, where the steering command is thought to be transmitted to pre-motor neurons (see *Compass output*).

During migration, the Bogong moth has to follow its desired heading over thousands of kilometres. In theory, this could be achieved by simply fixing the weights of the CPU4 neurons onto the CPU1 cells in a sinusoidal manner, such that the CPU4 output will be consistent over time and across the entire population of cells (Honkanen et al., 2019). The CPU1 neurons would then compare the ever-changing current heading to a fixed desired heading and compute a steering decision accordingly.

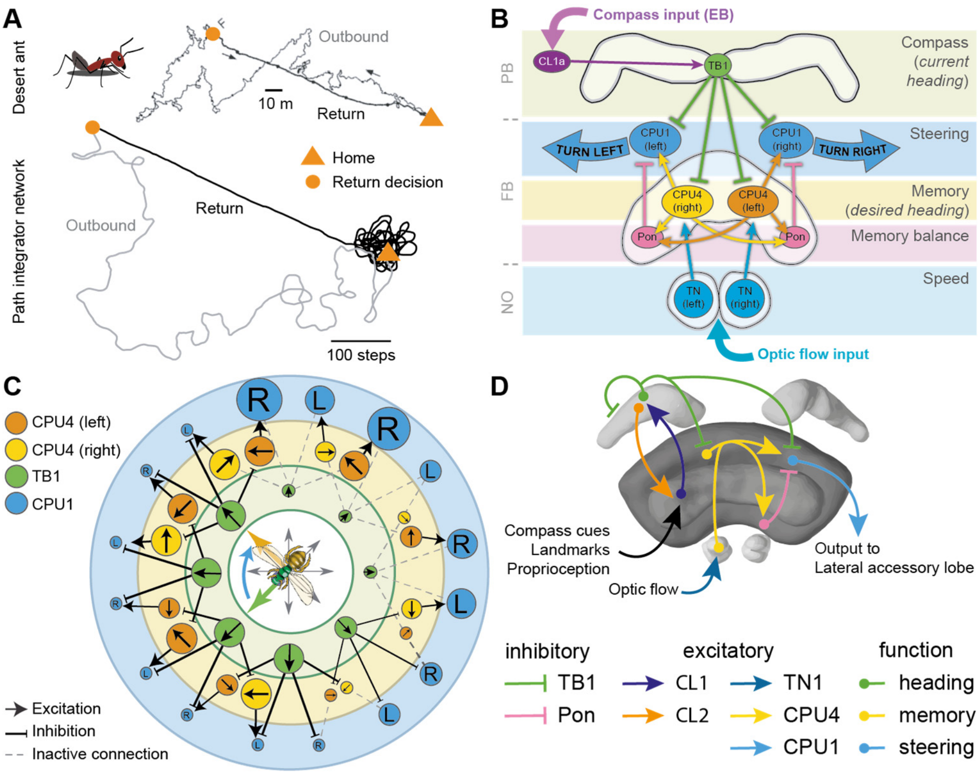

Figure 10: The path integrator network of the central complex.
(A) Path integration is a computation that accumulates the direction and walking speed of an animal, e.g. a desert ant, and converts it into a vector whose direction encodes the direction of the path's origin, and whose magnitude encodes the distance to the origin. Using the vector, the animal can return home in a straight line without having to follow the convoluted outbound route back to the nest. A model of the path integrator network shows the same behaviour. (B) The anatomically constrained neural network that underlies path integration. (C) A schematic diagram of all connections (except pontine cells, Pon) illustrating how the CPU4 memory network accumulates the vector as a population code across CPU4 neurons (orange and yellow). The current heading is encoded across the population of TB1 cells (green). (D) Overview over known central complex networks. The heading direction network gets compass, landmark and proprioceptive input in the ellipsoid body (EB) and forms a recurrent loop between the EB and the protocerebral bridge (PB). TB1 neurons in the PB stabilise the heading direction ring attractor. The path integrator network receives heading direction information via TB1 neurons, as well as optic flow input via TN1 neurons in the noduli. Both types of information are integrated and result in a home vector in the CPU4–Pon memory loop. CPU1 neurons compare the current heading (TB1) with the intended heading (CPU4), resulting in a motor command to adjust the insect's direction of movement. Panels (A-C) adapted from Honkanen et al., 2019.

Selecting appropriate behavioural strategies

In addition to estimating its own position and orientation in the environment and making steering decisions, the insect needs to make other behavioural decisions based on sensory information, such as how to interact with objects in its environment. Interestingly, ellipsoid body neurons integrate antennal movements, among other cues (Homberg, 1994). Cockroach central complex neurons have also been shown to process mechanosensory information from the antennae, which are involved in examining an object before selecting a behavioural strategy to deal with that object (Ritzmann et al., 2008). As one example, an antenna touching a wall elicits a turn away from that wall. However, cockroaches with lesions to the central complex (PB, FB and EB) failed to turn upon antennal contact and instead walked headlong into the wall (Harley and Ritzmann, 2010). It is worth mentioning that this abnormal behaviour also occurred in animals with lesions in the mushroom body and lateral accessory lobes (Harley and Ritzmann, 2010). This suggests that, while the central complex integrates the sensory information from the antennae to provide the spatial context for the turn, the behaviour itself is initiated downstream from the central complex. Interestingly, the central complex has also been suggested to be a pre-motor site. In a recent landmark study, a set of central complex neurons was found to predict changes in the forward and rotational velocity of freely walking cockroaches (Martin et al., 2015). Furthermore, stimulating the central complex altered thoracic reflexes such that the observed motor activity was consistent with turning. This shows that the processing networks of the central complex have a clear and direct impact on motor activity.

It should be noted that not only sensory information is processed in the central complex. Several studies showed that moving stripes and optic flow modulate the response of certain central complex neurons during flight but not during walking or resting, suggesting that activity in the central complex is modulated by the behavioural state of the fly (Bausenwein et al., 1994; Weir et al., 2014; Weir and Dickinson, 2015). The same was shown by extracellular recordings in tethered walking cockroaches (Bender et al., 2010). Furthermore, a subset of fan-shaped body neurons promote sleep in fruit flies, and suppress visually guided locomotion (Liu et al., 2016; Donlea et al., 2018). Finally, the hunger state of the fly also feeds into the ellipsoid body, such that starvation led to enhanced excitability of certain ring neurons, promoting increased locomotion and feeding (Park et al., 2016). Taken together, these findings show that all activity in the central complex is modulated by the behavioural and motivational state of the insect.

Compass output and motor control

The lateral accessory lobes

While the central complex processes spatial information and provides an internal representation of the animal's position in space, the lateral accessory lobes (LAL) have been implicated in issuing motor commands. The LALs are paired neuropils that are part of the lateral complex and lie immediately adjacent to the central complex (Figure 7). They are a major target for central complex output cells, including CPU1 neurons that provide output from the path integration network (Heinze and Homberg, 2009; Stone et al., 2017; Franconville et al., 2018). The LALs are therefore likely to be a major processing site of central complex output, and have been implicated in motor control. Harley et al. (2010) showed that cockroaches with lesions in the LAL had impaired turning and climbing behaviour, while animals with lesions in the central complex climbed normally (Harley et al., 2009). An additional link to motor control was found in the silkworm moth *Bombyx mori*. In order to find a female silkworm moth for mating, the male silkworm moth follows the pheromone plume of the female by zig-zagging through it. The underlying neural circuit has been described in detail and was found to include an unusual neuron type called a flip-flop neuron, which mediates this zig-zagging behaviour (Olberg, 1983; Kanzaki et al., 1994; Kanzaki and Mishima, 1996; Mishima and Kanzaki, 1999; Iwano et al., 2010). This neuron "flip-flops" between a highly active and a largely inactive state, and its flip-flopping activity correlates with neck motor neuron activity, which initiates a head turn that precedes the body turn (Kanzaki et al., 1994). The flip-flop neurons, as well as several other interneurons that are part of the flip-flop network, receive input in the LAL (Kanzaki et al., 1994; Namiki et al., 2014). While this network is best known for its role in odour-following behaviours, an early study showed that flip-flopping activity could also be elicited by light pulses (Olberg, 1983). The visual input to the lateral accessory lobes comes from the anterior optic tubercle (Pfeiffer et al., 2005; Pfeiffer and Kinoshita, 2012) and the lobula of the optic lobes (Namiki et al., 2014), as well as from the central complex. But can central complex output, such as steering commands computed by the path integrator, theoretically drive flip-flop neurons for efficient steering? I investigated this question in **Paper IV**.

6 The Bogong moth brain: the anatomical substrate of migration

There are many methods and tools that allow us to study the function of proteins, cells, neural networks and ultimately the brain, and they become all the more powerful if we combine them. However, in order to combine them in a meaningful way, we need an appropriate framework. In **Paper I**, I provide the anatomical framework in which all functional studies can be embedded and linked. Anchoring functional studies within the anatomical layout of the brain allows us to focus on specific brain areas that are crucial for navigation, for example the central complex, and investigate it in detail using a variety of methods.

Paper I provides an overview over all neuropils that can be found in the Bogong moth brain, both in males and females. To account for inter-individual variation in brain shape and size, as well as in neuropil shape and size, we produced a male standard brain. It is computed as the average of 10 individual brains and is thus a good representation of a 'typical' male Bogong moth brain.

Based on anti-synapsin wholemount stainings, 12 Bogong moth brains (10 male, two female) were reconstructed to obtain 3-dimensional models of all major neuropils. This allowed me to directly compare the neuropil volumes between males and females, and between individuals. I found only one striking, although expected, sexual dimorphism: the macroglomerular complex of the antennal lobes. Within the lepidoptera, the male macroglomerular complex is common. It consists of three enlarged olfactory glomeruli that specifically detect female pheromones. In all other respects, the Bogong moth brain follows the typical layout of lepidopteran brains, which differ from other insects in two main points: (1) the protocerebral bridge of the central complex is not fused (Honkanen et al., 2019), and (2) the Y-lobe of the mushroom body does not exist in other insects (Heinze and Reppert, 2012; Immonen et al., 2017; von Hadeln et al., 2018). These appear to be the only lepidoptera-specific hallmarks of brain morphology.

So far, within the lepidoptera, the only other brain that has been described in comparable detail is that of the Monarch butterfly. The Bogong moth and the Monarch butterfly share not only the overall layout of the brain, but also the fact that they both migrate over enormous distances, albeit on different continents and during different times of day. When comparing the brains of the two species, one difference becomes obvious: The optic lobes of the Bogong moth are less than half the size in relative volume than those of the Monarch butterfly (Figure 11), a difference that can be attributed to the two species' activity period rather than their migratory behaviour.

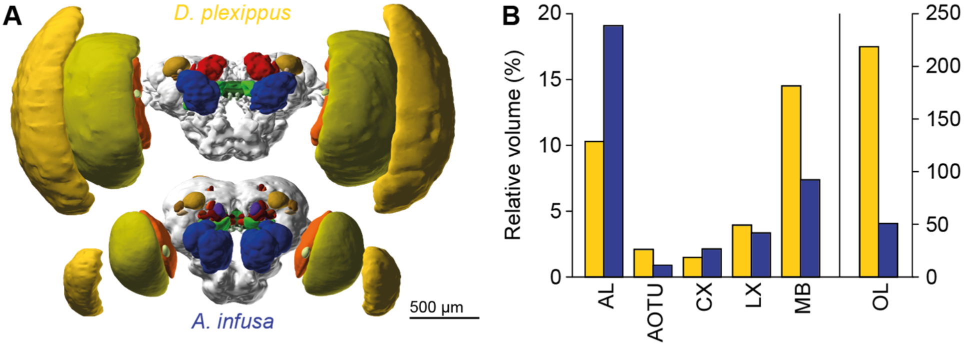

Figure 11: Comparison of Bogong moth and Monarch butterfly brain.
A: 3D reconstructions of brains of a male Monarch butterfly (*D. plexippus*) and a male Bogong moth (*A. infusa*). Scale bar = 500 µm. B: Relative volume comparison. The relative volume of a neuropil was obtained by normalising its absolute volume to the total volume of the central brain (minus the antennal lobe). Note the different y-axis for the optic lobe (OL). AL = antennal lobe, AOTU = anterior optic tubercle, CX = central complex, LX = lateral complex, MB = mushroom body.

When it comes to navigation, the brain region that is of special interest is the central complex. The Bogong moth central complex consists of four neuropils: The fan-shaped body, the ellipsoid body, the protocerebral bridge and the paired noduli. Based on anti-synapsin and anti-serotonin (5-HT) stainings, seven layers were identified in the fan-shaped body. The ellipsoid body has three identifiable layers, in addition to nine clearly visible slices (see Figure 7 in Paper I). The noduli consist of four subunits. The slices of the protocerebral bridge are less clear but can be identified with neurobiotin injections into single neurons. Taken together, these findings show that the Bogong moth central complex is a typical insect central complex as compared to, for example, the locust and the fruit fly (Honkanen et al., 2019).

Based on the detailed brain atlas of the Bogong moth presented in Paper I, it is now possible to specifically target the central complex with electrophysiological, immunohistochemical and molecular methods. However, the central complex is not the only brain area that is of interest with respect to navigation and migration. Other neuropils that are likely to process compass information are the optic lobes, the anterior optic tubercles and potentially the lateral accessory lobes. Knowing their relative locations within the brain simplifies targeting these neuropils with sharp electrodes as well as tetrodes. In the future, these methods will likely be instrumental in investigating the processing pathways of not only visual compass cues, but also of the magnetic sense.

We know that Bogong moths use the geomagnetic field in combination with landmarks to select a flight direction (Dreyer et al., 2018), but the sensory and neural basis of this ability are unknown. Magnetoreception in birds was proposed to be a visual mechanism, which is based on the light-sensitive photopigment cryptochrome. The hypothesis is that cryptochromes undergo a conformation change upon absorbing a photon, and together with the bound co-factor FAD form a radical pair. The nature of this radical pair depends on its orientation within the magnetic field, providing a potential magnetosensor. Like the Monarch butterfly, the Bogong moth has two cryptochrome genes, a vertebrate-like and an insect-like cryptochrome, giving rise to the possibility that the magnetic field may be sensed by light-sensitive cryptochromes in these migrating insects. If this is the case, neurons in the primary visual neuropils the optic lobes, the anterior optic tubercles and the ocellar neuropils may respond to changing magnetic fields. Additionally, in Monarch butterflies, the antennae were found to be necessary for orienting successfully in magnetic fields (Guerra et al., 2014), suggesting that neurons in the antennal lobes may also process magnetic field information. Independent of where the sensor is located, magnetic field information will eventually be integrated in the compass system and influence motor decisions, which leaves the central complex and the pre-motor neurons descending from the lateral accessory lobes and the posterior slope as prime targets for exploring this elusive sense.

In conclusion, the data presented in Paper I provides many starting points for future studies, as well as opportunities to embed existing data in this anatomical framework. However, this study does not reveal the possible existence of neuroanatomical specialisations that may underlie a migratory lifestyle. Is this lifestyle represented in the gross anatomical layout of the brain, or does it manifest on the level neural circuits? I investigated this question in Paper II.

7 A broader perspective on migratory and non-migratory brains

Neurons consume energy even at rest, as the membrane resting potential needs to be maintained. Thus, neural tissue is energetically expensive. However, adding more neurons increases the processing power of a brain, allowing it to deal more efficiently with external stimulation and respond with appropriate behaviours. The two opposing demands lead to an interesting trade-off between energy cost and adaptive benefit. It follows that brain areas which provide a benefit to the fitness of the animal should be invested in and become bigger, while brain areas that are less important can be afforded to decrease in size. This effect has been shown especially for primary sensory areas, such as the optic lobes and antennal lobes of insects. For example, diurnal moths tend to use primarily vision to find food and conspecifics, while nocturnal moths rely more on olfaction. In line with these behavioural differences, diurnal hawkmoths were found to have relatively larger optic lobes compared to their nocturnal relatives, while nocturnal hawkmoths had enlarged antennal lobes (Stöckl et al., 2016). Similarly, olfactory-driven species within the genus *Drosophila* were shown to have larger antennae and antennal lobes, while the more visual species had larger eyes and optic lobes (Keesey et al., 2019). This result was reflected in the behaviour of the flies, such that the 'visual' species had poor mating success in darkness and relied on colour in addition to odour cues when selecting a food source.

While these experiments showed that behavioural traits are reflected in the relative volumes of peripheral neuropils, it remained unclear whether the same is true for higher processing centres like the central complex. In **Paper II**, I therefore examined the volumes of all defined neuropils across a variety of moth species, with particular focus on two behavioural traits: circadian activity period and migratory behaviour. The species included in this study comprise two microlepidoptera (*Eriocrania semipurpurella* and *Lampronia capitella*), two butterflies of the family Nymphalidae (*Bicyclus anynana* and *Danaus plexippus*), three hawkmoths (Sphingidae: *Smerinthus ocellata*, *Macroglossum stellatarum* and *Deilephila elpenor*) and three noctuids (Noctuidae: *Mythimna pallens*, *Noctua pronuba* and *Agrotis infusa*), as well as the outgroup species *Rhyacophila nubila*, which belongs to the trichoptera, the sister group to the lepidoptera.

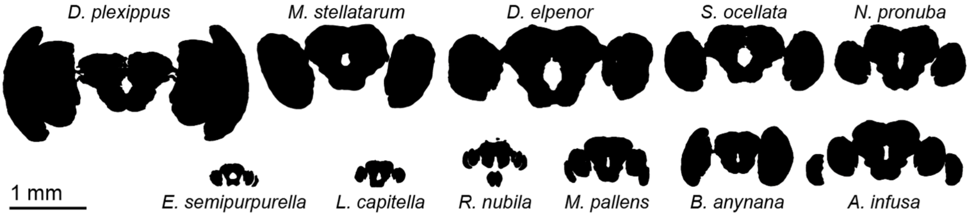

Figure 12: The brains of different lepidopteran species vary in size but not in shape. The brain of the Monarch butterfly (*D. plexippus*) is approximately 54 times larger than the brain of *E. semipurpurella*. However, the shapes of all brains are very similar across species. The brain of *R. nubila* (Trichoptera) is notably different, having a unfused gnathal ganglion.

Analogous to paper I, I stained moth brains against synapsin in order to produce detailed 3D reconstructions of the brains. I then extracted the absolute volumes of each neuropil and calculated the mean volumes per neuropil per species. In order to avoid distortions introduced by the big, peripheral brain areas that are known to vary strongly with activity period, I compared each neuropil to the combined volume of the central brain, that is, all central brain neuropils (excluding the antennal lobes) plus the unstructured protocerebrum.

While the brains of the 11 species varied strongly in size (Figure 12), they were very similar in shape and neuropil arrangement. I found that the central and lateral complex tend to be relatively smaller in larger species, while the mushroom bodies and sensory neuropils tended be relatively bigger. Inter-species variability was large in most neuropils, particularly in the primary sensory neuropils, as well as in the mushroom body. However, the compass neuropils – the central and lateral complexes, and the lower unit complex of the AOTU – showed low inter-species variability, which was comparable to the intraspecific variability in the Bogong moth, possibly reflecting the fundamental importance of these brain regions independent of lifestyle. When comparing neuropil volumes across species and correlating them to their activity period, I found differences between diurnal and nocturnal species that were consistent with previously reported results from hawkmoths (Stöckl et al., 2016). Interestingly, I also found some volume changes that may be linked to a migratory lifestyle. However, these changes were less clear and were only observed on the level of single neuropils, not when considering superregions. It became clear that changes linked to the sensory environment were reflected in the volumes of primary sensory processing centres, for example the optic lobes and antennal lobes, but also the upper unit of the anterior optic tubercle. In contrast, only neuropils that belong to higher integration centres, and more specifically to the compass pathway, could be linked to a migratory lifestyle.

At this point, the small number of species does not allow me to draw strong conclusions, as adding species may change the regressions considerably. To balance the dataset, it would be prudent to include additional big non-migratory species, as well as small migrants. In particular, the nocturnal migrant *Autographa gamma* (Noctuidae) is an interesting candidate to include, as it is one of the best-studied European migrants, but is substantially smaller than *A infusa* and *N. pronuba*. Since the two biggest species in my dataset are both migratory (*D. plexippus* and *M. stellatarum*), another useful addition is the big but non-migratory hawkmoth *Manduca sexta*. To allow for inter-family comparisons, it would also be helpful to add a non-migratory noctuid species. Finally, changes linked to a migratory lifestyle are yet to be disentangled from the effects of phylogenetic distance between the species, which may have a confounding effect on the results presented here.

Overall, the results presented in this manuscript show that changes related to a migratory lifestyle are more likely to be found on the level of neural networks, for example in changing synaptic connections between neurons, than in the gross anatomical layout of the brain. However, the approach presented here also shows that there is merit in comparing the overall neuroanatomy of brain regions, and volume changes can open new questions and provide the basis for future research.

8 Following the stars

We have now seen that the Bogong moth brain, as well as its central complex, is anatomically similar to that of other migratory and non-migratory insects, and there are few volumetric changes that correlate with a migratory lifestyle. However, changes may be found on the level of neural circuits and individual neurons. In **Paper III**, I targeted neurons in the central brain to reveal how they process one compass cue that is particularly relevant during nocturnal migration: the starry sky.

I used intracellular sharp-electrode electrophysiology to record from neurons in the central brain *in vivo* while stimulating the moth with the seasonally appropriate Australian night sky. An image of the sky was projected from above onto a plexiglass plate with a diffuser, which was situated 30 cm above the moth's head, filling approximately 100° of the moth's dorsal field of view (Figure 13A). The sky image could be rotated clockwise or counter-clockwise. As a control stimulus, the original sky image was broken up into blocks of 5x5 pixels, which were then randomised (Figure 13B). This stimulus thus provided a motion cue without being directional. After a successful recording, I injected the neuron with neurobiotin for morphological identification.

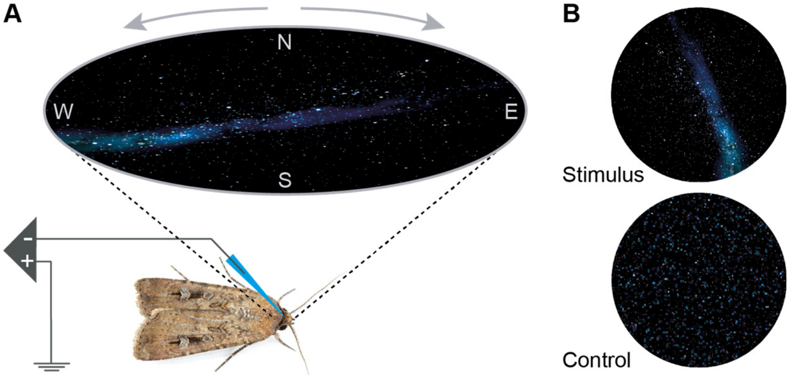

Figure 13: Electrophysiology setup and stimuli.
A: Schematic representation of the electrophysiology setup. The moth's head, thorax and wing joings are immobilised, and a small window is cut into the head capsule through which the recording electrode is inserted into the brain. A coated silver wire, inserted into the proboscis muscle, served as reference electrode.The starry sky stimulus is presented dorsally by projecting the stimulus or control image (B) onto a plexiglas plate situated above the moth. The stimulus can be rotated clockwise or counterclockwise and subtends approximately 100° of the moth's dorsal visual field. B: Starry sky stimulus and control. To create the control stimulus, the starry sky stimulus was subdivided into blocks of 5x5 pixels, which were then randomised.

Using this method, I was able to record responses to the night sky from 20 different neurons. Broadly speaking, these could be categorised into three subgroups: (1) unimodal excited (15 neurons), (2) unimodal inhibited (2 neurons), and (3) bimodal excited (3 neurons). While it was not always possible to anatomically identify the cells, those that I could identify unambiguously all belonged to group 1 and were either bilateral optic lobe cells or associated with the central complex (Figure 4, Paper III). Interestingly, the signal-to-noise ratio decreased from the optic lobes to the central complex, reflecting the different roles of those cell types (Figure 3, Paper III). In the optic lobes, neurons transmit almost exclusively visual information and can therefore afford to use their entire bandwidth for only one stimulus type. Central complex neurons process several types of information, including visual stimuli but also motivational state and proprioceptive feedback. Thus, it is likely that a central complex cell will not use its entire bandwidth for only one of these stimuli, an interpretation that is supported by my data.

Among the neurons that responded unimodally to the starry sky stimulus, three were associated with the CX: two CX input neurons (TU and PC-EG) and one CX output neuron (CPU2; Figure 14B-D). Of these cells, CPU2 cells are the best described physiologically and have previously been implied to play a role in the compass pathway. These neurons get input from the protocerebral bridge and the fan-shaped body, and project to both the ipsilateral and contralateral upper LAL, and in locusts cells of this type were characterised as being conditionally polarisation sensitive (Heinze and Homberg, 2009). Like in locusts, this cell appeared to have a high-activity and a low-activity state, indicated by a small but noticeable drop in the baseline as well as a small decrease in background activity (Bockhorst and Homberg, 2015). However, the response amplitude did not differ between the two states.

The two identified CX input neurons provide input to two different subregions. TU has output branches in the second layer of the fan-shaped body, and gets input in a small region in the superior lateral protocerebrum (Figure 14B-D). Previously described TU neurons of the locust brain also have wide ramifications in the lower LAL (Homberg et al., 1999, Heinze et al., 2013) which were not visible in the Bogong moth TU neuron presented here. The cell had a weak but distinct response to the starry sky, with a signal-to-noise ratio of approximately 10 imp/sec (Figure 15). It reached its activity peak when the moth was facing towards the North-West.

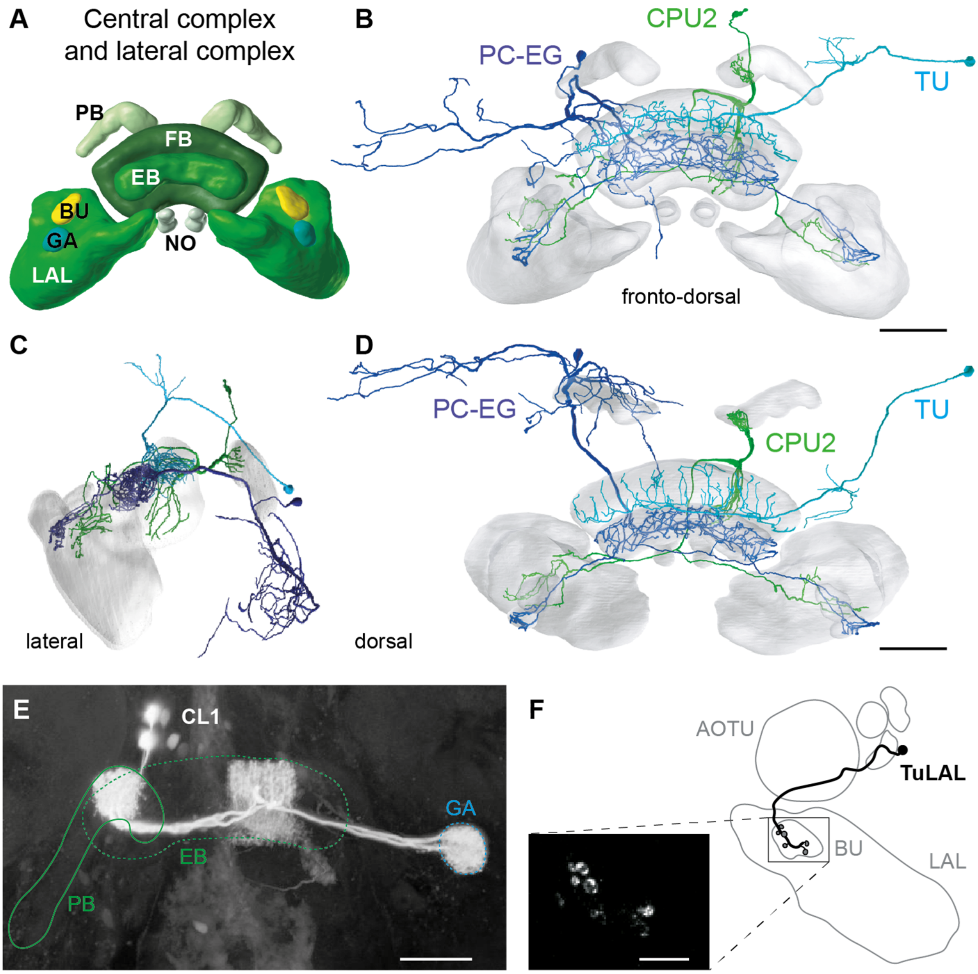

Figure 14: Morphologies of CX-associated neurons that respond to the starry sky.
A: Schematic view of the central and lateral complexes. The central complex (CX) consists of the fan-shaped body (FB), the ellipsoid body (EB), the protocerebral bridge (PB) and the paired noduli (NO). The lateral complex (LX) comprises the ipsi- and contralateral lateral accessory lobe (LAL), the bulb (BU) and the gall (GA). B-D: 3D reconstructions of TU (turquoise), CPU2 (green) and PC-EG (dark blue) neurons were registered into the Bogong moth standard brain and are shown fronto-dorsally (B), laterally (C) and dorsally (D). TU has sparse input branches in the superior lateral protocerebrum and projects to the second layer of the FB across its entire width. CPU2 gets input in one slice of the PB and one slice of the FB, and projects to the dorsal LAL. PC-EG has wide input branches in the posterior protocerebrum and provides output to the entire EB as well as the ipsi- and contralateral GA. The input and output branches of the three neurons do not overlap, except in the FB, where TU pre-synaptic branches and CPU2 dendritic arborisations may interact. Scale = 100 µm. E: Maximum projection of two neurobiotin-labelled CL1 neurons, with smooth (dendritic) branches in the GA and EB, and varicose (pre-synaptic) branches in neighbouring slices of the PB. Scale bar = 50 µm. F: Drawing of a neurobiotin-labelled TuLAL neuron. No smooth branches were visible in the AOTU. Large microglomerular pre-synapses are present in the BU (inset). Scale bar = 20 µm.

The PC-EG neuron has dendritic branches in a wide area of the posterior protocerebrum, and has pre-synaptic branches in the entire ellipsoid body, as well as both the ipsi- and contralateral bulbs of the lateral complex (14B-D). To my knowledge, this neuron type has not been described before, although neurons of a similar morphology have been found in locusts (von Hadeln et al., 2019). The cell responded very weakly to the starry sky, with a low signal-to-noise ratio and a very wide response angle of over 180° (Figure 15). It was maximally active when the moth was facing West.

Two other neurons yielded a clearly identifiable, but unexpected morphology. One was a TuLAL neuron (Figure 14F), which is a well-known part of the compass pathway in locusts and Monarch butterflies (Heinze and Reppert, 2011; el Jundi and Homberg, 2012). The other cell was a CL1 neuron (Figure 14E), which is an integral part of the CX heading direction network (Green et al., 2017; Turner-Evans et al., 2017). Surprisingly, neither neuron responded to the starry sky stimulus, or to any other directional cue that was presented during the experiment, and the distribution of spikes during each trial did not differ significantly from uniformity. Considering that the Bogong moth uses the starry sky as a directional cue, it is expected to be processed in the central complex heading network and therefore by CL1 neurons, among others. Skylight information would also be expected to be transmitted to the central complex via TuLAL cells, as is the case for compass and other visual information (Heinze and Reppert, 2011; el Jundi and Homberg, 2012; Omoto et al., 2017). Currently, both the CL1 and the TuLAL cells have only been recorded once in the Bogong moth, thus more recordings from these cell types are required to draw well-founded conclusions about their role in starry sky processing. However, if these results can be replicated, there are two possible explanations. The first possibility is that the compass and heading direction network may be strongly state-dependent. While the activity bump in the heading direction network persists in darkness and during periods of inactivity (Seelig and Jayaraman, 2015), it is unknown for how long a heading signal can be maintained in the absence of both external input and self-motion, and during prolonged periods of inactivity such as sleep. During an intracellular recording, the moth is restrained, making it difficult to judge its behavioural state and whether it is active at all. Thus, it is possible that the moth is simply in an inactive behavioural state, which may sufficiently diminish the responses of compass neurons. However, these data raise a second intriguing possibility: Starry sky information may not be transmitted to the central complex via the compass input pathway described above, but rather via a different and, so far, undescribed pathway. This potential pathway may involve cells such as TU1 and PC-EG, which project to the CX from areas of the unstructured protocerebrum that also receive visual input, among others (Hausen, 1982; Paulk and Gronenberg, 2008).

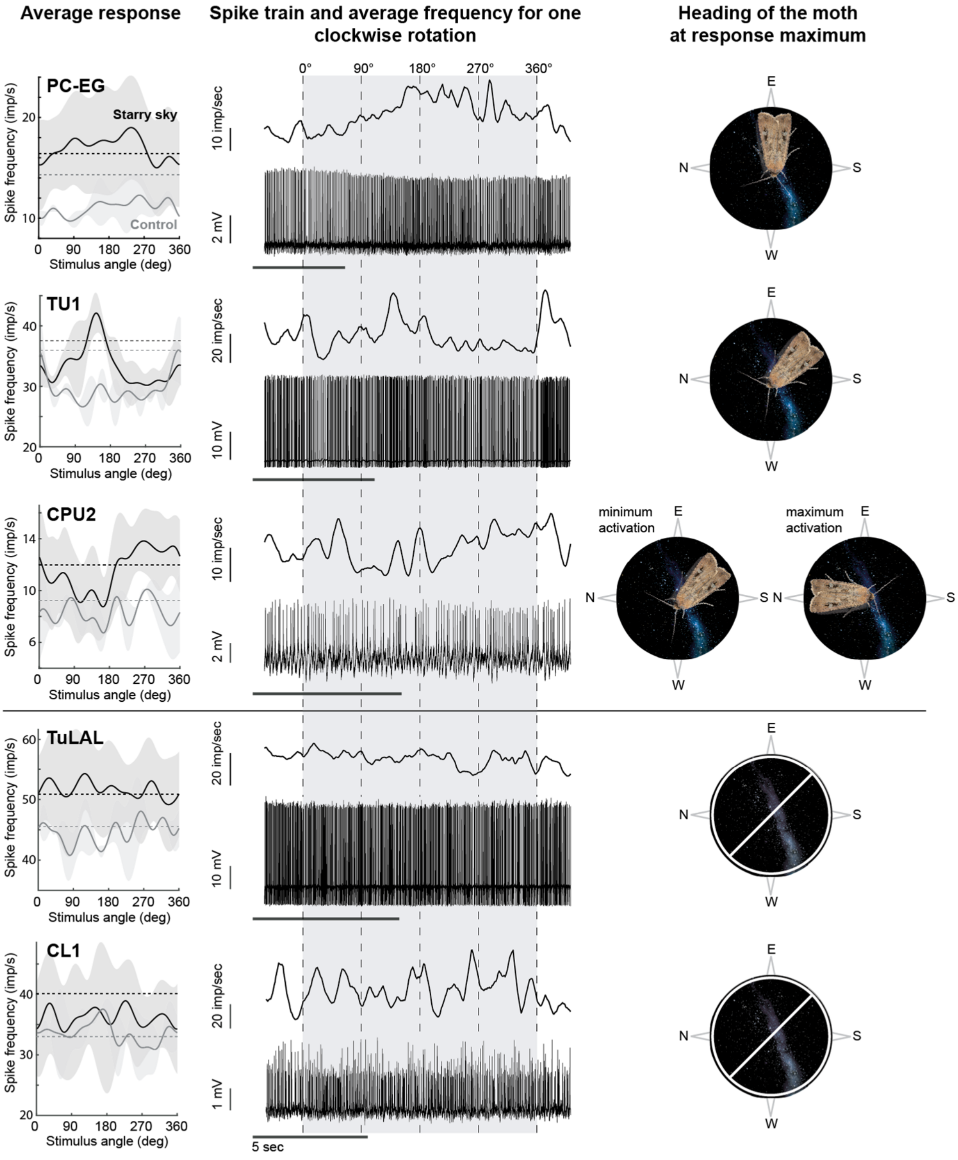

Figure 13: Central complex neuron responses to the starry sky stimulus.
PC-EG, TU1 and CPU2 neurons show a distinct response to the starry sky stimulus, but not to the control stimulus. In contrast, TuLAL and CL1 do not respond to the a rotation of the starry sky. Left column: Average responses of morphologically identified neurons in the Bogong moth central complex. Clockwise and counter-clockwise rotations are pooled for the starry sky stimulus (black line = mean response, dark grey area = standard deviation, dotted black line = pre-stimulation background activity) and the control stimulus (grey line, light grey area, dotten grey line). Middle column: Selected spike trains for one clockwise rotation (0° to 360°) of the starry sky stimulus, with the average spiking frequency for that trial plotted above. Right column: Heading of the moth relative to the starry sky stimulus at the neuron's maximum firing frequency.

While the exact neural processing of starry sky cues in the Bogong moth brain is still unclear, the neural responses presented in Paper III can be linked to a clear behaviour. Tethered moths that can rotate freely in a flight simulator will choose not only a stable flight direction, but this direction is their migratory heading (Figure 2; Paper III). This indicates that the moths use the starry sky as a true compass cue. At this point it is too early to speculate which part of the starry sky they use, but it is unlikely to be the rotational centre of the sky, since the projected sky image in the flight simulator was fixed. It is more likely that the Milky Way provides the directional information needed for selecting a flight direction. However, it is as yet unclear whether the moths can time-compensate the starry sky. Thus, the question whether Bogong moths use the starry sky as a time-compensated compass cue, or as a landmark used for a celestial snapshot, remains unsolved.

9 A model for modality-independent steering

When it comes to visual information and visual compass cues, the input pathways from the primary sensory neuropils to the central complex are well described. The same is true for several central complex networks, such as the heading direction network. We can now say with some certainty that every behavioural and motor decision is influenced by the spatial context computed in the central complex. However, surprisingly little work has focussed on how motor decisions are transferred from the central complex to thoracic motor centres. As the central complex does not give rise to descending neurons that might contact motor neurons directly, I asked how compass output signals are transformed into motor commands.

Central complex output neurons, such as CPU1 neurons, project to the lateral accessory lobes (LALs). As discussed in chapter 3, the LALs have been described as a pre-motor region, from which several descending neurons originate. One such descending neuron type is the flip-flop neuron, described in detail by Kanzaki and colleagues in silkworm moths. Flip-flop neurons are bistable neurons that have a high and a low activity state. Upon stimulation, the neuron flips from one state to the other (Kanzaki et al., 1994; Mishima and Kanzaki, 1999). Intriguingly, these activity changes correlate with changes in neck motor neuron activity, as well as with head turns during walking (Kanzaki and Mishima, 1996; Mishima and Kanzaki, 1998). Flip-flop neurons are therefore assumed to be part of a network that controls turning. In silkworm moths, activity changes in flip-flop neurons were elicited by odour puffs, mimicking a situation in which male moths navigate through a pheromone plume to find a female. However, the neurons also changed state in response to light flashes (Olberg, 1983), and morphologically similar neurons responded to acoustic stimulation in crickets (Zorovic and Hedwig, 2011). These findings indicate that the system may not be specific to odour information.

It is possible that central complex output neurons contact descending neurons, such as flip-flop neurons, in the LAL, providing a potential way of feeding spatial information into downstream motor circuits. In order to test this hypothesis, I constructed a basic computational model of a flip-flop neuron circuit (**Paper IV**). Computational modelling allows us to manipulate networks in ways that are difficult to achieve in real animals, especially in the case of insects that are not as genetically accessible as the fruit fly. Furthermore, this method allows us to challenge the network in different ways, for example by providing different types of input. To explore which components of the circuit are necessary and sufficient to underlie steering, I modelled the circuit using (a) rate-code neurons, and (b) leaky-integrate-and-fire neurons. Both are common neuron implementations in computational models, but they include different levels of biological detail.

In a rate-code neuron model, the behaviour of the neuron is specified directly, and in this case, the output is given by a sigmoid function applied to the sum of all inputs. For the flip-flop neuron, it was necessary to add the flip-flop behaviour as an additional function on top of the sigmoid, as a simple sigmoid function cannot capture this bi-stability. For the second model ('Nengo'), the behaviour of the neuron was constructed by combining smaller circuit components, meaning that instead of postulating a perfect flip-flop behaviour, the sub-components of the neuron were interconnected such that they approximated the desired bi-stability. This model was implemented in Nengo, a software that is based on the neural engineering framework (NEF) (Bekolay et al., 2014). Nengo provides an optimisation framework that finds the best way to connect a specified set of components to approximate a desired behaviour, turning the flip-flop neuron into an interconnected population of components whose connection weights are optimally adjusted to produce flip-flopping behaviour. The model presented here uses leaky-integrate-and-fire spiking neurons as sub-components. Thus, a population of Nengo spiking neurons approximates one rate-code neuron, and while the rate model computes the sum of all inputs, the Nengo model produces the weighted sum.

In a simulated odour plume, both models navigate efficiently towards the odour source (Figure 4, Paper IV). The trajectories qualitatively and quantitatively resemble those of real silkworm moths. However, the Nengo model approximated the real moth better than the rate model. Furthermore, in situations in which the input to the two sensors was very similar or equal, the Nengo model produced loops (Figure 4, Paper IV). Moths perform loops as a search strategy when they lose the odour plume (Cardé and Willis, 2008). Thus it appears that the Nengo model gives rise to a search behaviour that is intrinsic to the network.

Having confirmed that the models can approximate the observed behaviour of male silkworm moths, we then examined whether the same model could use input other than olfactory information to steer. To this end, we used output from the path integrator model (Stone et al., 2017) and fed it into the sensors of the flip-flop model. As the path integrator uses optic flow and visual compass cues to compute the current speed and current heading, respectively, this type of input was considered 'visual'. Both the rate model and the Nengo model steered the agent back home efficiently, using the same parameters as for the odour plume experiments. However, as the output of the path integrator is a steering command that signals small adjustments when the agent is even slightly off course, the difference between the left and the right sensor were very small. Re-scaling this difference to fall between 0 and 1 increased the success rate of both models dramatically, from 70% to 78% in the case of the rate model, and from 57% to 92% for the Nengo model. These findings show that this model of a basic flip-flop network can steer an agent independent of the sensory modality of the input, as long as the inputs to the two sides of the network are not too similar. In the case of central complex output neurons, such as CPU1, re-scaling could simply be achieved by increasing the weights of their synapses onto flip-flop neurons.

How do these findings relate to the Bogong moth? I recorded from a flip-flop neuron in the Bogong moth (Figure 16). Although it was not possible to identify this neuron anatomically, the physiology showed a clear bi-stability, and the neuron changed state in response to rotational optic flow. Given that this type of optic flow provides information about the speed of rotations, it may act as a visual feedback signal that indicates the start, duration and stop of a turn. During flight, this can provide visual control over compensatory turning and can stabilise steering based on the heading direction signal.

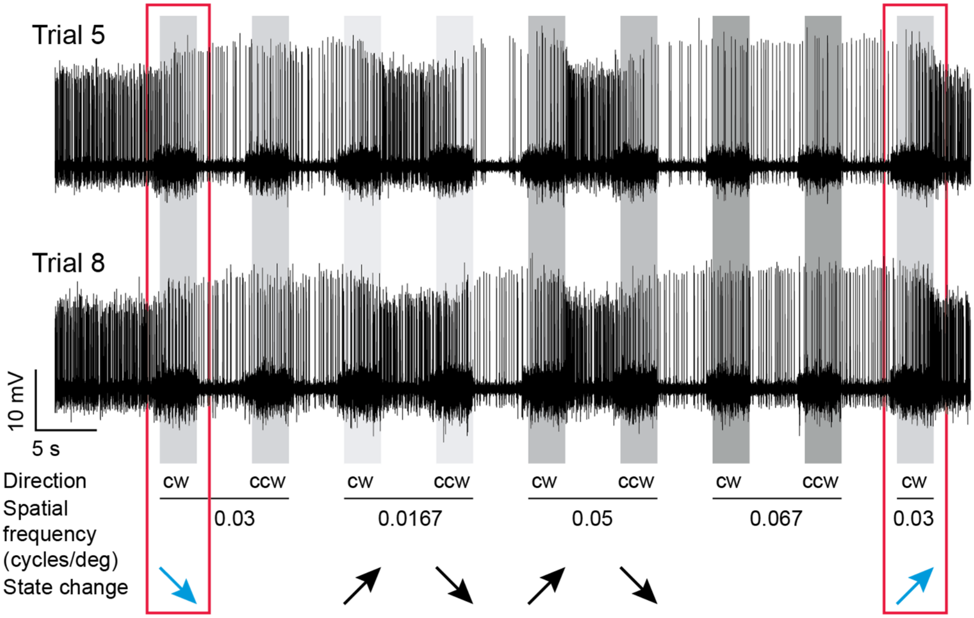

Figure 16: A Bogong moth flip-flop neuron changes state in response to rotational optic flow.
Two consecutive trials of a rotational optic flow series show flip-flopping in a Bogong moth neuron. The stimulus is a sine grating of different spatial frequencies, altering between clockwise and counter-clockwise rotations at 60 degrees per second. Note that the first and the last stimulus are identical, but the response to the stimulus are opposite. The neuron does not respond to the stimulus with the highest spatial frequency. While some branches of this neuron were stained in the lateral accessory lobes, the complete morphology could not be determined.

It has been suggested previously that the path integrator network can be adjusted to produce a migratory vector rather than a home vector (Honkanen et al., 2019). This could be achieved by fixing the synaptic weights of CPU4 neurons (memory) onto CPU1 neurons. This being the case, the CPU4 neurons represent a constant direction signal rather than accumulating a home vector, and the CPU1 neurons compare this constant signal to the current heading and adjust for deviations. Steering commands from CPU1 neurons could then be relayed to downstream motor centres via flip-flop descending neurons, which are known to project onto neck motor neurons and further into the thorax (Kanzaki and Mishima, 1996). Although only a minority of descending neurons originate in the LALs, there is evidence that descending neurons branch widely in the posterior slope (Namiki et al., 2018). Therefore, the posterior slope is likely an area where compass information interacts and is integrated with other sensory information, as well as with reflex commands (Schnell et al., 2017; Cande et al., 2018).

Conclusions and perspectives

Migratory moths, and in particular the Bogong moth, offer fascinating insights into the neural basis of navigation and migration. Not only are their brains accessible to electrophysiological recordings, but the neuroanatomy and neural circuits appear conserved enough to allow us to generalise from these moths to other insects. Combining data from many different insects, with different behavioural specialisations and ecological challenges, can lead to an integrated understanding of the neural circuits underlying behaviour in insects, and how they evolved to accommodate different environmental demands.

In this thesis, I showed that the Bogong moth brain has the anatomical layout of a typical lepidopteran brain, and does not appear to have any obvious anatomical specialisations that may underlie migration (Paper I). When comparing neuropil volumes across a range of moths and butterflies, it emerged that the volumes of primary sensory neuropils strongly correlated with the moths' activity period and thus their sensory environment, whereas migratory behaviour appeared to be linked exclusively to higher processing centres (Paper II). Disentangling lifestyle effects from effects of the phylogenetic distance between these species will be an important future challenge. Moving from the overall anatomy of the brain to specific neurons, I was able to show that certain neurons associated with the optic lobes and the central complex respond to a rotation of the starry sky (Paper III). This showed that the neural substrate for processing the starry sky, and potentially the Milky Way, as a compass cue is present in the Bogong moth. Starry sky responses from neurons that had not been recorded from before, as well as no response from known compass neurons, raise the possibility of a processing pathway for skylight cues that has not been described yet, and that runs in parallel with known pathways. Finally, I showed how compass output from the central complex may interact with thoracic motor centres, by building a computational model of a neural circuit in the lateral accessory lobes (Paper IV). The model was able to steer efficiently when driven by central complex output. Moreover, the model makes several testable predictions, which will drive future research and help close the gap between the compass network and downstream motor centres (Figure 17).

Considering the migration of the Bogong moth, several question remain unanswered. First and foremost, the processing pathways of skylight compass cues in the Bogong moth brain remain unclear. Additional recordings from central complex neurons are necessary to clarify if and how known heading direction cells, such as CL1, respond to the starry sky, and whether their responses may be state-dependent. Secondly, the starry sky stimulus is a complex naturalistic stimulus which contains the Milky Way, an elongated structure that is brighter on one end than the other, as well as having an intensity gradient and a colour gradient. Which of these properties are used by the moths to select a flight direction remains to be investigated. It is also currently unknown whether Bogong moths can time-compensate the rotation of the starry sky. This is a crucial question for understanding how different compass cues are integrated, and whether the moths indeed employ a ‘snapshot strategy’, as suggested in Dreyer et al. (2018). Finally, Bogong moths can use the geomagnetic field to navigate, but nothing is known about how this stimulus is perceived and processed in the brain. However, one prevailing hypothesis is that magnetic fields are sensed by light-activated photoreceptors (see chapter 6). This suggests that neurons in the primary visual neuropils – the optic lobes and/or the ocellar neuropils – as well as in the anterior optic tubercle may respond to changing magnetic field vectors. Independent of the location of the sensor, one would also expect to find responses to magnetic field cues in the central complex, as well as further downstream, in the lateral accessory lobes and descending neurons.

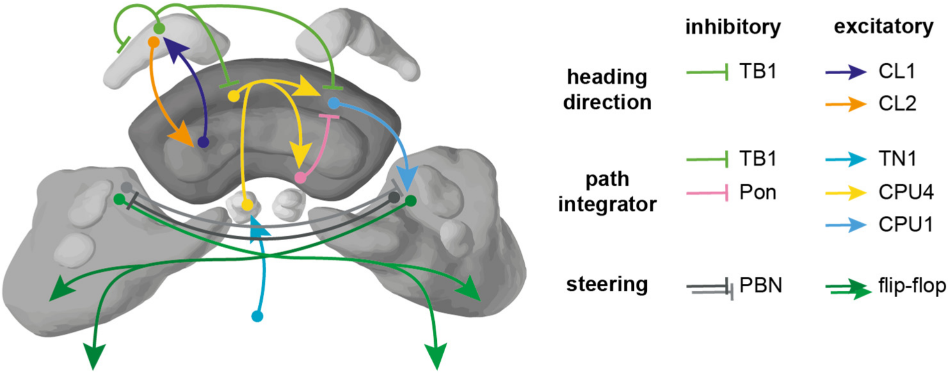

Figure 17: An integrated view of central and lateral complex networks.
Compass information as well as visual landmarks and proprioceptive cues feed into the heading direction network (CL1 and CL2). Heading direction information is fed into the path integrator via TB1 neurons, and integrated with optic flow information (via TN1) to result in a home vector, which is stored in a CPU4 memory loop. CPU1 neurons read out the home vector and compare it to the current heading (TB1). Any mismatches result in steering commands. CPU1 neurons project to the lateral accessory lobes, where they potentially interact with flip-flop descending neurons that mediate steering.

Answering these open questions will help us understand the neural processing that underlies navigation and migration in the Bogong moth, as well as giving us insights into insect compass navigation and steering in general. In particular, understanding how and where magnetic field information is integrated into the compass system may point us in the direction of a magnetosensor – a holy grail of animal navigation research that has long remained elusive. Generally, dissecting the neural networks that underlie orientation and navigation in different insect species allows us to understand small parts of the network that pertain to each species' ecology. In the future, putting all these findings together may lead to a complete understanding of the networks that process compass cues and underlie migration, from the sensory inputs to the behavioural output.

Finally, considering the ongoing decline of the Bogong moth, and given that this species is an important food source for many alpine birds and mammals, it is more important than ever to understand the ecology of the Bogong moth's migration, as well as the neural processes that underlie it. Aside from being an interesting scientific puzzle, answering these questions may help with conservation efforts. This will ultimately benefit not only the Bogong moth, but the entire ecosystem of the Australian Alps.

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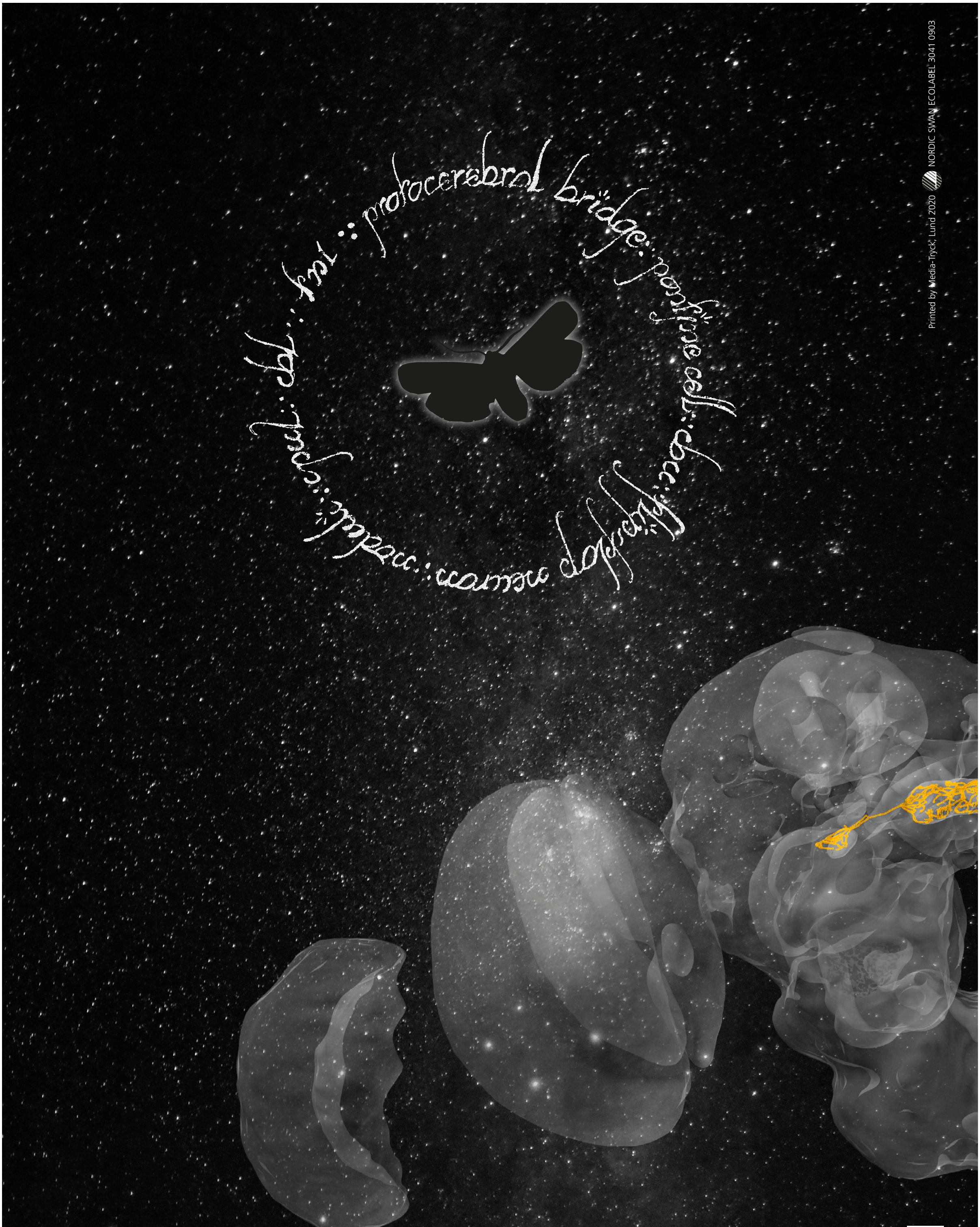

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