Neuronal basis of temporal polyethism and sky-compass based navigation in *Cataglyphis* desert ants

Die neuronale Grundlage von Alterspolyethismus und Himmelskompassnavigation in der Wüstenameise *Cataglyphis*

Doctoral thesis for a doctoral degree
at the Graduate School of Life Sciences
Julius Maximilians-Universität Würzburg
Section Integrative Biology

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Summary

Desert ants of the genus *Cataglyphis* (Formicinae) are widely distributed in arid areas of the palearctic ecozone. Their habitats range from relatively cluttered environments in the Mediterranean area to almost landmark free deserts. Due to their sophisticated navigational toolkit, mainly based on the sky-compass, they were studied extensively for the last 4 decades and are an exceptional model organism for navigation. *Cataglyphis* ants exhibit a temporal polyethism: interior workers stay inside the dark nest and serve as repletes for the first ∼2 weeks of their adult life (interior I). They then switch to nursing and nest maintenance (interior II) until they transition to become day-active outdoor foragers after ∼4 weeks. The latter switch in tasks involves a transition phase of ∼2-3 days during which the ants perform learning and orientation walks. Only after this last phase do the ants start to scavenge for food as foragers.

In this present thesis I address two main questions using *Cataglyphis* desert ants as a model organism:

1. What are the underlying mechanisms of temporal polyethism?
2. What is the neuronal basis of sky-compass based navigation in *Cataglyphis* ants?

Neuropeptides are important regulators of insect physiology and behavior and as such are promising candidates regarding the regulation of temporal polyethism in *Cataglyphis* ants. Neuropeptides are processed from large precursor proteins and

undergo substantial post-translational modifications. Therefore, it is crucial to biochemically identify annotated peptides. As hardly any peptide data are available for ants and no relevant genomic data has been recorded for *Cataglyphis*, I started out to identify the neuropeptidome of adult *Camponotus floridanus* (Formicinae) workers (manuscript 1). This resulted in the first neuropeptidome described in an ant species – 39 neuropeptides out of 18 peptide families. Employing a targeted approach, I identified allatostatin A (AstA), allatotropin (AT), short neuropeptide F (sNPF) and tachykinin (TK) using mass spectrometry and immunohistology to investigate the distribution of AstA, AT and TK in the brain (manuscript 2). All three peptides are localized in the central complex, a brain center for sensory integration and high-order control of locomotion behavior. In addition, AstA and TK were also found in visual and olfactory input regions and in the mushroom bodies, the centers for learning and memory formation. Comparing the TK immunostaining in the brain of 1, 7 and 14 days old dark kept animals revealed that the distribution in the central complex changes, most prominently in the 14 day old group. In the *Drosophila* central complex TK modulates locomotor activity levels. I therefore hypothesize that TK is involved in the internal regulation of the interior I–interior II transition which occurs after ∼2 weeks of age.

I designed a behavioral setup to test the effect of neuropeptides on the two traits: 'locomotor activity level' and 'phototaxis' (manuscript 3). The test showed that interior I ants are less active than interior II ants, which again are less active than foragers. Furthermore, interior ants are negatively phototactic compared to a higher frequency of positive phototaxis in foragers. Testing the influence of AstA and AT on the ants' behavior revealed a stage-specific effect: while interior I behavior is not obviously influenced, foragers become positively phototactic and more active after AT injection and less active after AstA injection. I further tested the effect of light exposure on the two behavioral traits of interior workers and show

that it rises locomotor activity and results in decreased negative phototaxis in interior ants. However, both interior stages are still more negatively phototactic than foragers and only the activity level of interior II ants is raised to the forager level. These results support the hypothesis that neuropeptides and light influence behavior in a stage-specific manner.

The second objective of this thesis was to investigate the neuronal basis of sky-compass navigation in *Cataglyphis* (manuscript 4). Anatomical localization of the sky-compass pathway revealed that its general organization is highly similar to other insect species. I further focused on giant synapses in the lateral complex, the last relay station before sky-compass information enters the central complex. A comparison of their numbers between newly eclosed ants and foragers discloses a rise in synapse numbers from indoor worker to forager, suggesting task-related synaptic plasticity in the sky-compass pathway. Subsequently I compared synapse numbers in light preexposed ants and in dark-kept, aged ants. This experiment showed that light as opposed to age is necessary and sufficient to trigger this rise in synapse number. The number of newly formed synapses further depends on the spectral properties of the light to which the ants were exposed to.

Taken together, I described neuropeptides in *C. floridanus* and *C. fortis*, and provided first evidence that they influence temporal polyethism in *Cataglyphis* ants. I further showed that the extent to which neuropeptides and light can influence behavior depends on the animals' state, suggesting that the system is only responsive under certain circumstances. These results provided first insight into the neuronal regulation of temporal polyethism in *Cataglyphis*. Furthermore, I characterized the neuronal substrate for sky-compass navigation for the first time in *Cataglyphis*. The high level of structural synaptic plasticity in this pathway linked to the interior–forager transition might be particularly relevant for the initial calibration of the ants' compass system.

Zusammenfassung

Wüstenameisen der Gattung *Cataglyphis* sind weit verbreitet in ariden Gebieten der paläarktischen Ökozone. Die von ihnen bewohnten Habitate reichen von landmarkenreichen Arealen im Mittelmeerraum, zu beinahe landmarkenfreien Wüstengebieten. Aufgrund ihres hochentwickelten Navigationssystems, welches größtenteils auf dem Himmelskompass basiert, wurden sie in den letzten 4 Jahrzehnten extensiv studiert und sind ein einzigartiges Modellsystem für Navigation. *Cataglyphis* weisen einen alterskorrelierten Polyethismus auf: Innendienstler dienen als Speichertiere für die ersten ∼2 Wochen ihres adulten Lebens (Interior I). Sie gehen daraufhin zu Brutpflege und Nestbau (Interior II) über bis sie nach ∼4 Wochen zu tagaktiver Furagiertätitkeit außerhalb ihres Nestes wechseln. Dieser letzte Übergang dauert ∼2-3 Tage und wird von den Ameisen genutzt, um Lern- und Orientierungsläufe durchzuführen.

In der vorliegenden Arbeit befasse ich mich vor allem mit zwei Fragen, die ich mit Hilfe von *Cataglyphis* als Modellorganismus beantworten möchte:

1. Welches sind die zugrunde liegenden Mechanismen des Alterspolyethismus?
2. Was ist die neuronale Grundlage von Navigation, die auf dem Himmelskompass basiert?

Neuropeptide sind bedeutende Regulatoren der Physiologie und des Verhaltens von Insekten und als solche vielversprechende Kandidaten im Hinblick auf die Regulation des Alterspolyethismus in *Cataglyphis* Ameisen. Neuropeptide werden aus

größeren Vorläuferproteinen herausgeschnitten und posttranslational stark modifiziert. Daher ist es wichtig, annotierte Peptide auch biochemisch zu identifizieren. Da für Ameisen kaum Peptiddaten zur Verfügung stehen und es zudem keine relevanten genomischen Daten für *Cataglyphis* gibt, identifizierte ich zunächst das Neuropeptidom adulter *Camponotus floridanus* (Formicinae) Arbeiterinnen (Manuskript 1). Daraus resultierte das erste Neuropeptidom, das für eine Ameisenart beschrieben wird—39 Neuropeptide aus 18 Peptidfamilien. In einer weiteren Studie identifizierte ich gezielt die Neuropeptidfamilien Allatostatin A (AstA), Allatotropin (AT), das kurze Neuropeptid F (sNPF) und Tachykinin (TK) mittels Massenspektroskopie und untersuchte die Verteilung von AstA, AT und TK im Gehirn mit Hilfe der Immunhistologie (Manuskript 2). Alle drei Peptide sind im Zentralkomplex lokalisiert, dem Gehirnzentrum welches sensorische Eingänge integriert und in einer übergeordneten Rolle Lokomotorverhalten steuert. AstA und TK sind zudem in den visuellen und olfaktorischen Eingangsregionen, sowie den Pilzkörpern, den Zentren für Lernen und Gedächtnisbildung, zu finden. Ein Vergleich der TK-Immunfärbung im Gehirn von 1, 7 und 14 Tage alten im Dunkeln gehaltenen Tieren zeigt, dass sich die Verteilung im Zentralkomplex verändert—dies ist besonders prominent in der 14 Tage alten Gruppe. In *Drosophila* moduliert TK im Zentralkomplex Lokomotoraktivität. Basierend darauf stelle ich die Hypothese auf, dass TK in der internen Regulierung des Übergangs von Interior I zu Interior II involviert ist, welchen die Tiere im Alter von ∼2 Wochen durchlaufen. Für eine dritte Studie konstruierte ich ein Verhaltenssetup um den Einfluss von Neuropeptiden und Licht auf die beiden Verhaltensmerkmale 'Lokomotoraktivität' und 'Phototaxis' zu testen (Manuskript 3). Der Test zeigte, dass Interior I Ameisen weniger aktiv sind als Interior II Ameisen, welche wiederum weniger aktiv sind als Furageure. Zudem sind Interior Ameisen negativ phototaktisch, verglichen mit einer häufiger zu beobachtenden positiven Phototaxis bei Furageuren.

Im Test zeigte sich auch, dass der Einfluss von AstA und AT stadiumsspezifisch ist: während das Verhalten von Interior I Tieren nicht offensichtlich beeinflusst wird, werden Furageure durch die Injektion von AT positiv phototaktisch, sowie aktiver und AstA-Injektion führt zu geminderter Lokomotoraktivität. Darüber hinaus testete ich den Lichteinfluss auf beide Verhaltensmerkmale in den Innendienststadien und zeige, dass er Lokomotoraktivität steigert und in einer geminderten negativen Phototaxis resultiert. Beide Innendienststadien sind jedoch weiterhin negativer phototaktisch als Furageure und nur die Lokomotoraktivtät von Interior II Ameisen wird auf das Niveau von Furageuren angehoben. Diese Ergebnisse stützen die Hypothese, dass Neuropeptide und Licht stadiumsspezifisch Verhalten beeinflussen.

Der zweite Aspekt dieser Thesis war es, die neuronale Grundlage der Himmelskompassnavigation in *Cataglyphis* aufzuklären (Manuskript 4). Die neuroanatomische Lokalisation der Himmelskompasssehbahn zeigt, dass die allgemeine Organisation dieser neuronalen Bahn der bei bisher untersuchten anderen Insekten stark ähnelt. Ich habe mich daraufhin auf Riesensynapsen im lateralen Komplex konzentriert, der letzten Verschaltungsstation ehe die Himmelskompassinformation in den Zentralkomplex übertragen wird. Ein Vergleich zwischen der Synapsenzahl in frisch geschlüpfte Ameisen und erfahrenen Furageueren zeigte einen Anstieg der Synapsenzahl von Innendienst zu Furaguer, was aufgabenabhängige synaptische Plastizität in der Himmelskompasssehbahn suggeriert. In einem weiteren Versuch verglich ich die Riesensynapsenzahlen lichtexponierter Tiere und dunkel gehaltener, gealteter Tiere. Dieses Experiment zeigte, dass der Zuwachs an Riesensynapsen durch den Lichteinfluss ausgelöst wird und keinen altersabhängigen Prozess darstellt. Zudem verändert sich die Anzahl der neu gebildeten Riesensynapsen in Abhängigkeit von den spektralen Eigenschaften des Lichts, dem die Ameisen ausgesetzt sind.

Zusammengefasst beschrieb ich in dieser Thesis Neuropeptide in *C. floridanus* und *Cataglyphis* und lieferte erste Evidenz, dass diese den Alterspolyethismus in *Cataglyphis* beeinflussen. Zudem zeigte ich, dass das Ausmaß in dem Neuropeptide und Lichtexposition Verhalten beeinflussen können, stadiumsspezifisch ist. Dies suggeriert, dass das System nur unter bestimmten Bedingungen auf externe Einflüsse reagiert. Diese Ergebnisse lieferten erste wichtige Einblicke in die neuronale Grundlage von Alterspolyethismus in *Cataglyphis*. Zudem charakterisierte ich erstmals das neuronale Substrat der Himmelskompassnavigation in *Cataglyphis*. Das hohe Maß an synaptischer Plastizität in dieser Sehbahn beim Übergang von Innen- zu Außendienst, könnte besondere Relevanz für die initiale Kalibrierung des Kompasssystems haben.

Contents

1 Introduction

1.1 Polyethism in eusocial insects

Social insects are among the most successful and advanced societies on earth (Hölldobler and Wilson, 1990, 2009). They adapted to inhabiting a huge variety of landscapes ranging from tundras and deserts hostile to life to more favorable rainforests or temperate zones (Hölldobler and Wilson, 2009). Key to their success was the evolution of eusociality, which is defined by three aspects: reproductive division of labor, overlapping generations and cooperative brood care (Reeve and Hölldobler, 2007; Hölldobler and Wilson, 2009). This, in the case of hymenopteran insect societies, means that there is mostly only one or few reproductively active queens and up to several thousands or even millions of sterile workers which cooperatively care the brood produced by the queen. The workers again divide the tasks inside the nest among each other (division of labor). While some animals care for the brood and queen, others act as repletes (living food storage) or garden a fungus and another part constructs the nest or forages. How labor is divided within the worker caste can be related to the age of the individuals (age or temporal polyethism), as it can be the case in social bees, wasps and many ant species, or determined by different morphs (polymorphism; physical polyethism) which so far has solely been shown for some ant species. However, age polyethism can also occur in polymorphic ant species (Hölldobler and Wilson, 2009). In almost all colonies exhibiting temporal polyethism young ants fulfill tasks inside the nest while the older workers leave the nest for defense or foraging.

Temporal polyethism in insect societies is an emergent phenomenon that is not based on strictly timed succession of certain tasks but on a plastic change of behavior of individual colony members related to their age. It is the plasticity within the system that allows the colony to flexibly respond to changing environmental conditions. The transition from indoor duties to outdoor foraging demands adapting to and orienting in a new environment and therefore is probably an individual's most impressive behavioral switch. This transition is accompanied by drastic behavioral and neuronal reorganizations and has been studied quite intensely with a focus on the basic regulatory mechanisms and neurobiology (reviewed in Robinson, 1992; Wehner, 2003; Galizia et al., 2012; Menzel and Benjamin, 2013; Pandey and Bloch, 2015; Fahrbach and van Nest, 2016). A rewarding model to study how age-related division of labor - especially the interior–forager transition - is regulated and which changes underly this switch on a neuronal level are *Cataglyphis* ants.

1.2 *Cataglyphis* desert ants

Cataglyphis (Formicinae) desert ants inhabit a large part of the palearctic ecozone where they mainly settled in desert-like, dry areas (for an overview see Knaden et al., 2012). Their nest lies underground and only foragers leave the nest to scavenge for food. *Cataglyphis* ants undergo a temporal polyethism which is described in detail for *C. bicolor* (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984; Wehner and Rössler, 2013) (see figure 1).

Newly eclosed ants (callow stage) are easily recognizable by their pale cuticle for ∼24 hours and it is only after this 1 day-period that the ants start to fulfill their indoor duties. They start off as repletes (interior I stage; ∼14 days)—a mostly inactive stage with low energy expenses. Repletes are considered the colonies' living

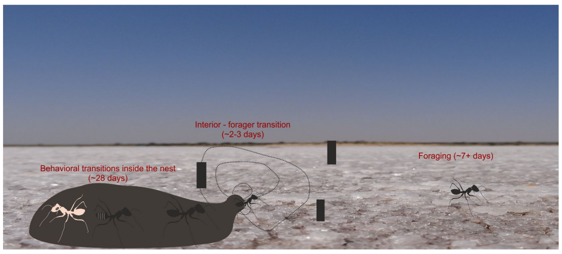

Figure 1: **Overview of the *Cataglyphis* temporal stages.** Ants stay inside the nest for the first ∼28 days of their life, undergoing behavioral transitions from callow (pale cuticle) to interior I (repletes), to interior II (nursing, nest construction). Subsequently, ants leave the nest for the first time to perform orientation and learning walks for ∼2–3 days. This phase is required for subsequent long-distance navigation based foraging. On average, foragers die after ∼7 days due to predation or heat stress.

food storage and their full crop makes them readily identifiable by their swollen gaster and stretched intersegmental membranes. Subsequently, the ants care for the brood and queen, and perform nest digging duties (interior II stage). Thus ants in this stage are actively moving around, carrying brood to the most suitable parts of the nest, expanding and maintaining the nest. After the ∼28 days lasting indoor phase interior workers become foragers. The interior–forager transition takes 2–3 days and involves extensive exploration of the nest surroundings in increasing distance to the nest entrance (Wehner et al., 2004; Stieb et al., 2012; Fleischmann et al., 2016). During this phase ants stay within close vicinity of the nest performing looped runs around it. These runs are interpreted as learning and orientation walks and have also been described in bees and wasps and further ant species (Zeil, 1993; Capaldi and Dyer, 1999; Nicholson et al., 1999; Hempel de Ibarra et al., 2009; Müller and Wehner, 2010). *Cataglyphis* ants do not use trail pheromones (Wehner, 1983) but are considered as almost purely visually guided navigators that solitarily leave the nest to forage with every foraging trip being an individual run (reviewed in Wehner, 2003). Learning walks are thus considered a crucial prerequisite to enable successful orientation during later foraging runs (Fleischmann et al., 2016).

Foragers leave the nest in the direction of the previous successful search and are able to return to the same area again (Wehner et al., 2004; Wolf et al., 2012). However, if not successful, they continue a meanderous crosswind search (Buehlmann et al., 2014) until they find food (mostly dead arthropods). Employing a path integration system the ants return to the nest in a straight line (see figure 2A)(reviewed by Wehner et al., 1996; Wehner, 2003, 2009). This path integration system allows the ants to consistently calculate a home vector based on directional and distance information the ants acquire during their outbound search (Wehner and Wehner 1990; reviewed in Wehner 2003, 2009) that can last up to 1 km in *C. fortis* (Buehlmann et al., 2014; Huber and Knaden, 2015). Step integration—the integration of stride number and length—is the basis for the ants' odometer, the measure for the distance walked (Wittlinger et al., 2006, 2007). In addition, self-induced optic flow can be used to minimize accumulating errors (Ronacher and Wehner, 1995). Directional information is primarily deduced from the sky-compass (see figure 2B). Predominant sky-compass cues used by *Cataglyphis* are the position of the sun, the concentric pattern of linear polarized skylight around it and the color gradient of the sky. The integration of directional and distance information results in a home vector available to the ants at any position of their foraging runs. However, path integration is not insusceptible to errors which easily accumulate during the long-distance foraging trips (Merkle and Wehner, 2008, 2010). To correct for those errors the ants are able to learn visual (Wehner et al., 1996; Collett et al., 1998; Bisch-Knaden and Wehner, 2003; Huber and Knaden, 2015) and olfactory landmarks (Steck et al., 2009, 2011; Buehlmann et al., 2015), especially in close vicinity of the nest or food site. Besides, the often constant wind direction in the ants' habitat can be used as an indicator for compass direction (Müller and Wehner, 2007; Buehlmann et al., 2014). While *Cataglyphis* has long been a model organism for path integration based navigation on a behavioral level, the neuronal

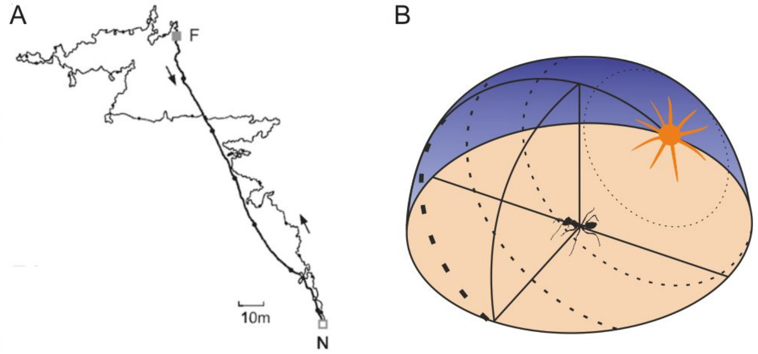

Figure 2: **Path integration in *Cataglyphis* is predominantly based on sky-compass information. A**. *Cataglyphis* leaves the nest (N; open square) solitarily in a meanderous manner (thin line) until it finds food (F; closed square). It then returns to the nest in a straight line (heavy line) (adapted from Wehner and Wehner, 1990). **B** The sky-compass serves as predominant directional cue. Its main components are the position of the sun, the concentric polarization pattern around it and the color gradient of the sky.

basis of temporal polyethism and sky-compass based navigation in these ants has only started to be studied in recent years.

1.3 Regulation of the timing of behavioral transitions

Since temporal polyethism is a common feature in insect societies, its regulating factors have been in the focus of interest for several decades. There are defined external regulating factors acting on the individual animal within a colony and stimulating it from transitioning from one task to another. These are environmental conditions like temperature, or food availability and distribution, as well as the condition of the colony, like colony size or number of foragers and brood (Kolmes, 1985; Gordon, 1989; Robinson, 1992; Mersch, 2016). Artificial manipulation of these factors in honeybee colonies, for example sudden reduction of the number of foragers or nurses, results in a quick restructuring of task allocation within the colony (Huang and Robinson, 1996), indicating a plastic system. Division of labor has further been suggested to be influenced by a number of internal factors as juvenile hormone (JH), biogenic amines, insulin or neuropeptides (see fig. 3).

The interior–forager transition is associated with a rise in the juvenile hormone (JH) level in honeybees (Jassim et al., 2000; Elekonich et al., 2001; Scholl et al., 2014) and ants (*Pogonomyrmex californicus*: Dolezal et al., 2012). Manipulation of JH levels delays or accelerates the interior–forager transition in honeybees (Robinson, 1987; Sullivan et al., 2000) and affects associated aspects of the transition, as for example the sensitivity to alarm pheromone (Robinson, 1987) or oocyte maturation (Guidugli et al., 2005; Nelson et al., 2007). Although these studies suggest JH to be involved, no direct causality between a rise in JH levels and behavioral transitions under natural conditions could be stated so far. In fact, JH rise seems to accompany the transition rather than triggering it in *Pogonomyrmex* ants (Dolezal et al., 2012). It further does not affect synaptic plasticity in the mushroom bodies (Scholl et al., 2014) which is associated with the transition from indoor to outdoor workers in honeybees and ants (Stieb et al., 2010; Muenz et al., 2015).

Considering the behaviors, that are suggested to change in order to cause both—interior I–interior II and interior II–forager—transitions, neuromodulators with the potential to regulate for instance neuronal activity and sensitivity, locomotor activity or phototaxis are interesting. Studies in the honeybee show that, indeed, biogenic amines can influence behavior (e.g. Thamm et al., 2010) and correlate with worker specialization (Wagener-Hulme et al., 1999) but their specific role in the interior–forager transition is not yet resolved.

One group of potent neuromodulators for the regulation of specific behavioral components are the highly diverse neuropeptides which are known to influence physiology and behavior as neuroactive substances in the nervous system or peptide hormones circulating in the hemolymph (see e.g. Ribeiro-da Silva and Hökfelt, 2000; Boonen et al., 2009; Nässel and Winther, 2010). Neuroactive peptide mes-

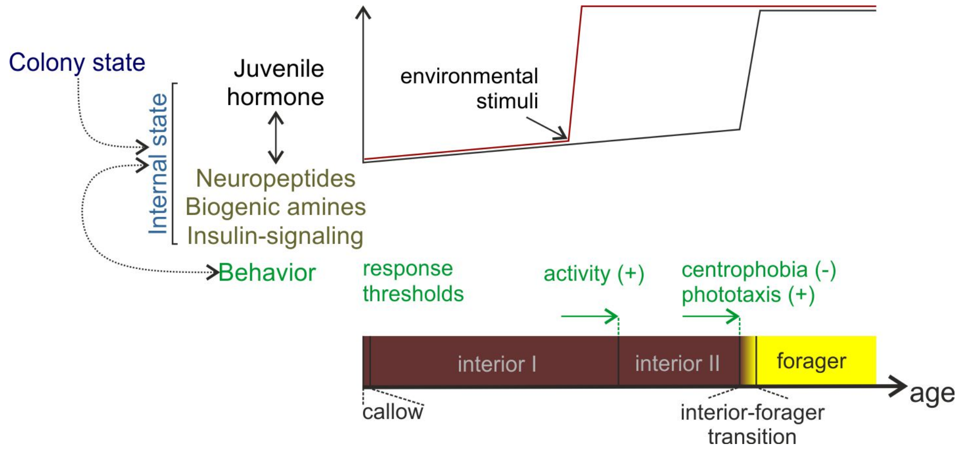

Figure 3: **Schematic model of the interplay of various internal and external factors influencing temporal polyethism in *Cataglyphis*.** In general, behavioral transitions are accompanied by a shift in response thresholds to relevant stimuli. The interior I–interior II transition is suggested to be accompanied by rising activity levels, while for the Interior II–forager transition centrophobia and phototaxis change. Rising JH titers go along with temporal polyethism and can be influenced by environmental stimuli, as for example light. Neuropeptides, biogenic amines and insulin signaling are supposed to regulate response thresholds e.g. in the context of feeding behavior, activity levels and phototaxis but can in turn be modulated by behavior. In addition, the animals' internal state is influenced by the colony state.

sengers are processed from precursor proteins (prepropeptides) at dibasic cleavage sites and underlie posttranslational modifications (PTM) which are essential for the bioactivity and stability of neuropeptides (reviewed in Rholam and Fahy, 2009). Unlike neurotransmitters, neuropeptides are not restricted to local cell-cell communication but, when released from the presynaptic dense-core vesicles, act more globally on the surrounding tissue (reviewed in Fricker, 2012). In insects they are involved in a huge variety of processes like feeding behavior, metabolism, reproduction, circadian rhythmicity, learning and memory or locomotor activity (reviewed in Nässel, 2000, 2002; Nässel and Winther, 2010).

In the given context the neuropeptide families allatostatin A (AstA), allatotropin (AT), short neuropeptide F (sNPF) and tachykinin (TK) might be of special interest. Functional studies of AstA show that it affects feeding behavior and foraging decisions in *Drosophila* (Hergarden et al., 2012; Wang et al., 2012; Hentze et al., 2015; Chen et al., 2016). Furthermore, AstA promotes sleep if activated in the same neurons that influence feeding behavior (Chen et al., 2016). Also, AstA is widely expressed in the honeybee brain (Kreissl et al., 2010) and injection of the peptide in the brain impairs appetitive olfactory learning (Urlacher et al., 2016).

AT is present in hymenopteran genomes but has so far only been identified in the honeybee ventral ganglia using immunohistology (Veenstra et al., 2012). Although no functional studies regarding AT have been conducted in Hymenoptera, it was shown to affect circadian rhythms via photic entrainment in *Manduca sexta* (Petri et al., 2002) as well as heartbeat rates in *M. sexta* and cockroaches (Veenstra et al., 1994). It further affects gut contraction in cockroaches where it is colocalized with serotonin in the abdominal ganglia (Rudwall et al., 2000). A neuromodulatory role of AT is suggested by its presence in the brain of for example the locust (Homberg et al., 2004), *M. sexta* (Zitnan et al., 1995) and cockroaches (Fusca et al., 2015). In cockroaches, the neuropeptide is colocalized with TK in the antennal lobe (Fusca et al., 2015).

sNPF was detected in the honeybee brain (Brockmann et al., 2009) and in both, the honeybee as well as the fire ant *Solenopsis invicta*, sNPF receptor (sNPFR) levels in the brain differ depending on the animals' nutrition level (Ament et al., 2011; Castillo and Pietrantonio, 2013). Besides, downregulation of sNPF leads to enhanced and longer sleep (Chen et al., 2013). In *S. invicta* sNPFR levels further are suggested to vary depending on the amount of brood in the colony and the physical stage (Castillo and Pietrantonio, 2013). In addition, the role of sNPF in *Drosophila* Kenyon cells (Johard et al., 2008) seems to be as functional neuromodulator for olfactory memory formation (Knapek et al., 2013). Further studies revealed that low sNPF levels in the *Drosophila* fanshaped body of the central complex result in an increased walking distance and speed (Kahsai et al., 2010). Here, it appears to interact with TK which, in the central complex, regulates general locomotor activity and central zone avoidance (Kahsai et al., 2010) and overall genetic knock-down of TK in the brain results in hyperactivity (Winther et al., 2006). TK is also present in *Drosophila* local interneurons of the antennal lobe where it modulates the dynamic range of the sensitivity to relevant odors (Ignell et al., 2009; Fusca et al., 2015). In honeybee foragers TK prepropeptide levels are higher expressed than in nurses (Takeuchi et al., 2003) but so far, a behavioral relevance was not shown.

The results regarding the pleiotropic roles of AstA, AT, sNPF and TK in insects suggest these neuropeptide families as potential key factors orchestrating *Cataglyphis* behavioral transitions. However, especially the interior–forager transition itself is supposed to trigger adaptive behavior and neuronal reorganization in *Cataglyphis*, in response to novel environmental information.

1.4 The neuronal basis of navigation in *Cataglyphis*

The main control center of insect behavior is the central brain (reviewed in e.g. Menzel and Benjamin, 2013) (see fig. 4 for an overview of the *Cataglyphis* brain). Here, sensory stimuli are received and processed to generate adequate behavioral output. First order processing centers for visual and olfactory information are the optic (reviewed in Strausfeld and Nässel, 1981) and antennal lobes (reviewed in Hansson and Anton, 2000; Kleineidam and Rössler, 2009; Galizia and Rössler, 2010), respectively. Higher order processing of this information takes place in the mushroom bodies and the central complex. Primary function of the mushroom bodies is suggested to be as centers for learning and memory formation (Heisenberg, 1998; Strausfeld et al., 1998; Menzel, 1999, 2001; Gerber et al., 2004; Hourcade et al., 2010; Scholl et al., 2015) while the central complex is considered to receive highly preprocessed multi-modal sensory input to control locomotor behavior, including navigation (Strauss, 2002; Strausfeld and Hirth, 2013; Pfeiffer and Homberg, 2014; Webb and Wystrach, 2016). Both neuropils are provided with visual information from the optic lobe: the anterior optic tract projects to the mushroom body visual input region (collar) in ants (Gronenberg, 2001; Yilmaz et al., 2016) and the central complex gets visual input via the sky-compass pathway in, for example, locusts (reviewed in Homberg, 2004; Homberg et al., 2011; Pfeiffer and Homberg, 2014) and bees (Pfeiffer and Kinoshita, 2012; Mota et al., 2011). As a center for long-term storage of information, the mushroom bodies are suggested to provide the substrate for landmark and panorama learning in *Cataglyphis* (Stieb et al., 2010), and previous studies were able to describe a substantial degree of plasticity in the mushroom bodies visual input region (collar) related to the interior–forager transition and age of the ants (Kühn-Bühlmann and Wehner, 2006; Seid and Wehner, 2009; Stieb et al., 2010, 2012). However, the neuronal substrate for processing sky-compass information in *Cataglyphis* was no subject of previous studies, although *Cataglyphis* ants have long been a model system to investigate behavioral components of sky-compass based navigation (reviewed in Wehner, 2003; Wehner and Rössler, 2013).

Main work on the anterior insect sky-compass pathway was conducted in the locust *Schistocerca gregaria* (reviewed in Homberg, 2004; Homberg et al., 2011; Pfeiffer and Homberg, 2014)(see fig. 5): Specialized photoreceptors in the dorsal rim area of the compound eye enable the detection of the polarization (POL) pattern of the sky (Labhart, 1980, 1986, 1988; Meyer and Domanico, 1999; Homberg and Paech, 2002). These photoreceptors exhibit POL opponency, i.e. they show maximal sensitivity towards a certain electric field-vector (e-vector) direction and maximal inhibition towards the respective e-vector direction rotated by 90° (Labhart, 1988). Since every ommatidium in the dorsal rim area is sensitive to a different e-vector orientation, the POL pattern of the sky elicits a certain firing pattern of receptor

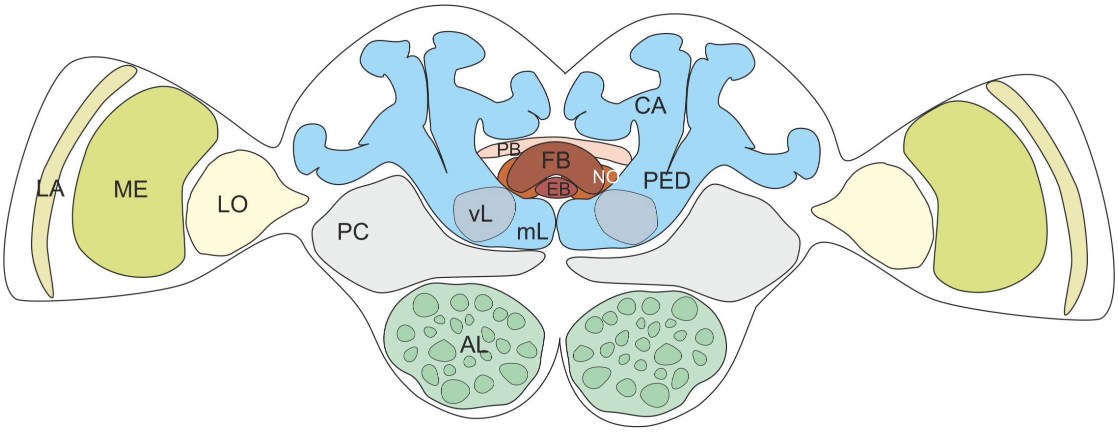

Figure 4: **Schematic overview of the *Cataglyphis* central brain.** Visual information received in the compound eye is first processed in the optic lobe consisting of lamina (LA), medulla (ME) and lobula (LO). The first olfactory processing center, the antennal lobe (AL) consists of definite units, called glomeruli. Higher order integration centers are the mushroom bodies and the central complex. The mushroom bodies consist of sensory input regions, the calyces (CA), the peduncle (PED) formed by Kenyon cells and the vertical lobe (vL) and medial lobe (mL). The central complex consists of the paired noduli (NO), the unpaired fanshaped body (FB), ellipsoid body (EB) and protocerebral bridge (PB). PC: protocerebrum.

neurons in this area depending on the position of the sun. Photoreceptors from the dorsal rim area project into the dorsal parts of the lamina and medulla (Blum and Labhart, 2000; Homberg and Paech, 2002). In a distinct layer of the medulla, POL information is interconnected with neurons from the main retina, providing information about further sky-compass cues (reviewed in Homberg et al., 2003; Pfeiffer et al., 2005; el Jundi et al., 2014). In the honeybee, this layer has further been shown to be in close vicinity to pigment dispersing factor (PDF)-positive neurons, thus potentially providing circadian input (Zeller et al., 2015). Further processing of the sky-compass information takes place in the anterior optic tubercle (Pfeiffer et al., 2005). The last relay station before final processing of the sky-compass information takes place in the central complex are giant synapses in the lateral complex (Träger et al., 2008; Mota et al., 2011). Giant synapses consist of a large cup-shaped presynaptic site which contacts several postsynaptic profiles via a large number of active zones. Tangential neurons provide GABA-ergic (γ-

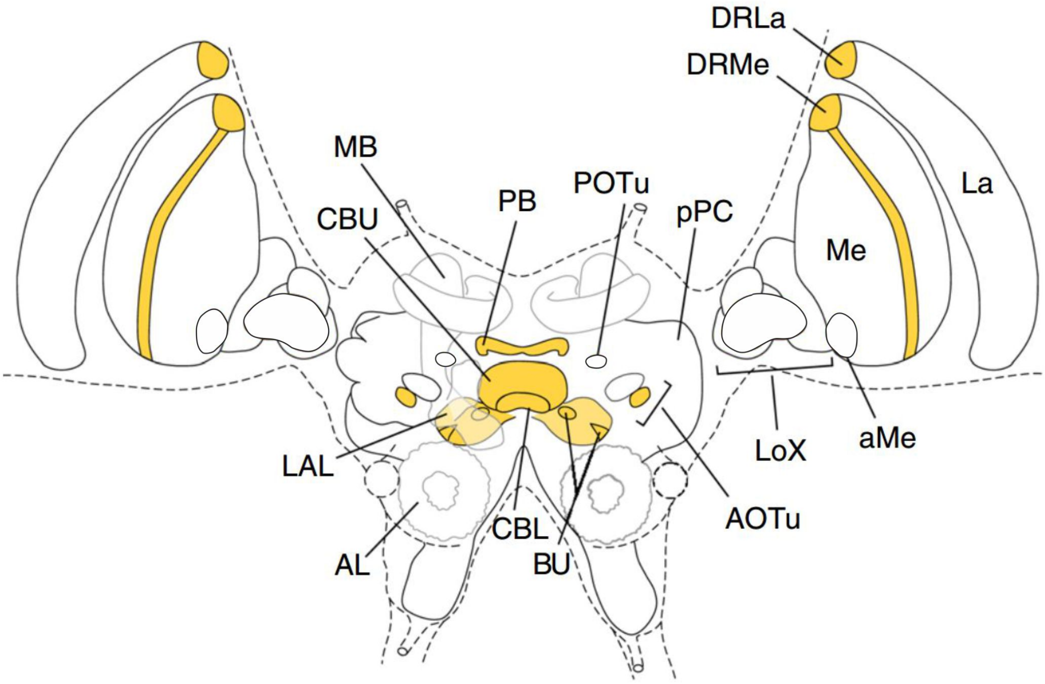

Figure 5: **Brain regions (highlighted in gold) involved in the processing of sky-compass information via the anterior pathway in the locust *Schistocerca gregaria*.** Polarized skylight is received via the dorsal rim area of the compound eye and further processed in the dorsal parts of the lamina (DRLa) and medulla (DRMe). In a distinct layer in the medulla (ME) polarized skylight information is suggested to be interconnected with sky-compass information from the main retina. Further processing centers of sky-compass information are the anterior optic tubercle (AOTu), the lateral accessory lobe (LAL), containing the medial and lateral bulbs (BU) and the central complex consisting of a lower (CBL) and an upper unit (CBU), protocerebral bridge (PB) and noduli. aME: accessory medulla, AL: antennal lobe, LoX: lobula complex, MB: mushroom bodies, POTu: posterior optic tubercle, pPC: posterior protocerebrum. (modified from el Jundi et al., 2014).

aminobutergic), thus inhibitory input to the ellipsoid body (former lower unit of the central complex; new nomenclature after Ito et al. 2014) (Träger et al., 2008). So far, in *Cataglyphis* POL sensitive UV-photoreceptors in the dorsal rim area of the compound eye (Herrling, 1976; Mote and Wehner, 1980; Labhart, 1986; Meyer and Domanico, 1999), and POL sensitive interneurons in the optic lobe (Labhart, 2000) are described.

During the transition phase from indoor to outdoor worker, the ants need to receive, process and learn information in a yet unknown environment. The position of the sun and with it the concentric POL pattern around it change over the course of the day. Furthermore, the sun's course depends on the geographic position and on the time of the year. Thus, the ants' sky-compass needs to be initially calibrated to these parameters when the ants leave the nest for the first time. This directional information also needs to be accounted with the distance information obtained from the step integrator. The resulting home vector is essential for navigation (Müller and Wehner, 1988). These drastic changes in behavioral demands from intranidal worker to forager correlate with changes in the neuronal structure of the mushroom body (Kühn-Bühlmann and Wehner 2006; Seid and Wehner 2009; Stieb et al. 2010, 2012; reviewed in Wehner and Rössler 2013). Therefore, the question arises whether the sky-compass pathway is subject to neuronal plasticity as well. A promising starting point is to investigate the conspicuously large giant synapses in the lateral complex (Träger et al., 2008).

2 Thesis outline

Cataglyphis ants have long been established as a model system to study vector navigation (reviewed in Wehner, 2003; Wehner and Rössler, 2013) and recent studies show that they provide an excellent model system to investigate the neuronal basis of their remarkable behavior, as well (Kühn-Bühlmann and Wehner, 2006; Seid and Wehner, 2009; Stieb et al., 2010, 2012).

In the present work I address two main problems:

1. Do neuropeptides act as internal drivers and modulators of behavioral transitions?
2. What is the neuronal substrate of sky-compass orientation and its plasticity?

To test whether neuropeptides influence *Cataglyphis*' behavior, they need to be identified in *Cataglyphis*. There is no access to *Cataglyphis* genome or expressed sequence tag (EST) data which is necessary to reliably predict neuropeptide sequences, masses and potential post-translational modifications (PTM). Therefore, in the first study I investigate the neuropeptidome of *C. floridanus*, another formicine ant, to later transfer this information to *Cataglyphis*. Based on neuropeptidomes of *A. mellifera* (Hummon et al., 2006), *Nasonia vitripennis* (Hauser et al., 2010) and genome annotations in *C. floridanus* (Nygaard et al., 2011) I mass spectrometrically identify the *C. floridanus* neuropeptidome and analyze the distribution of the respective neuropeptides throughout the nervous system.

In the second study, I describe the neuropeptide families AstA, AT, sNPF and

TK biochemically in *C. fortis*, assuming and confirming the same neuropeptide sequences as in *C. floridanus*. I use immunostainings to locate AstA, AT and TK in the central brain to see which brain regions they might influence. Further, I investigate potential age-related changes in the distribution pattern for TK immunohistologically.
The interior–forager transition is hypothesized to be highly influenced by changing activity levels and phototactic behavior. For the third study, I design a behavioral setup to test this hypothesis. Using this setup, I describe differences in activity and phototaxis levels in interior I, interior II and foragers. I further test the influence of light preexposure on interior workers. In addition, I manipulate the neuropeptides AstA and AT by injection into the brain to test their influence on behavior.

The interior–forager transition further triggers neuronal plasticity in the mushroom bodies (Kühn-Bühlmann and Wehner, 2006; Seid and Wehner, 2009; Stieb et al., 2010, 2012), the centers for learning and memory in the insect brain (reviewed in Heisenberg, 1998; Gerber et al., 2004), where landmark information was suggested to be processed (Mizunami et al., 1998; Stieb et al., 2010). However, *Cataglyphis* primarily relies on the sky-compass for navigation (reviewed in Wehner, 2003; Wehner and Rössler, 2013). Sky-compass information is processed in a distinct pathway in the insect brain (reviewed in Homberg, 2004; Homberg et al., 2011; el Jundi et al., 2014). In the fourth study, I neuroanatomically characterize the sky-compass pathway in the *Cataglyphis* brain using tracer injections and immunohistology. Focusing on giant synapses in the lateral complex, I investigate potential plasticity in the sky-compass pathway related to the interior–forager transition.

Manuscript 1

Neuropeptidomics of the carpenter ant *Camponotus floridanus*

Franziska Schmitt, Jens T. Vanselow, Andreas Schlosser, Jörg Kahnt, Wolfgang Rössler,
Christian Wegener

Journal of Proteome Research (2015) 14: 1504-1514

Abstract

Ants show a rich behavioral repertoire and a highly complex organization, which have been attracting behavioral and sociobiological researchers for a long time. The neuronal underpinnings of ant behavior and social organization are, however, much less understood. Neuropeptides are key signals that orchestrate animal behavior and physiology, and it is thus feasible to assume that they play an important role also for the social constitution of ants. Despite the availability of different ant genomes and *in silico* prediction of ant neuropeptides, a comprehensive biochemical survey of the neuropeptidergic communication possibilities of ants is missing. We therefore combined different mass spectrometric methods to characterize the neuropeptidome of the adult carpenter ant *Camponotus floridanus*. We also characterized the local neuropeptide complement in different parts of the nervous and neuroendocrine system, including the antennal and optic lobes. Our analysis identifies 39 neuropeptides encoded by different prepropeptide genes, and in silico predicts new prepropeptide genes encoding CAPA peptides, CNMamide as well as homologues of the honey bee IDLSRFYGHFNT- and ITGQGNRIF-containing peptides. Our data provides basic information about the identity and localization of neuropeptides that is required to anatomically and functionally address the role and significance of neuropeptides in ant behavior and physiology.

Manuscript 2

Neuropeptides in the desert ant *Cataglyphis fortis*: Mass spectrometric analysis, localization and age-related changes

Franziska Schmitt, Jens T. Vanselow, Andreas Schlosser, Christian Wegener

Journal of Comparative Neurology; in press

Abstract

Cataglyphis desert ants exhibit an age-related polyethism with ants performing tasks in the dark nest for the first ∼4 weeks of their adult life before they switch to visually based long-distance navigation to forage. While behavioral and sensory aspects of this transition have been studied, the internal factors triggering the behavioral changes are largely unknown. We suggest the neuropeptide families Allatostatin A (AstA), Allatotropin (AT), short neuropeptide F (sNPF), and Tachykinin (TK) as potential candidates. Based on a neuropeptidomic analysis in *Camponotus floridanus*, we used nanoLC-ESI MS/MS to biochemically identify these neuropeptides in *Cataglyphis fortis*. Furthermore, we show that all identified peptide families are present in the central brain and ventral ganglia of *C. fortis* while in the retrocerebral complex, only sNPF could be detected. Immunofluorescence staining against AstA, AT and TK in the brain revealed arborizations of AstA and TK positive neurons in primary sensory processing centers and higher-order integration centers, while AT immunoreactivity was restricted to the central complex, the antennal mechanosensory and motor center, and the protocerebrum. In artificially dark-kept ants, we found that TK distribution changed markedly in the central complex from days 1 and 7 to day 14 after eclosion. Based on functional studies in *Drosophila* this age-related variation of TK is suggestive for a modulatory role in locomotion behavior in *C. fortis*. We conclude that the general distribution and age-related changes in neuropeptides indicate a modulatory role in sensory input regions and higher order processing centers in the desert ant brain.

Manuscript 3

Locomotor activity and phototaxis are influenced by the neuropeptides allatostatin A and allatotropin and by light exposure in the desert ant *Cataglyphis noda*

Franziska Schmitt, Myriam Franzke, Wolfgang Rössler

Abstract

Cataglyphis desert ants undergo an age-related polyethism that culminates in a forager state with extraordinary navigational capabilities. Intranidal workers in this genus first serve as inactive interior I ants (repletes) (∼14 days) and thereafter as interior II (∼14 days) which care for the brood and queen and maintain the nest. Subsequent to the ∼28 days indoor phase is a further transition of ∼2–3 days during which the ants perform learning and orientation runs to then serve as vision-based long distance foragers for their last ∼6–7 days. Locomotor activity and phototaxis are two main behavioral components that change throughout the ants' life, potentially before the ants transition to the following stage. We show that the three stages exhibit different degrees of locomotor activity levels with a rise from interior I to interior II to foragers, and that interior workers generally are negatively phototactic while foragers are not. We further show that light exposure, which under natural conditions accompanies the interior–forager transition and a potent stimulator for neuronal reorganization in very young ants, changes interior behavior but does not trigger a forager-like behavior. We chose the two neuropeptide families allatostatin A (AstA) and allatotropin (AT) for a first approach to manipulate *Cataglyphis*' behavior. Injections of AT into the brain via the medial ocellar tract resulted in an increase of positive phototaxis and general activity levels, whereas AstA led to a decrease in activity levels. Injection of both neuropeptides did not influence interior I behavior, suggesting that the effects are age- or stage-specific.

Manuscript 4

Experience-related reorganization of giant synapses in the lateral complex: potential role in plasticity of the sky-compass pathway in the desert ant *Cataglyphis fortis*

Franziska Schmitt, Sara M. Stieb, Rüdiger Wehner, Wolfgang Rössler

Journal of Developmental Neurobiology (2016) 76(4): 390-404

Abstract

Cataglyphis desert ants undergo an age-related polyethism from interior workers to relatively short-lived foragers with remarkable visual navigation capabilities, predominantly achieved by path integration using a polarized skylight-based sun compass and a stride integrating odometer. Behavioral and physiological experiments revealed that the polarization (POL) pattern is processed via specialized UV-photoreceptors in the dorsal rim area of the compound eye and POL sensitive optic lobe neurons. Further information about the neuronal substrate for processing of POL information in the ant brain has remained elusive. This work focuses on the lateral complex (LX), known as an important relay station in the insect sky-compass pathway. Neuroanatomical results in *C. fortis* show that LX giant synapses (GS) connect large presynaptic terminals from anterior optic tubercle neurons with postsynaptic GABAergic profiles of tangential neurons innervating the ellipsoid body of the central complex. At the ultrastructural level, the cup-shaped presynaptic structures comprise many active zones contacting numerous small postsynaptic profiles. Three-dimensional quantification demonstrated a significantly higher number of GS (∼13%) in foragers compared with interior workers. Light exposure, as opposed to age, was necessary and sufficient to trigger a similar increase in GS numbers. Furthermore, the increase in GS numbers was sensitive to the exclusion of UV light. As previous experiments have demonstrated the importance of the UV spectrum for sky-compass navigation in *Cataglyphis*, we conclude that plasticity in LX GS may reflect processes involved in the initial calibration of sky-compass neuronal circuits during orientation walks preceding active foraging.

3 Manuscript I

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4 Manuscript II

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5 Manuscript III

Locomotor activity and phototaxis are influenced by the neuropeptides allatostatin A and allatotropin and by light exposure in the desert ant *Cataglyphis noda*

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Abstract

Cataglyphis desert ants undergo an age-related polyethism that culminates in a forager state with extraordinary navigational capabilities. Intranidal workers in this genus first serve as inactive interior I ants (repletes) (∼14 days) and thereafter as interior II (∼14 days) which care for the brood and queen and maintain the nest. Subsequent to the ∼28 days indoor phase is a further transition of ∼2–3 days during which the ants perform learning and orientation runs to then serve as vision-based long distance foragers for their last ∼6–7 days. Locomotor activity and phototaxis are two main behavioral components that change throughout the ants' life, potentially before the ants transition to the following stage. We show that the three stages exhibit different degrees of locomotor activity levels with a rise from interior I to interior II to foragers, and that interior workers generally are negatively phototactic while foragers are not. We further show that light exposure, which under natural conditions accompanies the interior–forager transition and a potent stimulator for neuronal reorganization in very young ants, changes interior behavior but does not trigger a forager-like behavior. We chose the two neuropeptide families allatostatin A (AstA) and allatotropin (AT) for a first approach to manipulate *Cataglyphis*' behavior. Injections of AT into the brain via the medial ocellar tract resulted in an increase of positive phototaxis and general activity levels, whereas AstA led to a decrease in activity levels. Injection of both neuropeptides did not influence interior I behavior, suggesting that the effects are age- or stage-specific.

Introduction

Cataglyphis ants, as many other social insect species, show an age-related division of labor (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984; Wehner and Rössler, 2013). Newly eclosed ants are callows for the first ∼24h. They can be easily recognized by their pale and soft cuticle. *Cataglyphis* ants then undergo two major life-stage transitions: the first from the interior I to the interior II stage, and the second from the interior II to the forager stage. Interior I workers are living food storages (repletes) which mainly remain in one part of the dark nest and are mostly motionless. They can be easily distinguished from other workers by their stretched intersegmental membranes and immobility. After about 14 days, *Cataglyphis* workers transition to fulfill duties like tending brood and queen, or maintaining the nest. On average, the ants live for a total of ∼28 days as indoor workers and then rapidly transition to day-active, visually guided outdoor foraging which they pursue for about 6-7 days.

This latter transition requires drastic changes in the ants' behavior. While interior workers are adapted to the dark nest surroundings, foragers have long been known for their sophisticated visually guided navigation (Wehner 1968; reviewed in Wehner 2003; Wehner and Rössler 2013). The transition includes a 2-3 days orientation phase during which the ants are suggested to acquire landmark and panorama information and calibrate their compass system (Wehner et al., 2004;

Stieb et al., 2012; Fleischmann et al., 2016). This is accompanied by the massive neuronal changes associated with first light exposure in these ants (Stieb et al., 2010, 2012; Schmitt et al., 2016a). While the consequences of this transition are well studied on a behavioral and neuronal level, the driving internal factors so far remained elusive.

The interior–forager transition has been studied intensely in Hymenoptera regarding regulating factors like the juvenile hormone (JH)–vitellogenin (VG) system (e.g. Jassim et al., 2000; Elekonich et al., 2001; Amdam and Omholt, 2003; Dolezal et al., 2012; Scholl et al., 2014; Pandey and Bloch, 2015) and insulin signaling (Ament et al., 2008). So far, these factors were not shown to cause behavioral transitions. A further group of molecules that might directly affect transitions are neuropeptides which are known to be important regulators of insect physiology and behavior (for a review see Nässel and Winther, 2010). Previous studies in the honeybee assessed stage-specific neuropeptide levels (Brockmann et al., 2009; Pratavieira et al., 2014) and in addition a recent study from our group analyzed the neuropeptide families allatostatin A (AstA), allatotropin (AT), short neuropeptide F (sNPF) and tachykinin in *Cataglyphis* ants (Schmitt et al., 2016b). In the present study we focus on two of these peptides: AstA and AT which are both distributed in the brain, suggesting neuromodulatory functions. We aim to show how they affect two behavioral traits which we hypothesize to change before the ants' transition: locomotion activity and phototaxis.

Interior I ants hardly move, while interior II ants care for the brood inside the nest, thus are more active, and foragers navigate for distances over more than 1000 m (Buehlmann et al., 2014; Huber and Knaden, 2015) in the open field to scavenge for food (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984; Wehner and Rössler, 2013). While we assume that phototaxis levels do not change at the interior I–interior II transition, foragers are deemed more positively phototactic than interior ants. This has already been shown for *C. bicolor* (Wehner et al., 1972) and honeybees (Ben-Shahar et al., 2003).

We therefore designed a behavioral experiment that allows to reliably assess the ants' activity and phototaxis levels. Light is a major environmental stimulus, that drastically alters neuronal networks (Stieb et al., 2010, 2012; Schmitt et al., 2016a) and behavior (Wehner et al., 1972; Stieb et al., 2012) in *Cataglyphis*, and JH levels (Scholl et al., 2014), gene expression and behavior in honeybees (Becker et al., 2016). Therefore, we exposed interior ants to light and tested potential changes in activity and phototaxis. As neuropeptides are considered potent neuromodulators that can change the sensitivity or activity of distinct groups of neurons (reviewed by Nässel, 2000), we also tested the effect of the two neuropeptides AstA and AT on *Cataglyphis* behavior via direct injections into the head capsule.

Material and methods

Animals

C. noda colonies were excavated in the Schinias national park (38°08'N, 24°01'E) near Marathon, Greece, in May 2015. They were kept in constant darkness at 29°C and ∼30-40% relative humidity with free access to water, and fed with honey water (1:2) and dead cockroaches. We categorized interior I ants as workers with a swollen gaster, stretched intersegmental membranes and sluggish movement. Interior II ants were identified as ants actively caring brood inside the dark nest box. To collect foragers, the colony was connected to a daylight-exposed arena where dead cockroaches and honey water were provided as the colony's sole food source.

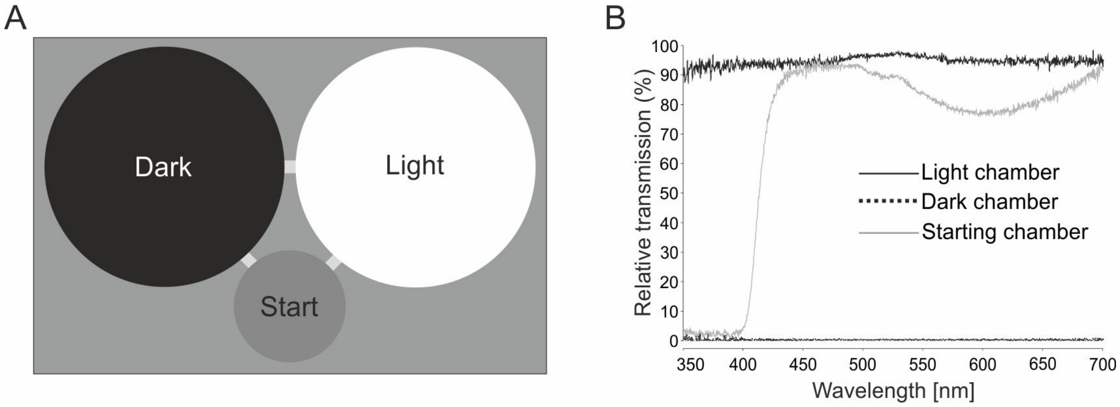

Figure 1: **Setup used for behavioral experiments.** The setup (**A**) consisted of 3 different connected chambers: a starting chamber (Start) covered by a darkened disc, transmitting only light above 400 nm (**B**, gray line), a light chamber (Light) covered by a disc transmitting all visible wavelengths and UV (**B**, black line), and a dark disc (Dark) covered by an optically opaque disc (**B**, dashed line). Dark and light disks were interchangeable. The connecting pathways consisted of closable, light-transmitting plugs.

Behavioral setup and testing procedure

To test for activity and phototaxis behavior we used a behavioral setup out of polyvinyl chloride (PVC) that consisted of 3 chambers connected by transparent, closable short passages (width: 0.7 cm; length: 1,7 cm) (see fig. 1). The two large connected test chambers (radius: 7.5 cm) could be covered by an optically opaque PVC plate (dark chamber) or a light-transmitting Plexiglas plate (light chamber). As *Cataglyphis* ants are sensitive to ultraviolet (UV) and green light (Mote and Wehner, 1980), we chose a Plexiglas plate transparent in the visible and UV-range. The small starting chamber (radius: 3.5 cm) was covered by a PVC plate only transmitting visible light.

The behavioral test started with putting a single ant into the starting chamber while the passages to both test chambers were still locked. Using single ants excluded social context as nestmate interaction might influence behavior. Usually, the ants were very agitated and explored the starting chamber. In preliminary experiments the ants needed a maximum of 60 sec to adapt to the new situation and environment and calm down. Therefore, we set a fixed time of 60 sec (acclimatization phase) until the passages to the test chambers were opened. Both passages were closed again after the ant left the starting chamber, so that it could only pass between the light and dark chamber. The following parameters were recorded for a total of 8 min (testing phase) after the acclimatization phase:

- time spent in starting chamber
- first choice (light or dark chamber)
- total time per test chamber
- number of crossings between the light and dark chamber

All behavioral tests took place under a UV-emitting daylight lamp (Solar Glo 125W, Exo-Terra). The temperatures ranged between 29 and 34 °C. To ensure that the ants' first choice did not depend on a right or left bias or is influenced by the environmental conditions in the room, the plates of the testing chambers were pseudo-randomly interchanged. Also, to prevent that the previously tested ant left any olfactory cues, all chambers were thoroughly cleaned with 70 % ethanol after each trial.

Light exposure

For light stimulation, ants were kept in subcolonies of 25–30 ants. 5 days light stimulation of interior I and interior II *C. noda* took place under a 12:12 h light:dark cycle. Based on their behavior and appearance interior I ants are more easily identifiable compared to interior II ants (see description in the introduction). Interior I ants were further used to test the effect of neuropeptides on behavior (see methods below). The procedure of harnessing the ants and injecting the peptide into the head capsule took about a total of 15 min. To test the effect of this comparably short light pulse on behavior, we exposed an additional interior I group to a 15 min light stimulus. This light exposure was given 24 h prior to testing and after the stimulus the ants were kept in darkness. All subcolonies had free access to water, dead cockroaches and honey water.

Peptide injection

Interior I ants and foragers are behaviorally and ontologically most distant from each other and easier to identify than interior II ants (see also methods above). We therefore started to manipulate these two stages. Prior to injection, the ants were immobilized on ice and subsequently harnessed in a Plexiglas holder. Using an acupuncture needle a hole was pricked into the medial ocellus in preparation for the peptide injection. 35 nl containing 1 mM AstA or AT (GeneCust, Luxembourg; peptide dilution as in Urlacher et al. 2016) in phosphate-buffered saline and 10 mM phenylmethylsulfonylfuorid (PMSF; gift of Elodie Urlacher), to prevent rapid degradation of the neuropeptides, were then injected into the ants' head capsule using the method introduced by Scholl et al. 2014. In short, the solution was injected through the punctured medial ocellus using glass capillaries (Borosilicate glass, O.D.: 1.0 mm I.D.: 0.3 mm, Sutter Instrument, Novato, USA) and a pneumatic picopump (Pneumatic PicoPump, World Precision Instrumetns, Ic., Sarasota, USA). After injection, animals were released from the Plexiglas holder and kept in a small box with access to food. The ants were tested 24 h after injection.

Data analysis

Data analysis was performed using Statistica 12 and 13 software (StatSoft (Europe) GmbH, Hamburg, Germany). As not all data were normally distributed (analysis of histograms and Kolmogorov-Smirnov Procedure, $p>0.05$), nonparametric tests were performed (Mann-Whitney U, $p>0.05$) to calculate differences in starting time, number of crossings per 100 sec and the relative amount of time the ants spent in the dark chamber. Differences in first choices between the groups was evaluated using a 2 x 2 contingency table (Fisher's exact, $p>0.05$). The same test was applied assessing differences in the relative proportion of ants that did not leave the starting chamber. To test whether the first choice of a group was distinct from a 50 : 50 distribution, a chi-square test ($p>0.05$) was performed using GraphPad (graphpad.com/quickcalcs/chisquared1, GraphPad Software, Inc. La Jolla, Ca, USA).

Animals that did not leave the starting chamber were only included in the analysis of the proportion of ants that have not left the starting chamber.

Since not all animals spent the same amount of time in the test phase, number of crossings was calculated relative to the test time and is labeled as 'crossings per 100 sec'. Percentage of time spent in the dark chamber was calculated by subtracting the time spent in the starting chamber from the full testing time of 8 min which resulted in the total time, the ants spent in the arena. Dividing the total time ants spent in the dark chamber by total time spent in the arena resulted in the relative time spent in the dark chamber.

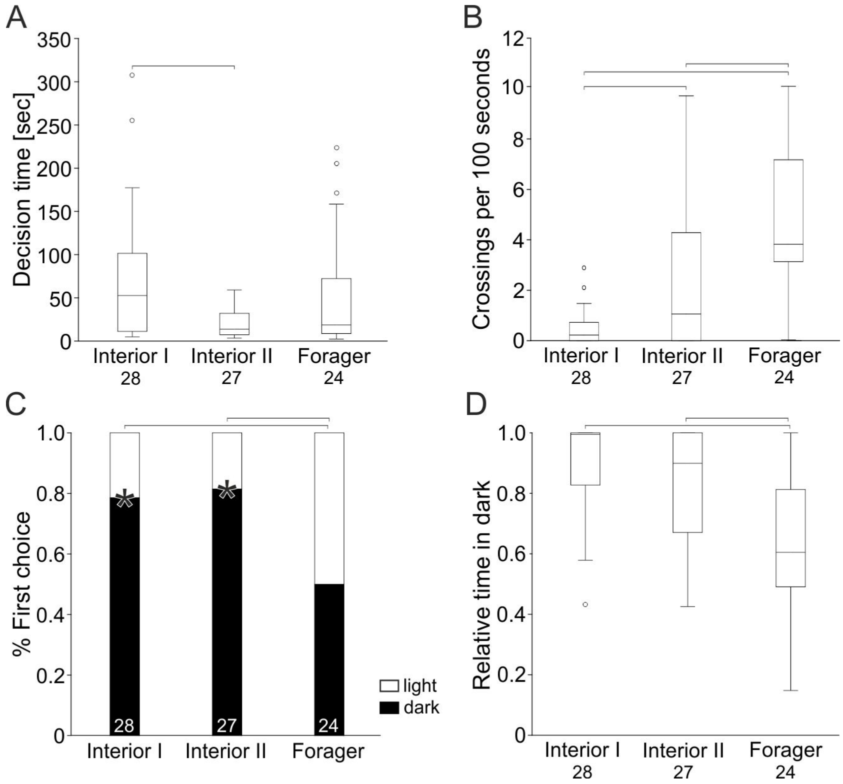

Figure 2: **Different behaviors of interior I, interior II and foragers in the setup. A.** Interior I animals took significantly longer to leave the starting chamber than interior II but there was no difference between interior workers and foragers. **B.** Interior I animals crossed less often between the light and dark chamber than interior II and foragers. Interior II crossed less often than foragers. **C.** Interior I and interior II walked into the dark chamber first while foragers chose equally between the light and dark chamber. Interior I and interior II chose the dark chamber more often than foragers. **D.** Interior I and interior II ants spent relatively more time in the dark chamber than foragers. * significant first choice for either light or dark chamber; lines above the bar indicate significant differences between groups.

Results

To test the manipulatory effects of light and neuropeptides on *Cataglyphis* behavior, we first had to design a reliable setup and protocol to test behavioral differences between stages. The setup employed allowed recording of four behaviors: starting or decision time, first choice, number of crossings, relative time in the dark chamber. After putting the ants in the starting chamber, they had 1 min to acclimatize. This time was sufficient for all tested ants to calm down. During this first minute, most ants started to explore their new environment and most of them already discovered the passage-plugs. When the passages were opened, most ants were quite quick to leave the starting chamber and enter one of the test chambers. However, in many cases the ants explored both passages before deciding for one. Often, ants entered the passages which are about one ant-length long and went back into the starting chamber backwards after experiencing the light condition in the respective test chamber.

Interior I workers needed significantly longer (52.7 sec) to decide than interior II ants (13.9 sec; $p<0.01$, $U=219$) (see fig. 2A), and changed significantly less between the two chambers (0.2 times) than interior II (1.1 times; $p=0.01$, $U=234.5$) and foragers (3.1 times; $p<0.001$, $U=42.5$). Foragers also crossed more often than interior II workers ($p<0.01$, $U=172.5$) (see fig. 2B). Both interior groups chose the dark chamber first (interior I: $p<0.01$; interior II: $p=0.001$) while foragers appeared indifferent to dark and light conditions ($p>0.05$)(see fig. 2C). Foragers had a higher probability to choose the light chamber first than both interior stages (interior I: $p<0.05$; interior II: $p<0.05$) and they also spent relatively less time in the dark chamber than interior ants (interior I: 99.5%; interior II: 89.9%; forager: 60.4%; interior I - forager: $p<0.001$, $U=90.5$; interior II - forager: $p<0.001$, $U=146$; see fig. 2D).

To test the effect of light exposure on activity levels and phototaxis in *C. noda*, interior I and interior II ants were exposed to light. Results from interior I, interior II and foragers described above are used as a reference. While for interior II ants only 5 days of light exposure was tested, interior I ants were preexposed to 5 days or 15 min of light and tested on the following day. Light exposure did not have an effect on the time interior I ants spent in the starting chamber (interior I no light: 52.7 sec, 15 min light: 18.4 sec, 5 days light: 20.2 sec; see fig. 3A, black boxes) but

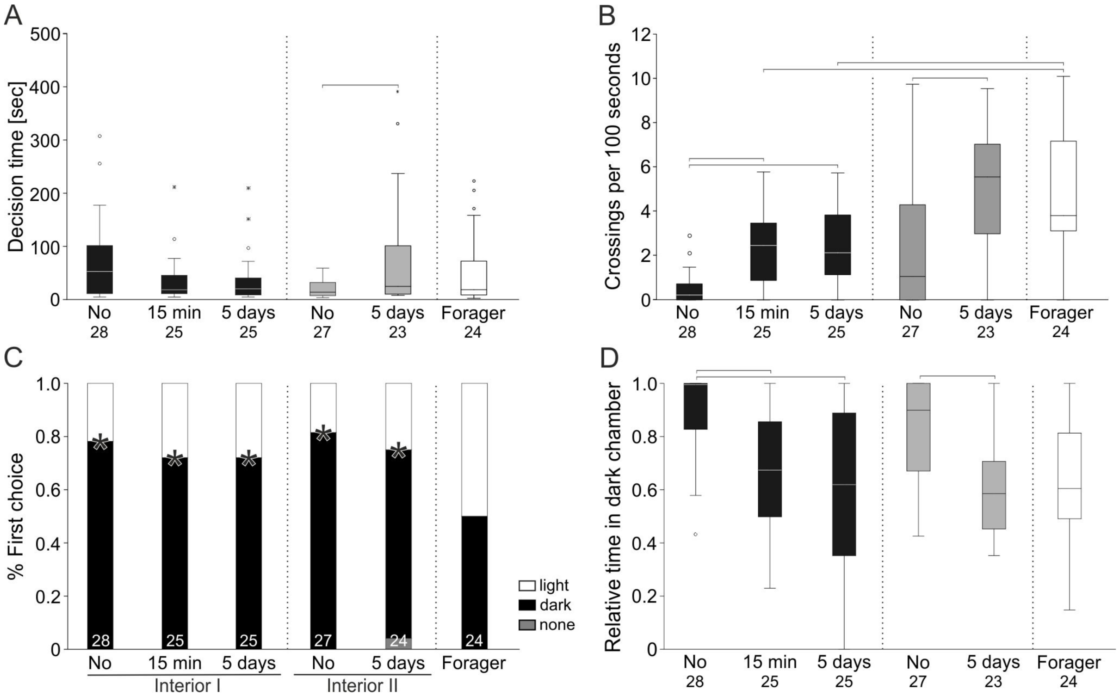

Figure 3: **Effect of light exposure on interior I and interior II ants.** **A, B, D**: black boxes represent interior I, gray boxes represent interior II ants, white boxes represent foragers. **A.** Light exposure did not affect the decision time of interior I but significantly effected decision time in interior II ants. **B.** Light exposure resulted in a higher number of crossings in both interior stages, but only in interior II ants the number of crossing was at the forager level. **C.** First choice of interior I and interior II ants after light exposure is the dark chamber. Light exposure did not have an effect on the first choice as compared to control interior I and interior II ants. **D.** Light exposure led to a decrease of the relative time interior I and interior II spent in the dark chamber, to the same level as forager. * significant first choice for either light or dark chamber; lines above the bar indicate significant differences between groups.

they crossed between the dark and light chamber significantly more often than control interior I ants following both light preexposure conditions (interior I no light: 0.2, 15 min light: 2.5, 5 days light: 2.1; no light – 15 min light: $p<0.001$, $U=96.5$; no light – 5 days light: $p<0.001$, $U=118.5$)(see fig. 3B, black boxes). The number of crossings however were still significantly lower than that of foragers (3.7; interior I 15 min light – forager: $p<0.01$, $U=162.5$; interior I 5 days light – forager: $p<0.01$, $U=156.5$)(see figs 3A and B, white boxes). 5 days of light preexposure in interior II ants resulted in a longer decision time (25.5 sec) as compared to control interior II animals (13.9 sec; $p<0.01$, $U=182.5$)(see fig. 3A, gray boxes) and in significantly more crossings between the test chambers (interior II no light: 1.1, 5 days light: 5.5; $p<0.001$; $U=142.5$)(see fig. 3B, gray boxes). Decision time as well as number of crossings of light exposed interior II ants did not differ from control foragers (see figs. 3A and B, white boxes). Light exposure did not result in any differences in the animals' first choice (see fig. 3C). All light exposed ants chose the dark chamber first ($p<0.05$) but spent relatively less time in the dark chamber compared to control interior I and interior II ants, respectively (interior I no light: 99.5%, 15 min light: 67.4%, 5 days light: 61.7%, interior II no light: 89.9%, 5 days light: 58.5%; interior I – interior I 15 min: $p<0.001$, $U=122.5$; interior I – interior I 5 days: $p<0.001$, $U=115$; interior II – interior II 5 days: $p<0.001$, $U=122$)(see fig. 3D, interior I: black boxes, interior II: gray boxes, foragers: white boxes). The relative time light exposed interior ants spent in the dark chamber did not differ from foragers (60.4%).

To test whether the neuropeptides AstA and AT affect *C. noda* behavior, the two peptides were co-injected with a peptidase-inhibitor (PMSF) into the medial ocellus of interior I workers and foragers. To control and correct for potential PMSF effects, one group was injected with PBS containing PMSF (control). In Interior I ants, the injection of AstA and AT did not have a significant effect on the time in the starting chamber (control: 20.4 sec, AstA-treated: 17.7 sec, AT-treated: 32.4 sec; see fig. 4A), the number of crossings between the dark and the light chamber (control: 1.4, AstA-trated: 1.4, AT-treated: 1.5; see fig. 4B), initial decision for one of the chambers (see fig. 4C) or the relative amount of time spent in the dark chamber (control: 56.5%, AstA-treated: 71.3%, AT-treated: 75.5%; see fig. 4D). AstA and AT animals chose the light chamber first ($p<0.05$), however the ratio did not differ from the control ants which were indifferent to light and dark.

In foragers, decision time was not influenced by peptide injection (control: 23.2

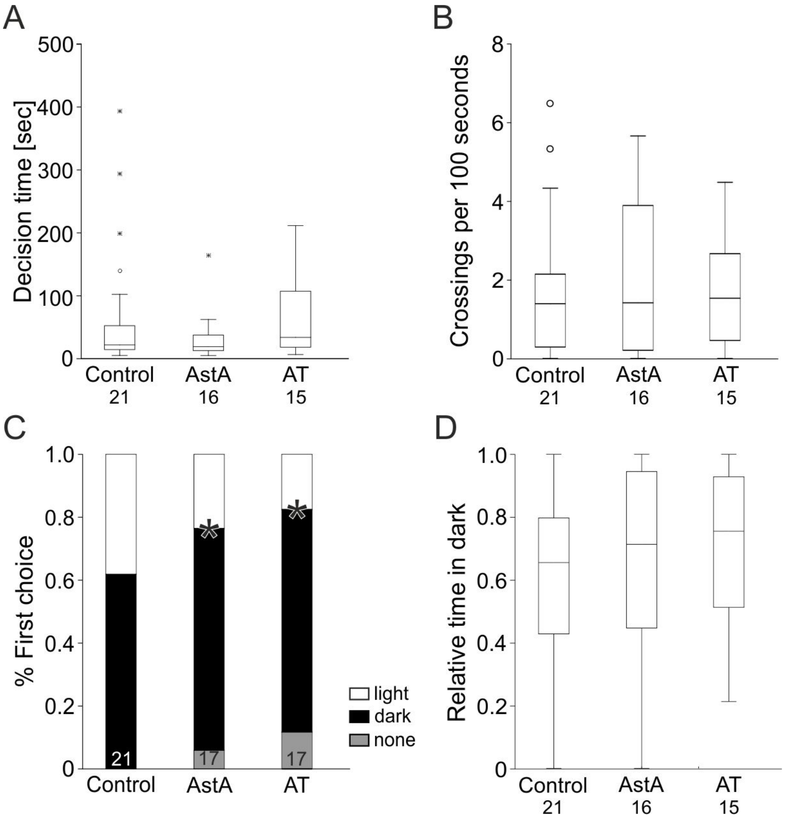

Figure 4: **Effect of allatostatin A (AstA) and allatotropin (AT) injection in interior I workers. A.** AstA and AT did not affect the decision time as compared to the control group. **B.** AstA and AT did not effect the number of crossings between the dark and the light chamber. **C.** AstA and AT injected animals chose the dark chamber first but not more often than the control animals. **D.** AstA and AT did not effect the relative amount of time the ants spent in the dark chamber. * significant first choice for either light or dark chamber.

sec, AstA: 72.7 sec, AT: 13.7 sec; see fig. 5A) but the AT treated group had a significantly higher number of crossings between the two test chambers than the control group (control: 1.3, AstA: 0.6, AT: 3.8; control – AT: p=0.01, U=85; see fig. 5B). A significant proportion of the AstA treated group did not leave the starting chamber (6 out of 15 animals) as compared to the control (2 out of 25; $p<0.05$). Those AstA-treated animals which left the starting chamber did not significantly chose for the light or the dark chamber, while the control group chose the dark chamber first (p=0.01; see fig. 5C). AT-injected animals decided for the light chamber ($p<0.05$) as opposed to the control ($p<0.001$). AstA-treated animals spent the same relative amount of time in the dark chamber (76.9%) as did control ants (84.1%). There is however a trend that AT-treated animals spent less time in the dark chamber (70.5%; $p=0.06$, $U=102.5$; see fig. 5D).

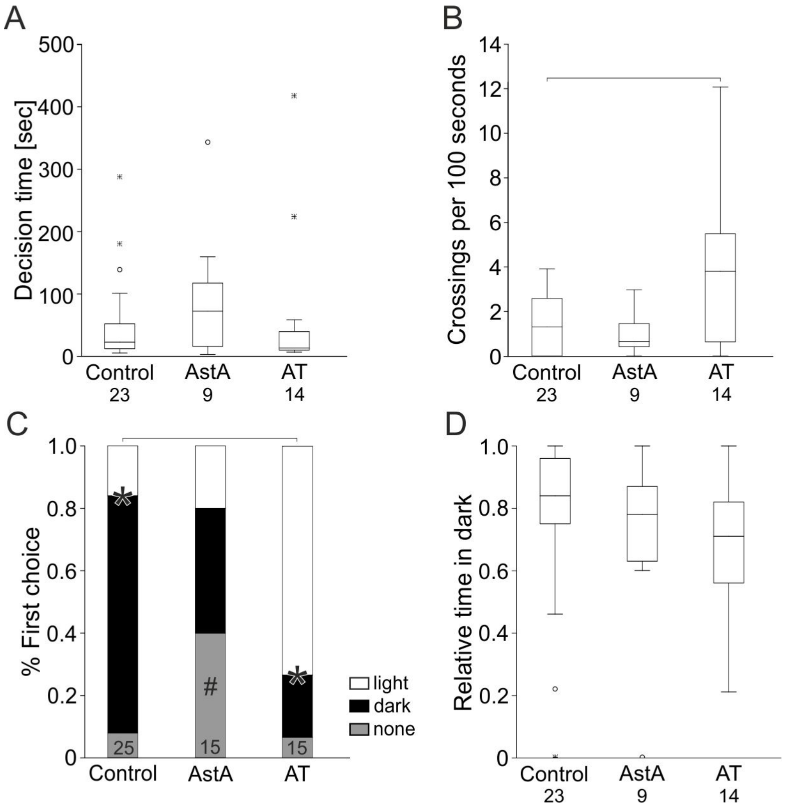

Figure 5: **Effect of allatostatin A (AstA) and allatotropin (AT) injection on forager behavior. A.** AstA and AT did not effect the decision time. **B.** AT injected foragers had a significantly higher number of crossings than control-injected animals. **C.** A significant proportion of AstA treated animals did not leave the starting chamber and those who did, were insensible for light and dark. AT treatment resulted in animals deciding for the light chamber first, while control animals significantly walked into the dark chamber. **D.** Time spent in the dark chamber was not different between the groups. However, there was a trend that AT animals spent less time in the dark chamber. * significant first choice for either light or dark; # significant proportion of animals did not leave the starting chamber; lines above the bar indicate significant differences between groups.

Discussion

Assessment of *Cataglyphis*' activity and phototaxis

In the present study, we used a newly developed behavioral setup to combine activity and phototaxis measurements in *C. noda*. Ants were tested in a situation that resembles the nest entrance: they were put in a small chamber and could then decide between light and dark conditions. To make sure, that the animals are motivated to leave the starting chamber, the light conditions differed from the two test chambers in that it is not completely dark (nest-interior) but also does not contain UV light (field conditions). We take two of the parameters tested (time in starting chamber and number of crossings) as a measure for activity and two of the parameters (initial choice and time spent in the dark chamber) as a measure for phototaxis levels. The results obtained from interior I, interior II and foragers clearly show that differences in behavioral traits can be detected using this setup with these four parameters. However, the single parameters cannot be interpreted independently from each other. All interior ants are negatively phototactic, thus the illuminated starting chamber is presumably stressful for these ants. Interior I ants, however, take longer to leave the starting chamber than interior II ants. While interior I are inactive repletes, interior II ants need to cover some distance within the nest to be able to maintain the colony and can thus be considered more active (Schmid-Hempel and Schmid-Hempel, 1984), therefore leaving the starting chamber more quickly. Foragers on the other hand do not appear to be negatively phototactic, as they have no preference in their first choice, and do not exhibit any significant trend in decision time. Interior II workers cross more often between the two chambers but in total do not spend more time in the dark chamber than interior I ants. This indicates that their elevated degree of activity increases the probability of them changing between the chambers but also that they might be more exploratory. However, there is no difference between the two interior stages in the relative time spent in the dark chamber, indicating that although the number of crossings cannot be interpreted decoupled from phototaxis as it involves entering an illuminated area, it can still be seen as a measure for activity. The high number of crossings and the relatively short time foragers spend in the dark chamber reflects the results from the parameters 'decision time' and 'first choice' as they exhibit the highest activity levels and spend only about 60% of their test-time in darkness.

The current results regarding phototaxis levels in interior workers and foragers are in line with previous results (Wehner et al., 1972; Ben-Shahar et al., 2003), although employing a different approach. In *C. bicolor*, interiors and foragers were tested for light and dark pattern preferences in a 360° arena (Wehner et al., 1972). While interior ants prefer dark patterns, foragers go into the direction of the brighter ones. Although phototaxis and pattern preference are difficult to tell apart, it is very likely that phototaxis plays a role in this experiment (Wehner et al., 1972). Experiments in the honeybee were based on a tunnel with a light source at the end and the time the animals needed to walk to the light source was measured (Ben-Shahar et al., 2003). While nurses never reach the end of the tunnel within 3 min, foragers always do, showing that honeybee foragers are positively phototactic as opposed to nurses. Therefore, a shift towards positive phototaxis from indoor to outdoor workers appears to be a reoccurring behavioral pattern in social insects.

While the present results in phototaxis are in line with previous studies, the activity levels of the different age-related behavioral stages in social Hymenoptera have, to our knowledge, not been compared so far. Our results indicate a steady activity rise from interior I to interior II to forager. Regarding the behavioral background of the ants, these findings fit very well to the hypothesis that locomotor activity levels change at both transitions while phototaxis only changes at the interior–forager transition.

The effect of light on activity and phototaxis in interior I and interior II ants

Light is a potent environmental stimulus associated with the interior–forager transition, especially in social insect species with a day-active foraging rhythm. Light exposure in ants and bees triggers neuronal reorganization in brain centers associated with the processing of visual stimuli which is supposed to be a relevant mechanism to enable successful vision based navigation (Stieb et al., 2010, 2012; Scholl et al., 2014; Schmitt et al., 2016a; Yilmaz et al., 2016). It further affects JH titers (Scholl et al., 2014) and gene expression (Becker et al., 2016) in nurse bees. To test whether light can trigger forager-like behavior in interior *C. noda*, they were exposed to 15 min (interior I) or 5 days (interior I and interior II) of light. Both light treatments (15 min and 5 days) affected interior I behavior to the same extent. While light preexposed interior II ants spend more time in the starting chamber than control interior II, all light exposed interior ants cross more often between the two test chambers compared to non light-exposed ants. However, light stimulation in interior I ants does not raise the number of crossings to the forager-level, indicating that there might be a stage-specific effect. Furthermore, our results suggest that light exposure does not trigger interior ants to initially chose for light conditions, although all preexposed interior groups do not significantly differ from foragers in the time they then spent in the dark chamber. The effect of light on interior behavior has previously been investigated in three studies conducted in social Hymenoptera (Wehner et al., 1972; Stieb et al., 2012; Becker et al., 2016). Wehner et al. (1972) tested *C. bicolor* for their preference to walk towards dark or bright areas, as explained above. In this approach, 3 days of light exposure in interior ants shifted their preference from walking towards dark areas to walking towards bright areas. This early study, however, did not differentiate between interior I and interior II ants. Becker et al. (2016) preexposed 1 and 7 days old honeybee nurses to five 45 min light pulses per day for five days. The bees' phototaxis level was subsequently assessed by measuring the time the animals need to walk towards a single light source in a dark arena. While phototaxis levels cannot be altered by light exposure in 1 day old bees, 7 days old bees become significantly positively phototactic after the treatment. In the third study, newly eclosed *C. fortis* were exposed to light five times a day (45 min each light pulse) for five days with activity analysis during and after the last light pulse per day (Stieb et al., 2012). The locomotor activity level of these ants was elevated on the 5th day of light exposure and only while the ants were exposed to light. Concluding the results of these previous studies and our present study, we hypothesize that light stimulation has stage-specific effects and does not result in generally higher activity levels or positive phototaxis. Instead, light conditions might be better accepted and lead to increased walking distances during light exposure but not in darkness. Under natural conditions, in *Cataglyphis* ants premature exposure to light might occur in polydomous (one colony is spread over several nests) colonies in which interior ants are carried from one nest to another one (Duelli, 1973, 1976; Fourcassie et al., 2000; Pfeffer and Wittlinger, 2016). The carried ants are able to use visual information to find back to the entrance of the nest they were extracted from (Duelli, 1976; Pfeffer and Wittlinger, 2016). In addition, some interior ants appear to be involved in the disposal of nest material outside the nest (Wehner and Menzel, 1969; Wehner et al., 1972; Fleischmann et al., 2016). These ants leave the nest in a straight line for very short distances, and immediately walk back to the nest. Thus, neuronal reorganization in response to visual stimulation as was shown in *Cataglyphis* (Stieb et al., 2010, 2012; Schmitt et al., 2016a) might be inevitable and also necessary in this situation as it enables successful homing. However, measuring JH levels in the honeybee after light exposure in young bees shows that there are limits to the extend in which the system can be accelerated. Although light preexposition rises the JH titer in young (4 and 7 days old) honeybees as well, it only reaches the foragers' titer in 10 days old bees, which are only a few days before starting their foraging career (Scholl et al., 2014). JH is well-studied and known to accompany and influence the interior–forager transition in bees (reviewed by Robinson, 1992; Pandey and Bloch, 2015) and ants (Dolezal et al., 2012). Similar results were also obtained, measuring gene-expression levels in 1 and 7 days old honeybees after light exposure (Becker et al., 2016). The upregulation of certain target genes after light stimulation in 7 days old bees is higher than in 1 day old light preexposed bees. In conclusion, light exposure appears to have differential stage-specific effects on behavior (Stieb et al., 2012; Becker et al., 2016), JH levels (Scholl et al., 2014) and gene transcription (Becker et al., 2016), while neuronal reorganization might be influenced independently from the animals' stage (Stieb et al., 2010, 2012; Scholl et al., 2014; Schmitt et al., 2016a). Especially with regard to the *Cataglyphis* transport system of interior ants in polydomous nests, having light as a sufficient and instantaneous trigger for interior ants to transition to the forager state might not be advantageous as this would disturb the age-related succession of the different stages which is the basis for successful division of labor in these ants.

Neuropeptidergic modulation of behavior in interior I workers and foragers

In addition to the stage-specific effect of light, we could see that the injection of AstA and AT have different effects interior I ants and foragers. Both, AstA and AT do not effect the behavior of interior I ants, compared to the control group, while foragers are influenced substantially. AT injection resulted in positive phototaxis, a trend to spend less time in the dark chamber than control animals, and a higher number of crossings between the two test chambers, indicating higher activity levels. In *C. fortis*, AT is present in the ventral ganglia and the brain, more specifically the fanshaped body of the central complex and paired neuronal connections in the antennal mechanosensory and motor center (former dorsal lobe in Hymenoptera; nomenclature after Ito et al. 2014) (Schmitt et al., 2016b). The central complex integrates sensory information and is the insect brain center involved in orientation and walking motivation, and control of locomotor output (reviewed in Strausfeld and Hirth, 2013; Pfeiffer and Homberg, 2014; Webb and Wystrach, 2016). As AT is present in the central complex it might directly influence locomotion activity. It is, however not clear how AT acts on phototaxis as it is not located in the optic lobe in *Cataglyphis* (Schmitt et al., 2016b) where it could directly modulate the light-sensitivity of neurons. So far, only few studies investigated the function of this neuropeptide in insects. In cockroaches, it interacts with the serotonergic system, influencing feeding behavior (Rudwall et al., 2000). In ants the serotonergic effect on feeding is confirmed (Falibene et al., 2012), but so far no data on the effect of AT on feeding or on colocalization of both neuromodulators in the nervous system are available. In honeybees, serotonin reduces positive phototaxis (Thamm et al., 2010) but no connection to AT has been tested so far. Whether and how AT and serotonin interact remains to be shown in future studies. However, influencing the serotonergic system might be one way how AT modulates phototaxis.

Almost half of the AstA-injected animals did not leave the starting chamber. However, the ones that entered the testing phase behaved the same as the control group. As for AT, functional studies of AstA are sparse, thus it can only be speculated about the pathways by which the neuropeptide influences behavior. However, immunolabeling in *C. fortis* shows that AstA neurons are closely colocalized with circadian pacemaker cells containing the output peptide of the circadian clock—pigment dispersing factor (PDF)(Schmitt et al., 2016b). In *Drosophila*, cells containing AstA and PDF are colocalized as well and it is suggested that AstA promotes sleep and reduces feeding behavior (Chen et al., 2016). Although AstA-injected ants that stayed in the starting chamber were not obviously sleeping, it might have been a similar effect that influenced the animals' behavior.

As no genetic tools are available for *Cataglyphis* yet, functional studies of neuroactive substances rely on the injection or feeding of the agent. This methodological approach does not allow for directed genetic up- or downregulation of e.g. neuropeptide genes in distinct neurons. Therefore, the specific interaction site often remains unknown. AstA is localized in sensory input regions of the brain, as well as higher order processing centers, including the mushroom bodies and the central complex (Schmitt et al., 2016b). Undirected peptide injection can thus influence several modalities in the brain. Urlacher et al. (2016) used a protocol similar to ours and showed that AstA-injection into the head capsule of honeybees impairs appetitive olfactory memory formation, while our results suggest that it effects activity levels or walking motivation. The distribution pattern of AstA in the *Cataglyphis*' (Schmitt et al., 2016b) and honeybee's brain (Kreissl et al., 2010) suggests that the peptide might affect several aspects of behavior. However, carefully designed future experiments will need to disentangle them.

Why do AT and AstA affect foragers but not interior I ants? The tested interior I ants were probably relatively old as over the time-span of the preceding 3 weeks no ants eclosed. However, their appearance indicated that their internal status was still 'interior I', meaning that the social colony factors most likely held them back from transitioning to interior II. Further, our testing protocol was aimed to disclose changes in the animals' activity and phototaxis levels but the figure of merit which we used to characterize the activity level 'number of crossings' was coupled to phototaxis. Thus, future experiments are necessary to exclude an influence of AstA and AT on the ants' locomotor activity levels. So far, the expression levels of neuropeptides in the nervous systems of different *Cataglyphis* behavioral stages are unknown. However, the levels of the neuropeptide tachykinin in the central complex of *C. fortis* are higher in newly eclosed ants compared to 2 weeks old ants (Schmitt et al., 2016b). Therefore, it is possible that the injection of additional neuropeptides does not affect behavior, if peptide levels are already high in a certain stage. Furthermore, it was shown in the fire ant *Solenopsis invicta* and in the honeybee that the receptor levels for sNPF are age- or task-related (Ament et al., 2011; Castillo and Pietrantonio, 2013). Thus, injection of a neuropeptide might have stage-specific effects, depending on both the amount of peptides and the amount of receptors.

Conclusion

This is the first study to provide evidence that neuropeptides can affect behavior in the context of temporal polyethism in a eusocial insect. We measured two important behavioral traits, locomotor activity levels and phototaxis, and show that they change as a function of temporal polyethism in *Cataglyphis*. Our data strongly suggest that locomotor activity and phototaxis can be influenced by light exposure which was also shown to affect neuronal structures in these ants (Stieb et al., 2010, 2012; Schmitt et al., 2016a) and hormonal (Scholl et al., 2014), and epigenetic levels in the honeybee (Becker et al., 2016). Moreover, we successfully manipulated activity levels and phototaxis using peptide injections. However, it became apparent that the effect of both—light and neuropeptides—might vary dependent on the animals' state. Here, we have successfully introduced a new kind of behavioral setup and performed initial experiments which clearly demonstrate its potential application for the investigation of the regulation of temporal polyethism in social insects.

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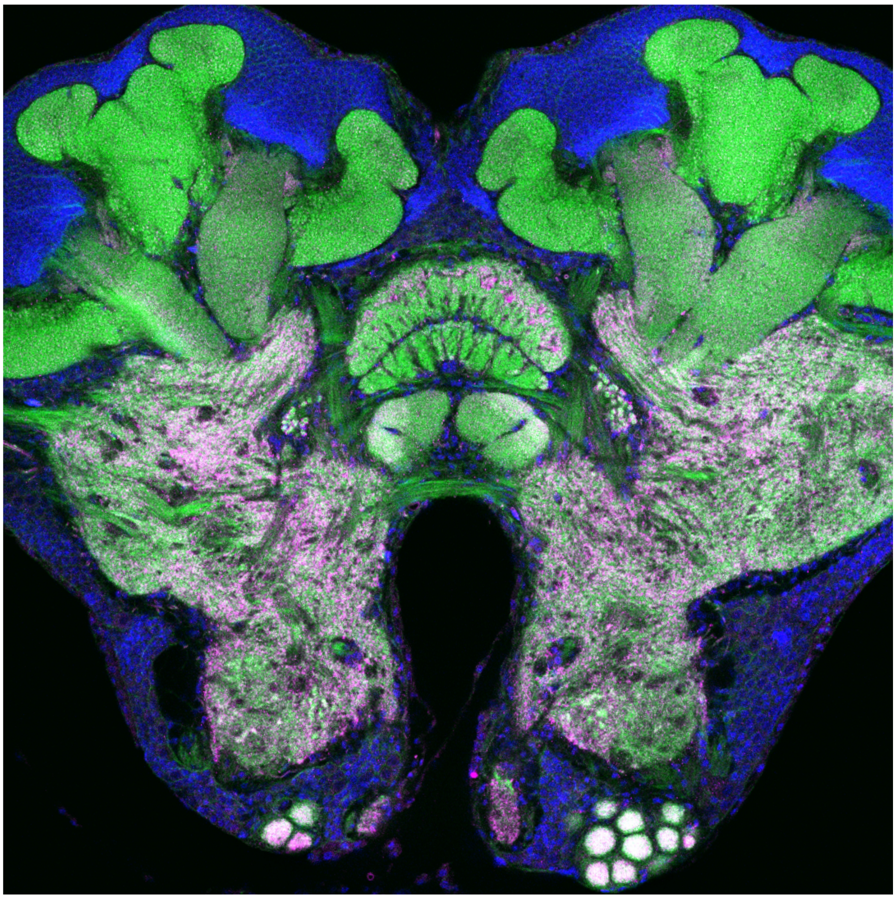
6 Manuscript IV



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7 Discussion

7.1 General discussion

In the present doctoral thesis the use of *Cataglyphis* as a model organism to study the neuronal basis of behavior is further established and extended. Introducing a new approach to transfer peptidomics information from a related formicine ant even opens up new opportunities to study the basis of the extraordinary level of behavioral plasticity of these ants. With ongoing age, *Cataglyphis* ants undergo two prominent behavioral transitions: from mostly inactive repletes (interior I) to locomotory active nurses (interior II) and from interior II workers in the dark nest to outdoor, day-active foragers (Schmid-Hempel and Schmid-Hempel, 1984; Wehner et al., 1972; Wehner and Rössler, 2013).
In this study I address two important objectives of *Cataglyphis*' behavior:

1. To understand the underlying mechanisms leading to a defined but flexible age-related succession of behaviors, focusing on the role of neuropeptides.
2. To understand the neuronal mechanisms underlying task- and experience-related plasticity in the *Cataglyphis* sky-compass pathway as a basis for successful navigation.

These two aspects are closely intermingled as neuropeptides can control physiology and behavior, leading e.g. to life-stage transitions (reviewed in Nässel, 2002;

Nässel and Winther, 2010) which then result in synaptic reorganization (see e.g. Groh et al., 2012; Hourcade et al., 2010; Stieb et al., 2010, 2012; Falibene et al., 2015). Synaptic reorganization in turn can trigger new neuropeptide levels, as e.g. elevated cAMP levels can increase peptide expression (reviewed in Goodman, 1990). However, to understand the complexity of this topic, a basic understanding of both aspects is necessary.

Neither genome nor EST data are available for *Cataglyphis* ants. Thus a prediction of neuropeptide sequences and potential posttranslational modifications with the according masses, and subsequent biochemical identification was conducted in the formicine ant *C. floridanus*. This resulted in the description of the first ant neuropeptidome (manuscript 1). Based on their function found in other insects, I considered the neuropeptide families AstA, AT, sNPF and TK prime candidates that might influence temporal polyethism in *Cataglyphis* and thus characterized them based on the *C. floridanus* neuropeptidome, assuming the same neuropeptide sequences. Using mass spectrometry and immunolocalization I biochemically identified these peptide families and described their distribution in the *C. fortis* nervous system with a focus on the central brain (manuscript 2). First age-related TK-immunostainings in *C. fortis* and manipulation experiments via the injection of AstA and AT in *C. noda* confirmed that these three peptide families can be considered relevant candidates to orchestrate behavioral transitions in *Cataglyphis* (manuscript 2 and 3). The major transition, entailing the most massive environmental changes in *Cataglyphis* ants, is the interior–forager transition. This transition is accompanied by synaptic plasticity in the mushroom bodies, presumably contributing to landmark learning (Kühn-Bühlmann and Wehner, 2006; Seid and Wehner, 2009; Stieb et al., 2010, 2012). As the sky-compass is the most relevant information employed in *Cataglyphis*' navigation, I characterized for the first time the sky-compass pathway in the *C. fortis* brain and showed that this pathway undergoes substantial synaptic plasticity (manuscript 4).

7.2 The regulation of temporal polyethism in *Cataglyphis*

7.2.1 Analysis of neuropeptides in *Cataglyphis* based on the *Camponotus floridanus* neuropeptidome

The *C. floridanus* neuropeptidome (manuscript 1) is the first one described in an ant species. For social insects in general the first neuropeptidome was published for the honeybee in 2006 (Hummon et al., 2006). On that basis, several functional studies were initiated in the following years regarding e.g. the role of neuropeptides in forager specialization (Brockmann et al., 2009), temporal polyethism (Brockmann et al., 2009; Pratavieira et al., 2014) or learning mechanisms (Urlacher et al., 2016). As neuropeptides undergo post-translational modifications which are essential for the bioactivity of the respective neuropeptide, the genome annotation of ant neuropeptides (Nygaard et al., 2011) does not reveal *bona fide* which neuropeptides are present in a specific stage and thus might be considered relevant for physiological processes or behavior (reviewed in Pauls et al., 2014). Therefore, biochemical identification using mass spectrometry is a crucial step.

I found the *C. floridanus* worker neuropeptidome to at least comprise a total of 39 neuropeptides out of 18 families. Although this neuropeptidomics study was able to detect more neuropeptides than previous attempts in the honeybee or *N. vitripennis* (Hummon et al., 2006; Hauser et al., 2010), especially large neuropeptides like the crustacean cardioaccelerating peptide (CCAP) or the neuropeptide F (NPF) still proved difficult to detect. Other peptides might only be active in different developmental stages or castes and may therefore have remained undetected in the present study (see manuscript 1 for a detailed discussion). However, these data provide a profound and important basis for future investigations regarding the role of neuropeptides in orchestrating the behavior of social insects.

I used this basis to identify neuropeptide candidates which I analyzed in a targeted approach employing mass spectrometry and immunohistology in *C. fortis* (manuscript 2). Both genera, *Camponotus* and *Cataglyphis*, belong to the formicine group and are closely related (Moreau et al., 2006; Hölldobler and Wilson, 2009; Gadau et al., 2012). In addition, neuropeptide sequences are highly conserved within insect species (Mirabeau and Joly, 2013) as they appear to be under strong stabilizing selection (Wegener and Gorbashov, 2008). Therefore, I assumed and confirmed that neuropeptide sequences correspond in both species. This also shows that—at least to a certain degree—genomic information is transferable between both related ant species. Furthermore, the ant genome information available via online databases improved in quality and quantity in recent years (e.g. for *Lasius niger*) and sequence comparison amongst more ant species will allow for a better evaluation of potential amino acid exchanges in a targeted sequence. This also opens up new genomic approaches towards a deeper understanding of the underlying mechanisms of behavior.

7.2.2 Neuropeptides act on behavioral traits linked to division of labor in *Cataglyphis*

Cataglyphis ants exhibit a distinct and well-described temporal polyethism (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984; Wehner and Rössler, 2013) and behavioral adaptations to the respective tasks can nicely be characterized as changes in locomotor activity, phototaxis or the sensitivity towards a specific stimulus related to a certain task. Neuropeptides potentially influencing these behaviors can—for a first approach—be narrowed down to AstA, AT, sNPF and TK which are successfully characterized biochemically in *C. fortis*. The four neuropeptide families were detected in the brain using mass spectrometry, hinting towards a neuromodulatory role. Furthermore, immunolabeling revealed that AstA, AT and TK are localized in the central complex. AstA and TK can additionally be found in the sensory input regions, the antennal and optic lobes, and in the mushroom bodies. The localization of neuropeptides in a respective brain region suggests them as potential modulators of neuronal sensitivity and activity in this neuropil (**?**)[reviewed in][]Naessel2002 (see manuscript 2 for a detailed discussion). Potential functional roles regarding *Cataglyphis* temporal polyethism are further investigated using immunohistology (TK; manuscript 2) and manipulation by direct injection of two of the neuropeptides into the head capsule (AstA, AT; manuscript 3).

Immunohistological staining in the *C. fortis* brain revealed age-related differences of the TK-distribution in the central complex. The central complex is the brain center supposedly controlling locomotor behavior in response to sensory signals and with reference to internal state in insects (e.g. Strauss, 2002; Bender et al., 2010; Guo and Ritzmann, 2013; Webb and Wystrach, 2016). Previous work in *Drosophila* suggests that different central complex neurons that are under neuropeptidergic control modulate locomotor behavior (Kahsai et al., 2010). In the *Drosophila* central complex, TK modulates locomotor activity levels (Winther et al., 2006; Kahsai et al., 2010) and spatial behavior like centrophobia (Kahsai et al., 2010). Kahsai et al. (2010) show, that distinct TK-expressing cells appear to modulate different aspects of locomotor behavior in the central complex. Whether this corresponds with the observation on a differential age-related distribution of TK in the central complex in *Cataglyphis* needs to be shown in future experiments. Furthermore, TK-expression is not restricted to the central complex in *Cataglyphis*. It is further localized in the antennal lobe of all insect species investigated in this respect (Nässel, 1999, 2002), including *Cataglyphis*, and is suggested to modulate olfactory sensitivity in the *Drosophila* antennal lobe (Winther et al., 2006). According to immunohistology in the *Cataglyphis* brain it might further influence the neuronal circuitry of the mushroom bodies and optic lobes. Age-related changes of the TK-expression in these neuropils, as suggested for the honeybee mushroom bodies (Takeuchi et al., 2004), cannot be excluded or confirmed at this stage.

AstA and AT-injection in *C. noda* interior I and foragers, the two behavioral stages ontologically most distant from each other, revealed a potential stage-specific effect of these neuropeptides. AT increases the activity of foragers and shifts their phototaxis towards a more positive level, while AstA lowers their activity. Both neuropeptides, however, did not affect the behavior of interior I ants. The regulation of temporal polyethism in social insects as *Cataglyphis* is suggested to be orchestrated by many factors (see fig. 3 of the introduction) and thus the right time-point in the animals' ontogeny might be crucial to successfully manipulate behavior. A similar, potentially stage-specific effect on behavior can be seen after light exposure as it triggers different levels of locomotor activity changes in interior I and interior II ants (see also manuscript 3 and discussion below). While light is a potent stimulus for synaptic reorganization in all social Hymenoptera species tested so far (*C. fortis*: Stieb et al. 2010, 2012; *Apis mellifera*: Scholl et al. 2014; *Camponotus rufipes*: Yilmaz et al. 2016), it appears to only accelerate behavior rather than trigger animals to immediately transition to the respective subsequent stage. This is supported by the fact that light exposure in honeybees has a stronger effect on JH titers and the levels of gene expression, the older the bees are and thus the closer they are to transitioning to the foraging stage (Scholl et al., 2014; Becker et al., 2016)(for a more detailed discussion see manuscript 3). Temporal polyethism in social insects exhibits a high degree of flexibility, as has been shown by experiments removing nurses or foragers from honeybee colonies

(Huang and Robinson, 1996), or studies with single-cohort colonies in ants (Dolezal et al., 2012) and bees (Jassim et al., 2000): if nurses or foragers are removed from a colony, foraging onset is delayed or accelerated, respectively, while in single-cohort colonies, animals of the same age fulfill different tasks. However, these artificial disruptions of the colony structure are massive and presumably involve changes of several factors, externally as well as internally.

To disentangle the various parameters influencing behavior at every stage, careful and integrative approaches will be necessary. These should involve measuring of potential changes, using e.g. immunolabeling or quantitative mass spectrometric analysis of peptide levels with subsequent manipulative studies: down– or upregulation of the respective peptide, followed up by analysis of behavioral changes. Furthermore, age-related polyethism cannot only be accompanied by varying titers of neuroactive substances but also by differing receptor levels as could be shown for sNPF in honeybees (Ament et al., 2011) and ants (Chen and Pietrantonio, 2006; Castillo and Pietrantonio, 2013). To still add to the level of complexity, the diverse group of potential factors is known to influence each other. For example insulin-signaling is modulated by neuropeptides (Lee et al., 2008), biogenic amine (Schulz et al., 2002) and gene transcription levels (Minakuchi et al., 2009) can be influenced by JH, and neuropeptides are known to have allatoregulatory potential (reviewed in Verlinden et al., 2015).

In this thesis I make a first and significant step in describing potential neuropeptide candidates involved in the regulation of *Cataglyphis* behavior. I give first evidence that AstA, AT and TK are involved in the timing and regulation of behavioral transitions. The change in TK distribution in the central complex, most prominent 14 days after adult eclosion, hints towards a role in the interior I–interior II transition. The effect of AstA and AT injection on the behavior of shows that the two neuropeptides might be involved in the interior–forager transition.

7.2.3 Stage-specific effects of light exposure in *Cataglyphis* ants

While the first part of this thesis was aimed to determine potential neuropeptides changing in the ants prior to transitions, thus triggering them, the second part considered potential behavioral and neuronal changes following the interior–forager transition. The most prominent change for a new forager is visual stimulation and the necessity to process visual information in order to successfully navigate. One important outcome of this thesis is that precocious light exposure appears to have different stage-specific effects on neuronal circuits, the hormonal system and behavior. It is not yet clear how far precocious light exposure can accelerate natural ontogeny but it clearly has stage-specific effects on the locomotor activity level of *Cataglyphis* ants, resulting in forager-like locomotor activity in interior II but not in interior I ants (manuscript 3). Furthermore, light–preexposure, has a stronger effect on JH levels and on changes in gene expression in older nurse bees than it has in very young nurse bees (Scholl et al., 2014; Becker et al., 2016). However, neuronal circuits in the mushroom body calyx (*Cataglyphis*: Stieb et al. 2010; honeybees: Scholl et al. 2014) and the sky-compass pathway (for a detailed discussion see below and manuscript 4) are fully reorganized and appear to be equal to the forager-state after the same precocious light exposure in newly emerged ants and bees. Under natural conditions, premature light exposure of interior ants in *Cataglyphis* mainly occurs in polydomous colonies, when an interior worker is carried to another part of the nest by a more experienced worker (Duelli, 1973, 1976; Fourcassie et al., 2000). Disturbing the carrier–carried couple shows that the unexperienced interior ant is able to use visual information to go back to the nest, it was extracted from (Duelli, 1976; Pfeffer and Wittlinger, 2016). In addition, some ants in the pre-forager stage leave the nest in a straight line, deposit waste at a radius of several centimeters and go back to the nest entrance in a straight line (Wehner and Menzel, 1969; Fleischmann et al., 2016). This shows, that under certain circumstances, ants are exposed to light prematurely in their natural habitat. In these cases, vision can be considered as an important basis for orientation and navigation leading the ants back to their nest, yet only over short distances (Wehner and Menzel, 1969). In contrast, light exposure should not necessarily trigger forager-like behavior in young interior ants as this would disrupt the colony structure which is based on age-related polyethism (for a more detailed discussion on the differential effects of light on *Cataglyphis* and honeybees, see manuscript 3).

7.3 Neuronal basis of sky-compass based navigation in *Cataglyphis*

While precocious light exposure appears to have limited effects on *Cataglyphis* behavior, it was shown to significantly influence the visual part of the *Cataglyphis* mushroom body input region, the collar (Stieb et al., 2010). *Cataglyphis* memorizes visual and olfactory landmarks, if they are available, to adjust their vector (Wehner et al., 1996; Collett et al., 1998; Bisch-Knaden and Wehner, 2003; Steck et al., 2009, 2011; Huber and Knaden, 2015). Based on data from the cockroach (Mizunami et al., 1998), the mushroom bodies are suggested to serve landmark learning in these ants (Stieb et al., 2010, 2012). However, their main navigational tool is the path integration system which is predominantly based on the sky-compass for directional information (reviewed in Wehner, 2003; Wehner and Rössler, 2013). Sky-compass orientation is a common feature in insect orientation and navigation. Many insects use it for simple course stabilization: a sky-compass system guides them into a certain direction, as for example short-distance ball rolling dung beetles (Byrne et al., 2003) or long-distance migrating monarch butterflies (reviewed in Merlin et al., 2012). Central place foragers like *Cataglyphis*, however, heavily rely on time-compensated sky-compass information about where in space their nest entrance is located (reviewed in Wehner, 2003; Wehner and Rössler, 2013). While course stabilization requires a fixed preset direction in which the animals move, central place foragers constantly calculate the mean direction they move into during an outbound run, and follow the reversed direction of that vector when homing (reviewed in Wehner, 1984). Although the behavior differs, processing of sky-compass information appears to take place in very similar neuronal pathways across insects. First full electrophysiological and anatomical description of the sky-compass pathway has been accomplished in the locust (reviewed in Homberg et al., 2011; Pfeiffer and Homberg, 2014). Further studies revealed that this pathway is conserved within insect species (reviewed by Merlin et al., 2012; el Jundi et al., 2014) based on data from the cricket (Blum and Labhart, 2000; Labhart et al., 1984; Sakura et al., 2008), monarch butterfly (e.g. Heinze and Reppert, 2012; Heinze et al., 2013), honeybee (Mota et al., 2011; Zeller et al., 2015) and bumblebee (Pfeiffer and Kinoshita, 2012). Neuroanatomical data of the sky-compass pathway from *Cataglyphis* (manuscript 4) further adds support for this. All insect species studied so far have a specialized area in the compound eye enabling polarization vision (dorsal rim area; Labhart, 1999). This information is then projected into the optic lobe (Blum and Labhart, 2000; Labhart, 2000; Homberg and Paech, 2002), where it is most likely interconnected with neurons providing information about sky-compass cues received via the main retina of the compound eye (reviewed in el Jundi et al., 2014), and clock neurons providing a potential neuronal substrate for time-compensation (Zeller et al., 2015). Further processing takes place in the anterior optic tubercle, the lateral complex and the central complex (reviewed in Homberg, 2004; el Jundi et al., 2014; Pfeiffer and Homberg, 2014). This study is the first providing detailed information about the entire sky-compass pathway in a central place foraging ant.

Cataglyphis ants are excellent navigators, able to accurately pinpoint their nest using the sky-compass as main source of directional information (reviewed in Wehner, 2003; Wehner and Rössler, 2013). However, their navigation system is error prone and the bigger the errors are that accumulate, the more likely the ants are to get lost, especially when reliable visual landmarks are sparse as in North African salt flats (Merkle and Wehner, 2008, 2010). As the sky-compass changes over the course of the day, and depends on the season and the geographic position the observer is located in, an initial calibration to a reliable reference system of the underlying neuronal pathway is crucial. In this thesis, I showed that light input, as opposed to age, is necessary and sufficient to trigger task-related synaptic reorganization of giant synapses in the lateral complex. This suggests that first light exposure causes reorganization of both visual pathways (see Stieb et al. 2010 for mushroom body pathway). I hypothesize that in the natural *Cataglyphis* ontogeny, this process takes place during learning and orientation walks at the interior–forager transition (Wehner et al., 2004; Stieb et al., 2012; Fleischmann et al., 2016). This phase is clearly necessary for landmark learning (Fleischmann et al., 2016) with the neuronal correlate for this process most likely being located in the mushroom bodies (Stieb et al., 2010, 2012). While, so far, it has not been shown that the orientation phase is causal for neuronal reorganization in the mushroom body or the sky-compass pathway, it is evident that learning processes are reflected in synaptic reorganization in the mushroom bodies of the honeybee (Hourcade et al., 2010) and leaf cutting ants (Falibene et al., 2015). Learning and orientation walks exhibit a distinct pattern which makes ants performing it clearly distinguishable from foragers or digging ants (Fleischmann et al., 2016) and they appear to be widespread, especially in central place foragers (ants: Müller and Wehner 2010; Nicholson et al. 1999, honeybees: Capaldi and Dyer 1999, wasps: Zeil 1993). I therefore hypothesize that the function of this particular behavior is the initial behavioral and neuronal calibration of the sky-compass to an earthbound reference, and, at the same time, learning and memorization of landmarks and panorama.

The enormous size of the giant synapses in the lateral complex (5-10 μm) might enable precise transmission of the sky-compass information to the central complex during foraging runs. Similarly large synapses were only found in the vertebrate auditory system, the calyx and the endbulb of Held (Ryugo et al., 1996; Schneggenburger and Forsythe, 2006). These axosomatic synapses support particularly fast and reliable signal transmission to enable a high precision in directional hearing (reviewed in Schneggenburger and Forsythe, 2006; Felmy and Künzel, 2014). A similar role has been suggested for insect giant synapses (Träger et al., 2008) and in *Cataglyphis*, it might be important for exact tuning of the sky-compass during foraging excursions (for a more detailed discussion, see manuscript 4).

While first light-exposure and orientation runs are suggested to be crucial for the initial calibration to the geographic position and season, it is not yet clear, how daily compensation of the compass is accomplished. The sun's position can change by up to ∼53° per hour (data for North African noontide in June), which is a time-span the ants are not unlikely to spend on a foraging trip. If the neuronal substrate was not able to compensate for this movement, the ants would invariably get lost after their first foraging trip, especially in a landmark-sparse environment like the North-African salt flats. Intracellular recordings, e.g. in the locust anterior optic tubercle and descending neurons connecting the central brain to thoracic ganglia, show that sky-compass information is indeed time-compensated (Pfeiffer and Homberg, 2007; Träger and Homberg, 2011). Input from the circadian system might be provided at the input stage in the medulla, as suggested from data in the honeybee (Zeller et al., 2015), as well as at the output stage via a posterior sky-compass pathway. This posterior pathway proceeds via the accessory medulla, which is considered as the insects' circadian pacemaker (Stengl and Homberg, 1994), the posterior optic tubercle and the protocerebral bridge of the central complex (el Jundi and Homberg, 2010). In the protocerebral bridge, sky-compass information from the anterior sky-compass pathway and from the posterior pathway could be integrated. However, both potential connections of the sky-compass pathway with the inner clock remain to be shown in *Cataglyphis* ants and in other insects.

Cataglyphis is able to flexibly weigh sky-compass and landmark information and, depending on the situation, decides which to follow (reviewed in Wehner et al., 2016). However, in addition to both visual pathways (mushroom body and sky-compass), the ants also process olfactory and anemotactic information (reviewed in Wehner, 2003; Wehner and Rössler, 2013; Wehner et al., 2016). Since *Cataglyphis* relies on all of them for successful navigation, the brain should provide a platform where preprocessed information from all modalities can be integrated and lead to a final decision. The central complex is the brain center controlling leg (Cruse, 1990) and head movements (Varga and Ritzmann, 2016), and navigation (reviewed in Strauss, 2002; Pfeiffer and Homberg, 2014; Webb and Wystrach, 2016) and would thus represent an adequate control center where information channels could be integrated. Visual and olfactory landmark information could be stored in the mushroom bodies (Mizunami et al., 1998; Hourcade et al., 2010). A connection between the mushroom bodies and the central complex, however, has only been suggested, so far (reviewed in Strausfeld and Hirth, 2013) but has not been proven. Anemotactic information is received via the Johnston's organ on the antennae and receptor neurons innervate the antennal and mechanosensory motor center, posterior protocerebral lobe and the subesophageal ganglion (honeybee: Ai et al. 2007; *Drosophila*: Kamikouchi et al. 2006; Yorozu et al. 2009), but a potential interconnection with mushroom body or central complex neuronal circuits remains to be shown. Future experiments will show, where in the *Cataglyphis* brain the sources of information relevant for successful and flexible navigation are integrated to result in an adaptive navigational decision.

7.4 Outlook

In this thesis I provided first insights into the complex topic of the neuronal basis of *Cataglyphis*' polyethism. Previous studies, mainly in the honeybee, primarily focused on JH and suggested it as the prime driver of temporal polyethism (reviewed in Robinson, 1992; Pandey and Bloch, 2015). This present study opens up a new point of view and shows that neuropeptides can act on two behavioral components suggested to change prior to the interior–forager transition: activity and phototaxis. Our findings imply that AstA levels are expected to decrease, while AT levels are expected to increase in the late interior II stage, prior to the onset of foraging. Further quantitative studies assessing neuropeptide levels are needed to confirm this hypothesis. In addition, more detailed investigations will also help to find out how the different internal factors might interact with each other.

The current findings suggest four neuropeptide families to be involved in the regulation of *Cataglyphis*' polyethism: AstA, AT, sNPF and TK. AstA, AT and TK could be shown to potentially influence *Cataglyphis* behavior. sNPF might be involved in the regulation of feeding behavior via the insulin pathway, as results in ants (Chen and Pietrantonio, 2006; Castillo and Pietrantonio, 2013), honeybees (Ament et al., 2008, 2011) and *Drosophila* (Lee et al., 2008) suggest. Furthermore, AstA (Chen et al., 2016) and AT (Rudwall et al., 2000) were shown to also be involved in the regulation of feeding behavior in insects. Therefore, testing e.g. the sucrose response following the manipulation of the neuropeptide titers could provide insights into how these peptides influence this behavior.

However, testing the effect of neuropeptides on behavior is just one future objective. The interior I–interior II and the interior–forager transition are likely to be accompanied by changing sensitivity levels towards pheromones, odors or visual stimuli. It is well established that neuropeptides can be potent modulators of neuronal sensitivity (reviewed in Nässel, 2002). Using, for example, extracellular recordings or calcium imaging while applying them directly on the neuronal substrate would not only show how they modulate neurons, e.g. whether a neuropeptide excites or inhibits neuronal activity, but also where in the brain and how behavioral outcome is modulated.

This thesis also provides valuable new insights into the processing of sky-compass information in the *Cataglyphis* brain. An interesting topic for future studies will be where and how landmark and sky-compass information are processed, stored and potentially integrated. Recent studies suggested that landmark information in *Cataglyphis* is stored in the mushroom bodies (Stieb et al., 2010, 2012) while the central complex is the last processing center for sky-compass information (reviewed in Pfeiffer and Homberg, 2014; Webb and Wystrach, 2016). To generate reliable output for navigation, both components need to be integrated. So far, a neuronal connection from the mushroom bodies to the central complex was only suggested (Strausfeld and Hirth, 2013). However, behavioral experiments showed that desert ants are able to flexibly weigh landmark and sky-compass information to determine their final course (reviewed in Wehner et al., 2016). This suggests that both information streams, although preprocessed independently from each other, are integrated in the brain. The location in the brain, the underlying mechanism and to what extent this connection is important for the calibration of the system will be investigated in future studies.

Time-compensation of sky-compass information is essential for successful navigation, especially in central place foragers as *Cataglyphis*. To understand where in the brain circadian input is provided to the sky-compass pathway, neuroanatomical investigations of the interface between circadian clock neurons and visual pathways in the brain are necessary. In addition, manipulation of the circadian input while at the same time recording of neuronal activity in neuropils processing visual information will help to assess how information processing is influenced by the circadian clock. The initial calibration of the sky-compass system is assumed to happen during the interior–forager transition phase (Stieb et al. 2010; Fleischmann et al. 2016; manuscript 4) and it is in this early outdoor stage when the ants need to couple the movement of the sun to a stable, earthbound system.

Abbreviations

AstA	Allatostatin A
AT	Allatotropin
CCAP	Crustacean cardioaccelerating peptide
EST	Expressed sequence tag
e-vector	electric field-vector
JH	Juvenile Hormone
NPF	neuropeptide F
PDF	Pigment dispersing factor
POL	Polarization
PTM	Posttranslational modification
sNPF	short Neuropeptide F
sNPFR	short Neuropeptide F receptor
TK	Tachykinin

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Curriculum vitae

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EDUCATION

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Nov 2012 – Aug 2013	**Master thesis**: "Genome annotation, mass spectrometric characterization and distribution of neuropeptides in the ants *Camponotus floridanus* and *Cataglyphis fortis*" Zoology II, University of Würzburg Supervision: Prof. Dr. Wolfgang Rössler Prof. Dr. Christian Wegener
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Jul 2007	Abitur (German University entrance qualification) Celtis-Gymnasium, Schweinfurt

Teaching experience

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since 2014	Mentoring of Bachelor and Master students

Research and field stays

May 2015	Field stay in Greece: Evaluation of new experimental site to study *Cataglyphis noda*
Nov – Dec 2014	Ecological Neuroscience Group; Australian National University (Australia) Research stay: The sky-compass pathway in the brain of *Myrmecia* ants.
June 2014	Field stay in Tunisia: Data acquisition within the PhD-thesis
Nov 2013	Ecological Neuroscience Group; Australian National University (Australia) Research stay: Tracking of individual *Myrmecia* ants using differential GPS.
June 2012	Field stay in Tunisia: Data acquisition for Master's practical course (Master F1)

Würzburg, 26.09.2016

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List of publications

PEER REVIEWED ARTICLES

Schmitt F, Vanselow JT, Schlosser A, Wegener C, Rössler W. In Press. Neuropeptides in the desert ant *Cataglyphis fortis*: Mass spectrometric analysis, localization, and age-related changes. Journal of Comparative Neurology. Doi: 10.1002/cne.24109

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Schmitt F, Vanselow JT, Kahnt J, Schlosser A, Rössler W, Wegener C. 2015. Neuropeptidomics of the carpenter ant *Camponotus floridanus*. Journal of Proteome Research 14:1505-1514. Doi: 10.1021/pr5011636

ORAL PRESENTATIONS

Schmitt F. 2014. Neuronal basis of behavior in the desert ant *Cataglyphis fortis*. Seminar 'Advances in *Cataglyphis* research', Jena, Germany.

Schmitt F, Stieb SM, Wehner R, Rössler W. 2015. Processing of sky-compass information in the brain of the desert ant *Cataglyphis fortis*. International Symposium and Workshop on "Frontiers in Insect Behavior, Social organization, and Evolution.

Schmitt F. 2015. Timing of behavioral transitions and initial calibration of the sun-compass pathway. Annual Meeting of the Sfb 1047 'Insect Timing'. Frauenchiemsee, Germany.

Schmitt F. 2016. Die neuronale Basis des Verhaltens bei der Wüstenameise *Cataglyphis fortis*. Seminar 'Advances in *Cataglyphis* research', Berlin, Germany.

Schmitt F, Rössler W. 2013. Investigations of the neuronal control of behavior in *Cataglyphis fortis*. Research School of Biology, Zeil-lab: Ecological neuroscience. Australian National University, Canberra, Australia.

Schmitt, F, Wehner R, Rössler, W. 2013. Investigation of the polarization vision pathway in the desert ant *Cataglyphis fortis*. Meeting of the Sfb 1047 'Insect Timing'. Tauberbischofsheim, Germany.

POSTER PRESENTATIONS

Schmitt F, Wehner R, Wegener C, Rössler W. 2015. Timing of behavioral transitions and initial calibration of the sky-compass pathway in *Cataglyphis* ants. Annual Meeting of the Sfb 1047 'Insect Timing'. Frauenchiemsee, Germany.

Schmitt F, Wegener C, Rössler W. 2015. Neuropeptides in the regulation of worker ontogeny in the desert ant *Cataglyphis fortis*. 11th Göttingen meeting of the German Neuroscience Society. Göttingen, Germany.

Schmitt F, Wehner R, Wegener C, Rössler W. 2014. The neuronal basis of behavior in the desert ant *Cataglyphis fortis*. Annual Meeting of the Sfb 1047 'Insect Timing'. Ellwangen, Germany.

Schmitt F, Wegener C, Rössler W. 2014. Discovering neuropeptides in the ant *Cataglyphis fortis* – a novel approach on the investigation of neuropeptides. 11th Congress of the International Society for Neuroethology, Sapporo, Japan.

Schmitt F, Rössler W, Wegener C. 2014. The neuropeptidome of *Camponotus floridanus*. International Symposium and Workshop on "Frontiers in Insect Behavior, Social organization, and Evolution. Würzburg, Germany

Schmitt F, Stieb SM, Wehner R, Rössler W. 2013. Light-induced plasticity of giant synapses in the lateral accessory lobe of the desert ant, *Cataglyphis fortis*. 10th Göttingen meeting of the German Neuroscience Society. Göttingen, Germany.

Stieb SM, Schmitt F, Wehner R, Rössler W.2011. Synaptic plasticity in visual and olfactory brain centers of the desert ant *Cataglyphis fortis*. Insect Homing: Mechanisms and Models. Bielefeld, Germany