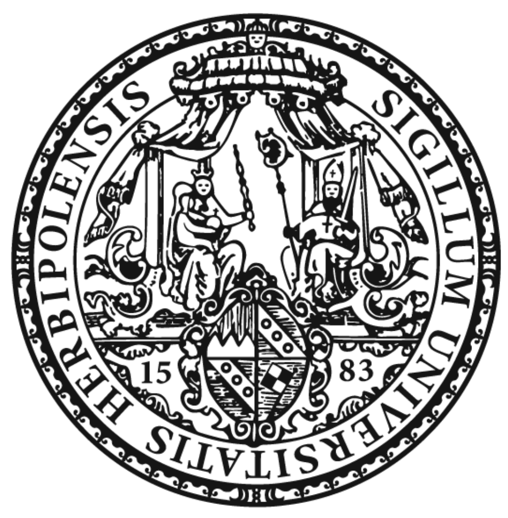
Timing of Sensory Preferences in *Camponotus* Ants

Zeitliche Anpassung sensorischer Präferenzen in *Camponotus* Ameisen



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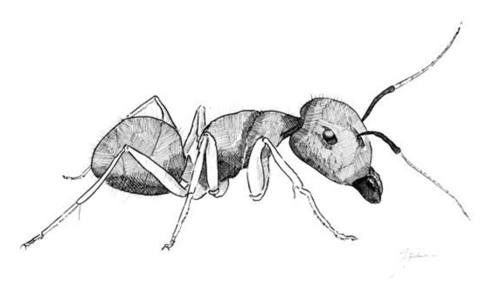

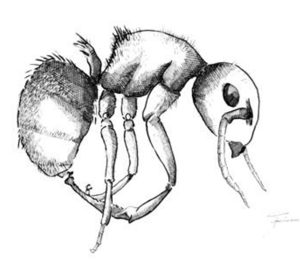

--- *Camponotus rufipes and Camponotus mus* ---

Author contributions

The experiments reported in the chapters I and II were performed in collaboration with project C1 (Prof. Dr. Flavio Roces, Dr. Stephanie Mildner) within the Collaborative Research Centre CRC1047 \`Insect Timing´. The field work reported in chapter II was additionally conducted in collaboration with Dr. Oliver Geissler (Zoology II, University of Würzburg). The work reported in chapter III was done in collaboration with project B3 (Dr. Johannes Spaethe) and B6 (Prof. Dr. Wolfgang Rössler) within the Collaborative Research Centre CRC1047 \`Insect Timing´. The EM tomography reported in chapter IV was performed in collaboration with Prof. Dr. Christian Stigloher (Zoology I).

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8	AL	CG			

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Summary

Ants belong to the most successful insects living on our planet earth. One criterion of their tremendous success is the division of labor among workers that can be related to age (age– or temporal polyethism) and/ or body size (size–related polymorphism). Young ants care for the queen and brood in the nest interior and switch to foraging tasks in the outside environment with ongoing age. This highly flexible interior–exterior transition probably allows the ant workers to properly match the colony needs and is one of the most impressive behaviors a single worker undergoes during its life. As environmental stimuli are changing with this transition, workers are required to perform a new behavioral repertoire. This requires significant adaptions in sensory and higher–order integration centers in the brain, like the mushroom bodies. Furthermore, foragers need proper time measuring mechanisms to cope with daily environmental changes and to adapt their own mode of life. Therefore, they possess a functional endogenous clock that generates rhythms with a period length of approximately 24 hours. The species–rich genus of *Camponotus* ants constitute a rewarding model to study how behavioral duties of division of labor were performed and modulated within the colony and how synaptic plasticity in the brain is processed, as they can divide their labor to both, age and body size, simultaneously.

In my PhD thesis, I started to investigate the behavioral repertoire (like foraging and locomotor activity) of two sympatric *Camponotus* species, *C. mus* and *C. rufipes* workers under natural and under controlled conditions. Furthermore, I focused on the division of labor in *C. rufipes* workers and started to examine structural and ultrastructural changes of neuronal architectures in the brain that are accompanied by the interior–exterior transition of *C. rufipes* ants.

In the first part of my thesis, I started to analyze the temporal organization of task allocation throughout the life of single *C. rufipes* workers. Constant video–tracking of individually labeled workers for up to 11 weeks, revealed an age–related division of labor of interior and exterior workers. After emergence, young individuals are tended to by older ones within the first 48 hours of their lives before they themselves start nurturing larvae and pupae. Around 52% switch to foraging duties at an age of 14–20 days. The workers that switched to foraging

tasks are mainly media–sized workers and seem to be more specialized than nurses. Variations in proportion and the age of switching workers between and within different subcolonies indicate how highly flexible and plastic the age–related division of labor occurs in this ant species. Most of the observed workers were engaged in foraging tasks exclusively during nighttime. As the experiments were conducted in the laboratory, they are completely lacking environmental stimuli of the ants´ natural habitat.

I therefore asked in a second study, how workers of the two closely related *Camponotus* species, *C. rufipes* and *C. mus*, adapt their daily activity patterns (foraging and locomotor activity) under natural (in Uruguay, South America) and controlled (in the laboratory) conditions to changing thermal conditions. Monitoring the foraging activity of both *Camponotus* species in a field experiment revealed, that *C. mus* workers are exclusively diurnal, whereas *C. rufipes* foragers are predominantly nocturnal. However, some nests showed an elevated daytime activity, which could be an adaption to seasonally cold night temperatures. To further investigate the impact of temperature and light on the differing foraging activity patterns in the field, workers of both *Camponotus* species were artificially exposed to different thermal regimes in the laboratory, simulating local winter and summer conditions. Here again, *C. mus* workers display solely diurnal locomotor activity, whereas workers of *C. rufipes* shifted their locomotor activity from diurnal under thermal winter conditions to nocturnal under thermal summer conditions. Hence, the combination of both, field work and laboratory studies, shows that daily activity is mostly shaped by thermal conditions and that temperature cycles are not just limiting foraging activity but can be used as zeitgeber to schedule the outside activities of the nests.

Once an individual worker switches from indoor duties to exterior foraging tasks, it is confronted with an entirely new set of sensory information. To cope with changes of the environmental conditions and to facilitate the behavioral switch, workers need a highly flexible and plastic neuronal system. Hence, my thesis further focuses on the underlying neuronal adaptations of the visual system, including the optic lobes as the primary visual neuropil and the mushroom bodies as secondary visual brain neuropil, that are accompanied with the behavioral switch from nursing to foraging. The optic lobes as well as the mushroom bodies of light–deprived workers show an `experience–independent´ volume increase during the first two weeks of adulthood. An additional light exposure for 4 days induces an `experience–dependent´ decrease of synaptic complexes in the mushroom body collar,

followed by an increase after extended light exposure for 14 days. I therefore conclude, that the plasticity of the central visual system represents important components for the optimal timing of the interior–exterior transitions and flexibility of the age–related division of labor. These remarkable structural changes of synaptic complexes suggest an active involvement of the mushroom body neuropil in the lifetime plasticity that promotes the interior–exterior transition of *Camponotus rufipes* ants. Beside these investigations of neuronal plasticity of synaptic complexes in the mushroom bodies on a structural level, I further started to examine mushroom body synaptic structures at the ultrastructural level. Until recently, the detection of synaptic components in projection neuron axonal boutons were below resolution using classical Transmission Electron Microscopy. Therefore, I started to implement Electron Tomography to increase the synaptic resolution to understand architectural changes in neuronal plasticity process. By acquiring double tilt series and consecutive computation of the acquired tilt information, I am now able to resolve individual clear–core and dense–core vesicles within the projection neuron cytoplasm of *C. rufipes* ants. I additionally was able to reveal single postsynaptic Kenyon cell dendritic spines (~62) that surround one individual projection neuron bouton. With this, I could reveal first insights into the complex neuronal architecture of single projection neuron boutons in the olfactory mushroom body lip region. The high resolution images of synaptic architectures at the ultrastructural level, received with Electron Tomography would promote the understanding of architectural changes in neuronal plasticity.

In my PhD thesis, I demonstrate that the temporal organization within *Camponotus* colonies involves the perfect timing of different tasks. Temperature seems to be the most scheduling abiotic factors of foraging and locomotor activity. The ants do not only need to adapt their behavioral repertoire in accordance to the interior–exterior switch, also the parts in the peripheral and central that process visual information need to adapt to the new sensory environment.

Zusammenfassung

Ameisen gehören zu den erfolgreichsten Insekten unserer Erde. Hauptverantwortlich für ihren enormen Erfolg ist die Arbeitsteilung der Arbeiterinnenkaste. Ameisenarbeiterinnen können sich ihre Aufgaben abhängig ihrer Körpergröße teilen (Größenpolymorphismus), indem unterschiedlich große Tiere verschiedenen Aufgaben in der Kolonie nachgehen. Zusätzlich kann die Arbeitsteilung aber auch altersbedingt sein (auch genannt Alters– oder zeitlicher Polyethismus): Junge Ameisen kümmern sich um die Königin und Brut innerhalb des Nestes, bevor sie mit zunehmendem Alter das Nest verlassen und zu Futtersammlerinnen (Furageuren) werden. Der extrem anpassungsfähige Wechsel von Innen– zu Außendiensttieren ist einer der erstaunlichsten Verhaltensweisen, die Arbeiterinnen an den Tag legen und ermöglicht es ihnen, den unterschiedlichen Bedürfnissen ihrer Kolonie nachzugehen. Der Übergang der Ammentätigkeit zum Furagieren ist mit beträchtlichen Veränderungen der sensorischen Umgebung der einzelnen Arbeiterinnen verbunden und erfordert eine Verhaltensanpassung an diese neuen Gegebenheiten. Wenn sich die Verhaltensweisen der Arbeiterinnen ändert, führt das zu Anpassungen der sensorischen und höheren Verschaltungszentren in bestimmten Gehirnarealen. Eines dieser sensorischen Verarbeitungszentren sind die Pilzkörper. Außerdem müssen Furageure in der Lage sein, tägliche Veränderungen ihrer Umwelt wahrzunehmen, um ihre Verhaltensweisen stets optimal an die sich ändernde Umwelt anzupassen. Dafür brauchen sie eine funktionierende innere Uhr, die rhythmisch mit einer Periodenlänge von ca. 24 Stunden läuft. Die artenreiche Gattung der *Camponotus* Ameisen ist ein geeigneter Organismus, um die Verhaltensweisen die mit der Arbeitsteilung der Arbeiterinnenkaste einhergehen, zu untersuchen, da sowohl der Größenpolymorphismus als auch der Alterspolyethismus zeitgleich in dieser Gattung auftauchen können. Dadurch eignen sich *Camponotus* Ameisen auch hervorragend, um strukturelle Veränderungen synaptischer Komplexe im Gehirn, die sich durch die Arbeitsteilung ändern können, zu untersuchen.

In meiner Doktorarbeit habe ich damit angefangen, die Verteilung von bestimmten Aufgaben (Ammen und Furageure) von *C. rufipes* Arbeiterinnen zu untersuchen. Mithilfe von Videoaufnahmen über elf Wochen, konnte ich sowohl eine altersabhängige, als auch

eine größenabhängige Arbeitsteilung zwischen Ammen und Furageuren für diese Art zeigen. Frisch geschlüpfte Tiere wurden innerhalb der ersten 48 Stunden von anderen Ammen umsorgt, bevor sie selbst zu Ammen wurden und Aufgaben wie Brutpflege übernommen haben. Nach rund 14–20 Tagen sind 53% der Ammen zu Furageuren gewechselt. Zusätzlich zu der altersabhängigen Arbeitsteilung konnte ich zeigen, dass die Körpergröße der Ammen deutlich breiter gestreut ist als die der Furageure, was in einer höheren Spezialisierung der Furageure resultiert. Proportionale Unterschiede des Alters und der Größe der Tiere, die diesen Wechsel vollzogen haben zeigen, wie hoch flexibel und anpassungsfähig die Arbeitsteilung innerhalb der Arbeiterinnenkaste sein kann. Die meisten der beobachteten Furageure waren außerdem fast ausschließlich nachtaktiv. Da ich diese Experimente im Labor durchgeführt habe, fehlt es komplett an der natürlichen sensorischen Umgebung der Tiere.

In dem zweiten Teil meiner Doktorarbeit habe ich mich damit beschäftigt, ob sich tägliche Aktivitätsmuster (Furagier– und Bewegungsaktivität) von Arbeiterinnen zweier nah verwandter *Camponotus* Arten (*C. rufipes* und *C. mus*) unter natürlichen Bedingungen (in Uruguay, Südamerika) und unter kontrollierten Bedingungen (im Labor), in Abhängigkeit von den abiotischen Faktoren Licht und Temperatur, verändern können. Meine Ergebnisse zeigen, dass *C. mus* Arbeiterinnen unter natürlichen Bedingungen strikt tagaktiv sind, wohingegen *C. rufipes* Arbeiterinnen vornehmlich nachts furagierten. Ein paar *C. rufipes* Nester zeigten allerdings eine erhöhte Furagieraktivität tagsüber, was auf die saisonal kalten Nächte zurückzuführen sein könnte. Um den Einfluss von Licht und Temperatur, der sich auf die Furagieraktivität im Feld gezeigt hat, genauer untersuchen zu können, wurden Arbeiterinnen beider *Camponotus* Arten verschiedenen Licht– und Temperaturbedingungen im Labor ausgesetzt. Auch hier zeigten Arbeiterinnen der Gattung *C. mus* eine strikt tagaktive Bewegungsaktivität, wohingegen *C. rufipes* Arbeiterinnen von tagaktiv unter Winter Temperaturbedingungen zu nachaktiv unter Sommer Temperaturbedingungen wechselten. Die Kombination aus den Ergebnissen der Feld– und Laborstudien zeigen deutlich, dass die generelle Aktivität der beiden Arten hauptsächlich durch Licht und Temperatur beeinflusst wird und dass Temperaturzyklen nicht nur ein limitierender Faktor der Furagieraktivität sind, sondern auch als Zeitgeber dienen können um Aktivität generell zu regulieren.

Wenn der Übergang von Innen– zu Außendiensttieren stattgefunden hat, ändert sich die komplette sensorische Umgebung der Furageure. Um diese Veränderungen verarbeiten zu können, brauchen Arbeiterinnen ein hoch anpassungsfähiges und flexibles neuronales System. Daher beschäftigte ich mich in meiner Doktorarbeit außerdem mit den zugrundeliegenden neuronalen Anpassungen der visuellen Verarbeitungsregionen im Gehirn, wie die optischen Loben und die Pilzkörper, die mit dem Wechsel von Ammen zu Furageuren einhergehen. Ich konnte zeigen, dass die optischen Loben und die Pilzkörper von im Dunkeln gehaltenen Arbeiterinnen eine `Erfahrungs–unabhängige´ Volumenszunahme innerhalb der ersten zwei Wochen nach dem Schlupf zeigen. Eine folgende Lichtexposition von vier Tagen führte zu einer `Erfahrungs–abhängigen´ Abnahme der synaptischen Strukturen im Pilzkörper, die allerdings durch eine länger anhaltende Lichtexposition von 14 Tagen wieder anstieg. Diese Plastizität des zentralen visuellen Nervensystems repräsentiert eine wichtige Komponente für die optimale zeitliche Anpassung des Wechsels von Ammen zu Furageuren und die enorme Flexibilität der altersabhängigen Arbeitsteilung. Außerdem scheinen die Pilzkörper durch diese beeindruckenden strukturellen Veränderungen der synaptischen Komplexe aktiv an dieser neuronalen Plastizität beteiligt zu sein und daher den Übergang von Innen– zu Außendiensttieren in *C. rufipes* Ameisen zu unterstützen. Neben meinen Untersuchungen zur neuronalen Plastizität synaptischer Komplexe im Pilzkörper auf der strukturellen Ebene, habe ich damit begonnen, diese Plastizität der neuronalen Komplexe auch auf Ultrastruktur Ebene zu untersuchen. Durch die zu geringe Auflösungsmöglichkeit der klassischen Transmissions Elektronenmikroskopie, konnten bisher einzelne synaptischer Komponenten in den axonalen Endigungen der Projektionsneurone nicht detektiert werden. Deswegen habe ich damit angefangen, die Methode der Elektronen Tomographie zu etablieren um die Auflösung synaptischer Komplexe zu verbessern. Mit dieser höheren Auflösung ist es möglich, bauliche Veränderungen der synaptischen Komplexe in Plastizitätsprozessen besser zu verstehen. Mit der Durchführung von `double tilt´ Serien und der anschließenden Verarbeitung der erhaltenen Bildinformation, war es mir möglich, einzelne `clear–core´ und `dense–core´ Vesikel innerhalb des Zytoplasmas der Projektionsneurone von *C. rufipes* Ameisen detektieren. Außerdem konnte ich mit dieser Methode einzelne postsynaptische dendritische Dornen der Kenyon Zellen (~62) identifizieren, die ein einzelnes Endknöpfchen eines Projektionsneurons umgeben. In diesem Teil meiner Arbeit konnte ich erste Einblicke in die komplexe neuronale Bauweise einzelner Endigungen der Projektionsneurone in der

olfaktorischen Region der Pilzkörper zeigen. Die hochauflösenden Bilder synaptischer Komplexe auf dem Ultrastruktur Level, die man mit der Elektronen Tomographie erzielen kann, bringen das Verständnis baulicher Veränderungen innerhalb der neuronales Plastizität voran.

In meiner Doktorarbeit konnte ich zeigen, dass die zeitliche Organisation verschiedener Aufgaben innerhalb der Kolonien von *Camponotus* Ameisen einer perfekten Zeitplanung bedarf. Hier scheinen die abiotischen Faktoren Temperatur und Licht den größten Einfluss auf die Furagieraktivität und die generelle Aktivität zu haben. Die Ameisenarbeiterinnen müssen nicht nur ihre Verhaltensweise nach dem Übergang von Ammen zu Furageuren anpassen, es müssen sich auch die Teile des Gehirns, die für die Verarbeitung visueller Reize zuständig sind, dieser neuen sensorischen Umgebung anpassen.

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General Introduction

Social Hymenoptera, like ants, bees, wasps and termites build up large colonies consisting of several thousands or even millions of individuals. Their vast ecological success mostly relies on the evolution of eusociality, termed after E. O. Wilson (1971), defined by three criteria: overlapping generations, cooperative support of brood and queen and reproductive division of labor. In this reproductive division of labor, mostly one or few reproductive queens and several thousand up to millions of infertile workers are involved to cooperatively tend for the brood produced by the queen. Workers then further divide different tasks among each other (division of labor) by (1) performing coincidently different activities by (2) groups of specialized workers. This enables a more efficient working paradigm than performing tasks by unspecialized individuals (Oster and Wilson, 1978; Porter and Tschinkel, 1985; Jeanne, 1986a; b; Gordon, 1989). The functional specialization of workers in a colony of eusocial insects is called polyethism and can be related to age (age or temporal polyethism) and/ or body size (polymorphism) of single individuals (Hölldobler and Wilson, 2009). Age–based division of labor is common in most eusocial insect species: young individuals stay inside the nest as nurses and with ongoing age become foragers (Wilson, 1971). As some ants (less than 15%) and nearly all termites also divide their labor regarding to the workers´ body size (ants: Kaspari and Byrne, 1995; Anderson and McShea, 2001, termites: Wheeler, 1986, 1991), both age– and size–related division of labor can occur at the same time in ant colonies (reviewed in Hölldobler and Wilson, 1990).

In Hymenoptera, division of labor among the worker caste is one of the most aspiring phenomena that is restricted to age and/ or body size related flexible adaptations of the behavioral repertoire of single workers. The highly flexible interior–exterior transition probably allows the individuals to properly match actual colony needs and is one of the most impressive behaviors a single worker undergoes during its life. As environmental stimuli are changing with the interior–exterior transition, workers are forced to perform a completely new repertoire of behavioral tasks. The species–rich genus of *Camponotus* ants constitute a rewarding model to study how behavioral duties are performed and modulated at the colony and individual level, as some species are already known for their age–related and/ or size

related division of labor. Of special interests are the two closely related *Camponotus* species, *C. rufipes* and *C. mus,* which show an overlap in their population area but prefer different climate zones. Therefore, they represent interesting study animals to investigate how they cope with varying environmental conditions.

The Model Organism *Camponotus* ants

Colonies of *C. mus* ants prefer to build their nests within stone walls, dead wood or roof trusses (personal communication by F. Roces and O. Geissler) in the more temperate climate zones of South America. By contrast, *C. rufipes* colonies are more common in subtropical and tropical regions of South America (Kusnezov, 1952, 1963; Jáffe and Sanchez, 1984) and mainly build up their nests in open grasslands (Hansen and Akre, 1990). Moreover, both species have been described to build adjacent nests and life sympatrically in some areas in South America (Fig. 1, Goni et al., 1983). However, so far little is known about the colony structure of *C. rufipes* and nearly nothing of *C. mus*.

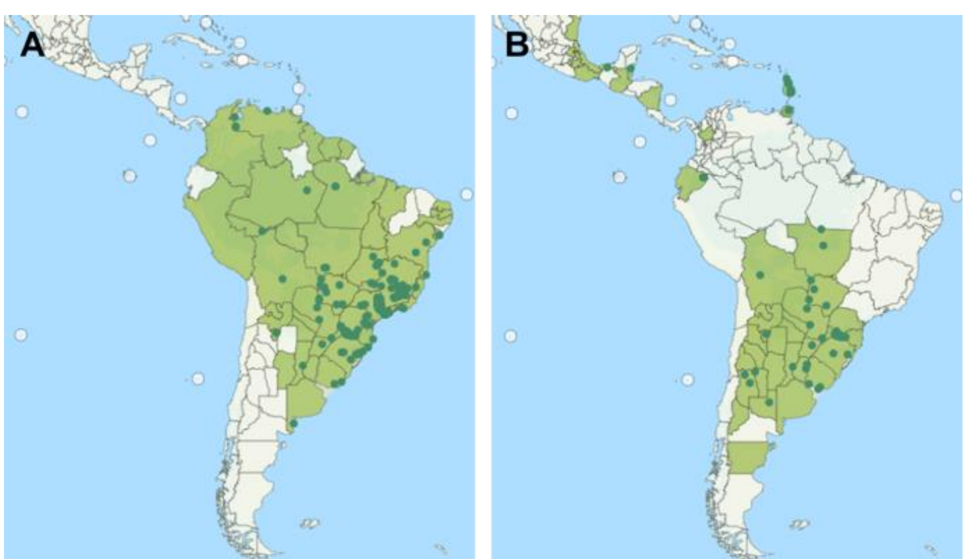

Figure 1: Distribution of *Camponotus rufipes* (A) and *C. mus* (B) in South America. Areas of distribution are represented in green. Coordinates were *C. rufipes* or *C. mus*, respectively, have been collected are represented as green dots. Modified after antweb.org

An earlier study conducted in *C. rufipes* workers compared young (<45 days) and old (>45 days) workers without observing any differences in their frequency of performing nursing and foraging tasks (Soares et al., 2008). As the transition from interior duties to exterior tasks mainly takes place within the first three weeks of an ant worker's life (Hölldobler and Wilson, 1990), the investigated age stages of the workers are maybe too broad to detect an age–related division of labor. Thus, a more detailed study to investigate whether and when

C. rufipes worker ants undergo an age–related transition from interior to exterior work needs to be conducted. To examine whether *C. rufipes* workers are also able to divide their labor related to different body sizes of the worker caste requires the discrimination of different sized workers. A standardized classification for differently sized individuals is published for various ant species, resulting in different morphs that are commonly termed minor, media, majors and soldiers (Wilson, 1968, 1980; Oster and Wilson, 1978; Hölldobler and Wilson, 1990; Waser, 1998). A size–related division of labor in *C. rufipes* workers was first described by Jáffe and Sanchez (1984), where they observed minor ants performing all investigated tasks, whereas major ants mainly defend the nest and supply the colony with nectar via trophallaxis.
Another special feature among *Camponotus* ants are their periodically foraging activity patterns at different daytimes. *C. rufipes* ants forage actively during the night (referred to as \`nocturnal´; Jáffe and Sanchez, 1984) with some occasional daytime (referred to as \`diurnal´) foraging activities (Del–Claro and Oliveira, 1999; Fagundes et al., 2005). Contrary, workers of *C. mus* solely exhibit diurnal foraging activity, with an activity peak two hours before light onset (Del–Claro and Oliveira, 1999; Müller, 2012). To date, little is known about the underlying mechanisms and factors that are accountable for the behavioral switch from diurnal to nocturnal foraging activity and vice versa in *C. rufipes* workers and why workers of the closely related *C. mus* species forage strictly diurnally.

For most ant species, foraging activity in a daily and seasonal manner is mainly affected by biotic factors like food availability (Bernstein, 1976; Briese and Macauley, 1980; Marsh, 1985), inter– and intraspecific competition (Galle, 1986; Fellers, 1987; Savolainen and Vepsäläinen, 1988; 1989; Andersen, 1992) and presence of brood (Bernstein, 1976). Besides, also abiotic factors, mainly temperature (Gordon, 1983a; Marsh, 1985, 1988; Porter and Tschinkel, 1987), but also humidity (Whitford and Ettershank, 1975), light intensity (Christian and Morton, 1992) and wind (Briese and Macauley, 1980; Marsh, 1988) have been shown to affect foraging activity. Temperature strongly and directly schedules foraging activity due to its influence on walking speed (Shapley, 1920; Barnes and Kohn, 1932; Drees et al., 2007), water loss (Lighton and Feener Jr, 1989), respiration (Jensen and Nielsen, 1975) and oxygen consumption (MacKay and Sassaman, 1984). Therefore, thermal conditions are of special importance for ants, because foraging activity in ectothermic animals is restricted to certain times of the day when surface conditions are physiologically tolerable (Marsh, 1988; Cerdá et al., 1998). A seasonal based shift of foraging activity is shown for different

Mediterranean ant species, with increasing nocturnal activity during summer times to avoid critical temperatures (Cerdá et al., 1998). Heat–tolerant species are mostly diurnal with low changes in daily foraging activity, whereas nocturnal species are more heat–intolerant (Cerdá et al., 1998). Species with a lower tolerance for heat are more dominant and occupy food sources when non–dominant (heat–tolerant) species forage at the same time (e.g. Cerdá et al., 1997, 1998; Bestelmeyer, 2000; Cerdá and Retana, 2000; Retana and Cerdá, 2000; Albrecht and Gotelli, 2001; Thomas and Holway, 2005). Furthermore, foraging activity in two sympatric *Myrmecia* species was affected by temperature and inter–specific competition, with the dominant species foraging at cooler temperatures (Jayatilaka et al., 2011).

Also the two closely related *Camponotus* species, *C. mus* and *C. rufipes*, are exposed to the same daily and seasonal thermal changes, but mainly differ in their foraging activity (Del–Claro and Oliveira, 1999). Therefore, workers of both species must be able to precisely time their foraging activity to the given environmental factors to avoid intolerable and hazardous conditions. This timing of foraging activity is mainly based on time measuring mechanisms, that enables living organisms to cope with periodically environmental changes and to adapt their own mode of life (Sharma, 2003). Time measuring mechanisms are accomplished by a circadian clock system, generating rhythms with a period length of approximately 24 hours.

Circadian Rhythms in Insects

Endogenous Clocks

Circadian rhythms (lat.: circa= around; dies= day; `around one day´) are biological processes that are self–sustained under constant conditions, are entrainable by environmental cues (e.g. light, temperature, humidity), and are temperature compensated, meaning their endogenous period cycles independently over a physiological range of temperatures (Pittendrigh, 1960). Many physiological and biological processes are controlled by circadian rhythms, like activity, feeding, mating and pupal eclosion (Brady, 1974; Saunders, 2002). Endogenous clocks are found across all kingdoms, ranging from cyanobacteria, plants, and insects to mammals (Lakin–Thomas, 2000; Bell–Pedersen et al., 2005). The underlying molecular mechanism is highly conserved between the taxa (reviewed in Danks, 2003; Paranjpe and Sharma, 2005) and is well described for insect species and characterized in the fruit fly *Drosophila melanogaster* (reviewed in Hardin, 2011). The circadian system consists of a central pacemaker that can be entrained by environmental cues via input and output pathways, which process information from the pacemaker to various biochemical, physiological and behavioral proceedings (Dunlap et al., 2003; Bell–Pedersen et al., 2005). In insects, the main pacemaker of the circadian clock is associated with the optic lobes, located in neurons between the medulla and the central brain (Helfrich–Förster et al., 2007). The underlying molecular basis to generate stable rhythms consists of a complex negative feedback loop (reviewed in Hardin, 2011), that runs self–sustained even under constant conditions without any zeitgebers (Giebultowicz, 2001).

As the period length of the endogenous clock differs from the 24h environmental cycle, the internal timekeeper has to be synchronized each day by external stimuli, termed zeitgeber (reviewed in Johnson et al., 2003; Roenneberg et al., 2003; Pittendrigh, 1960). The most prominent zeitgebers are changes in light and temperature values (Edmunds, 1988; Roenneberg and Foster, 1997). The daily light–dark (LD) cycle is assumed to be the most dominant and reliable zeitgeber (Devlin, 2002). Under the absence of LD cycles daily thermal and humidity cycles are also able to entrain circadian rhythms (Hastings et al., 1991; Saunders, 2002). Furthermore biotic factors like availability of food (Aschoff, 1986; Mistlberger, 2009; Stephan, 2002; Mildner and Roces, 2017), presence of prey and predators, or social interactions (Hastings et al., 1991; Mildner and Roces, 2017) can serve as zeitgebers. Locomotor activity rhythms provide easily measurable output signals of the

circadian clock. To demonstrate the proper function of the endogenous clock, locomotor activity rhythms need to be recorded under the absence of zeitgebers. If an environmental factor can serve as a zeitgeber, the locomotor rhythm should be entrained by the phase shifted putative zeitgeber cycle. The locomotor activity was truly entrained by a zeitgeber, if the period length and phase–relationship of the behavioral rhythm and the period and phase of a particular zeitgeber cycle, are synchronized (Aschoff, 1979).

Timing Aspects in Ant Colonies

Individuals of social insects need to cope with changing colony structures, like colony size, inter individual interactions, the time of the year, food availability, predation pressure and climatic conditions (Robinson, 1992). Therefore circadian rhythms are believed to play an important role in the maintenance of the social structure of colonies (Bloch and Robinson, 2001). Timing of activities within social insect colonies can occur at both, a daily and annual/ seasonal basis. Ants use annual cycles for a proper survival of their colony, as nurturing and caring of larvae and pupae is restricted to the warm periods of the year and queens stop oviposition during the colder months of the year (Kipyatkov, 1993). Spontaneous rhythms of development and diapause can occur in ant species, reared under laboratory conditions, suggesting the impeccable function of an endogenous clock (*Camponotus*: Hölldobler, 1961; Kipyatkov, 1995). Under natural conditions in the field, decreasing ambient temperature and shortening of the day length are the main zeitgebers inducing diapause in ants (Kipyatkov, 1993; 1995; 2001). Besides oviposition and hibernation, reproduction and mating are two other annually occurring phenomena within an ant colony. Mating flights of sexuals (queens and males) coincide with rainy seasons, temperature, time of the day and cloud coverage (e.g.: Boomsma and Leusink, 1981; Peng et al., 2013; Rwegasira et al., 2015; Nene et al., 2016). Unmated, virgin queens are reported to be rhythmic, but lose their rhythmicity after successful mating (McCluskey, 1967; McCluskey and Carter, 1969). Arrhythmicity of mated queens could help them to avoid predators and support them to store energy as they need to rear the first larvae and pupae on their own with just the food reserves stored in their bodies (Wheeler, 1933).

Workers of ant species also need to deal with daily rhythms, like adjusting their foraging activity to abiotic factors like temperature (Gordon, 1983a; Marsh, 1985, 1988; Porter and Tschinkel, 1987), humidity (Whitford and Ettershank, 1975), wind (Briese and Macauley, 1980; Marsh, 1988) and light intensity (Christian and Morton, 1992) as well as to biotic

factors like food availability (Bernstein, 1976; Briese and Macauley, 1980; Marsh, 1985) and the potential presence of predators or prey (Galle, 1986; Fellers, 1987; Savolainen and Vepsäläinen, 1988, 1989; Andersen, 1992). Other exterior activities like nest maintenance, waste management and patrolling are also performed in a daily manner in *Pogonomyrmex* ants (Gordon, 1983b, 1986). Under natural conditions in the field, it is almost impossible to measure circadian rhythms in the nest interior without perturbations of the nest. Until now, just one endogenous rhythm of nurses under laboratory conditions was studied in *Camponotus* ants (Roces and Núñez, 1989, 1996; Roces, 1995). *C. mus* showed a bimodal endogenous rhythm of the preferred temperature to translocate their brood piles, by moving their brood in between 27.5°C and 30°C. Contrary, workers of the closely related species *C. rufipes* choose a stable temperature throughout the day (Roces and Núñez, 1995). Gaining the optimal rearing temperature is of high importance for insects as it affects their development, has tremendous impact on their learning abilities, and facilitates the proper growth of important brain neuropils (Tautz et al., 2003; Jones et al., 2005; Becher et al., 2009; Weidenmüller et al., 2009; Falibene et al., 2016).

Behavioral tasks and circadian rhythms are controlled and regulated by all *Camponotus* workers individually. Environmental cues like biotic and abiotic factors, as well as the time of the day and the season are perceived by the individual ants, processed and interconnected in sensory integration centers in the brain, like the optic lobes and the mushroom bodies. Therefore, the brain of *Camponotus* ants and its underlying neuronal plasticity are of outstanding interest to understand neuronal processes, as they are crucial for sensory perception, learning abilities, the control of behavioral tasks and the maintenance of circadian rhythms.

Visual Integration Centers in an Insects´ Brain

With the nurse to forager transition, environmental factors (e.g. light, temperature, olfactory cues) are changing dramatically. The most prominent factor that is changing with this behavioral transition is probably visual cues, attributable to the changing light stimuli. To ideally cope with these changes, workers need to be able to process the gathered stimuli via their compound eyes, distinct structures located at the periphery of the ants' head. They contain thousands of subunits, termed ommatidia, each of them serving as a single eye containing photoreceptor cells. The photoreceptor cells are interconnected with the primary visual brain neuropil, the optic lobes (OL) via nerve bundles (reviewed in Strausfeld, 1989). In Hymenoptera, the OLs are subdivided into three distinct structures: the lamina (LA), medulla (ME) and lobula (LO; reviewed in Strausfeld, 1989). LA neurons branch into the ME, the largest neuropil of the OLs, where they are interconnected to several ME interneurons (Strausfeld and Obermayer, 1976). The neurons of the LA respond mainly to changes in light intensity, summation and lateral inhibition (Strausfeld, 1989; Gronenberg, 2008), whereas the ME processes information about color and may extract motion from the received visual input (Gronenberg, 2008). LO neurons probably process both, motion and color information (Strausfeld, 1989; Paulk et al., 2008). Projections emerging from the ME and LO, run anteriorly through to the protocerebrum, building up collaterals in the ipsi– and contralateral input region of the secondary visual brain neuropil, the mushroom bodies and run across the brains´ midline, again innervating the input region of the contralateral mushroom bodies and terminate in the contralateral ME. This prominent visual tract is called anterior superior optic tract (asot) and was first described in the honeybee (Kenyon, 1896; Mobbs, 1984; Ehmer and Gronenberg, 2004) but is also described in some *Camponotus* ant species (Gronenberg, 2001). In *Camponotus*, this tract is tightly packed with 15–20 thin fibers, in *Atta* and *Pogonomyrmex* ants the asot contains about 12–15 single neurons, whereas in honeybees and paper wasps 30 neurons were successfully labeled (Gronenberg, 2001). In the honeybee, neurons emerging from the LO do not contribute to the asot, but run as a broad strand from the proximate LO to the mushroom bodies. Their axons fuse with the fibers of the asot in close proximity to the visual input region of the mushroom bodies and cannot be further distinguished from the neurons of the ME (Gronenberg, 2001).

Structure of the Mushroom Bodies

Every living being needs the ability to receive cues and signals (mostly olfactory and visual cues) from the surrounding environment in the context of their own internal state of motivation, and to process the collected information to generate an appropriate behavioral response. Within the insect brain this ability has at least partly been attributed to higher brain centers that are highly flexible in shape and size and commonly known as mushroom bodies (MB, lat.: *corpora pedunculata;* Dujardin, 1850). The MBs are paired neuropils in each adult brain hemisphere, consisting of hundreds to up to many thousands of densely parallel–projecting neurons, the Kenyon cells (KC, after Kenyon (1896)). The number of KCs varies between different insect taxa: the fruit fly *Drosophila melanogaster* possesses about 2,000 (Aso et al., 2009), the locust *Schistocercea* about 50,000 (Leitch and Laurent, 1996), the scarab beetle *Popillia japonica* about 80,000 (Farris, 2008), the carpenter ant *Camponotus rufipes* about 130,000 (Ehmer and Gronenberg, 2004), the cockroach (*Blattodea*) up to 175,000 (Neder, 1957) and the honeybee *Apis mellifera* up to 184,000 (Witthöft, 1967; Strausfeld, 2002). In Hymenoptera, the intrinsic KCs can be divided into three subpopulations, depending on their size and position (Fig. 2). The inner compact cells with a small diameter (~4–5 µm), the inner noncompact cells with a larger diameter (~6–7 µm) and the outer compact cells again with a small diameter (~4–5 µm) (Mobbs, 1982; Strausfeld, 2002). While the outer compact cells are located at the outer rim of the calyx, the inner compact cells are pushed to the sides of the calyces by the inner noncompact cells. Differing in their shape and size, all three subpopulations of KCs have in common, that their dendritic arborizations form the MB calyces, whereas the descending fibers (axons) run in a parallelly arranged manner, shaping the pedunculus (Fig. 2). The calyces represent the main input region of the MB and receive input from sensory projection neurons (PN) of primary sensory neuropils, like the antennal lobes and OLs (Strausfeld et al., 1998). Additionally, they are further innervated by GABAergic feedback neurons (*Apis mellifera*: Bicker et al., 1985; Grünewald, 1999; Ganeshina and Menzel, 2001).

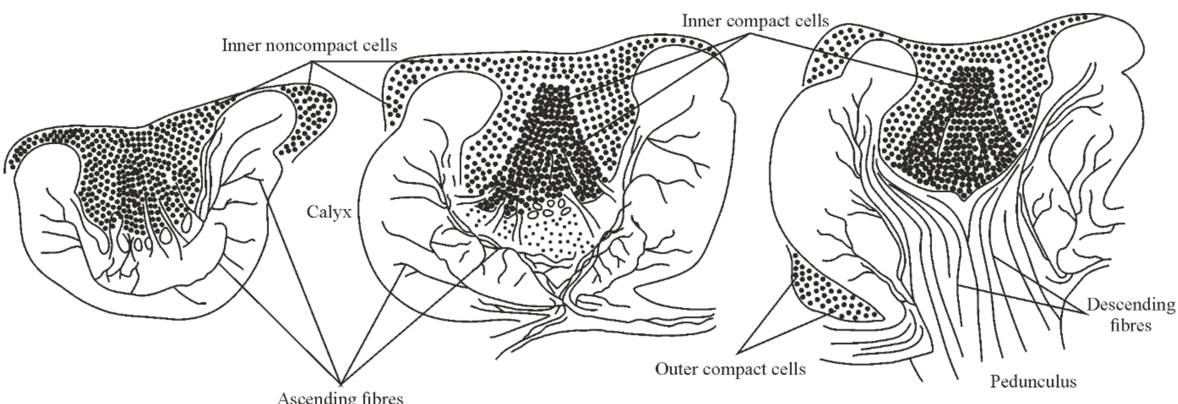

Figure 2: Different subpopulations of Kenyon cells. The inner compact cells possess a small diameter and are pushed to the periphery of a single calyx by the inner noncompact Kenyon cells (KCs). The outer compact cells are located outside of the calyx and have a large diameter. The calyx, known as main input region of the mushroom bodies (MB), are build up from ascending fibers from the primary olfactory and visual brain centers. The pedunculus, which is connecting the calyces with the main output region of the MBs, the vertical and medial lobes, is arranged by thousands densely parallel–projecting descending fibers. Pictures for schematic drawing adapted from Vowles, 1955.

In the honeybee, the different KC subtypes innervate different areas within the MB calyces that are anatomically different: the lip, collar and the basal ring (Mobbs, 1982; Strausfeld, 2002). The lip and collar region are innervated by the noncompact KCs (Mobbs, 1982), the basal ring gets input from the inner compact KCs and all three subneuropils are innervated by the outer compact KCs (Mobbs, 1982; Strausfeld, 2002). As sensory afferents of PNs innervate the three regions differently, they are further distinguishable by their function: the lip region receives olfactory input from the primary olfactory center, the antennal lobes, whereas the collar gets input from the primary visual center, the OL and both modalities are represented in the basal ring (Mobbs, 1982; Strausfeld, 2002). As inner compact KCs are mostly lacking in ant species, it is not astonishing that the basal ring is relatively small in size and similar in texture to the lip and collar and can only be visualized in the two genera *Aphaenogaster* and *Pachycondyla* (Gronenberg, 1999, 2001; Gronenberg and Hölldobler, 1999) by tracing its output connections (Gronenberg, 1999). In many ant species it seems that the collar is pushed outwards, while the lip and the basal ring are fused (Gronenberg, 2001).

In the MB calyces, PN terminals (`boutons´) are surrounded by dendritic spines of KC dendrites and form synaptic complexes, termed microglomeruli (MG; Yasuyama et al., 2002; Frambach et al., 2004; Groh et al., 2004, 2006; Leiss et al., 2009; Stieb et al., 2010). These synaptic microcircuits, first described by Trujillo–Cenóz and Melamed in 1962 and Steiger in 1967, receive mainly cholinergic input from PNs and, additionally, some modulatory input from extrinsic γ–aminobutyric acid (GABA)ergic (*Apis mellifera*: Bicker

et al., 1985; Grünewald, 1999; Ganeshina and Menzel, 2001; *Gryllus bimaculatus*: Frambach et al., 2004), dopaminergic (*Apis mellifera*: Blenau et al., 1999) and octopamin–containing (*Apis mellifera*: Hammer, 1993; Kreissl et al., 1994) neurons. Within one MG, an individual PN bouton is wrapped by numerous actin–rich, spine–like dendritic endings of the KCs. Each dendritic spine forms synapses with the KC spines with the PN bouton as indicated by clearly visible presynaptic active zones (*Apis mellifera*: Ganeshina and Menzel, 2001; Groh et al., 2012; *Drosophila melanogaster*: Leiss et al., 2009). Various studies using f–actin phalloidin labeling in the calyx of termites, honeybees, flies and ants showed that highly motile f–actin is accumulated in the KC dendritic spines (Groh and Rössler, 2011). The sensory subdivision of the calyces is also maintained when fibers of the PN run through the pedunculus and the medial and vertical output lobes (Fahrbach, 2006; Gronenberg, 2008). The peduncle connects the input of the MB with the main output region, the vertical and medial lobes represent the major MB output regions. Electron microscopic studies indeed revealed some input synapses in the lobes (Schürmann, 1973). In honeybees, the vertical lobe is distinguishable by four distinct layers (Strausfeld, 2002). The dorsal part of layer I consists of descending neurons from the basal ring, the collar sends its arborizations to the second layer, the third layer corresponds to neurons forming the lip and the fourth layer is innervated by axons from the outer compact KCs (Strausfeld, 2002).

Neuronal Plasticity within the Mushroom Body Neuropil

As described before, the worker caste of different ant species is known to undergo an age– and in some cases size–related division of labor. Especially the switch from interior tasks as a nurse to exterior duties as a forager, is accompanied with changes of the sensory input and of the behavioral repertoire. These enormous changes need to be met by neuronal centers like the MBs. To date, the active involvement of the MBs in structural changes that are mostly associated with ongoing age, the onset of foraging behavior, interspecific interactions and experience of sensory cues has been extensively studied. Various studies have shown that the MB calyces undergo a tremendous volume increase during the lifespan of an individual honeybee or ant worker (*Apis mellifera*: Durst et al., 1994; Groh et al., 2004, 2006; Fahrbach and Dobrin, 2008; *Cataglyphis fortis*: Stieb et al., 2010, 2012). In honeybees, an initial volume increase of the MB calyces during the first week of adult maturation suggests an `experience–independent´ internal maturation program hinting towards a prolonged post–eclosion neuronal development (Ismail et al., 2006; Muenz et al., 2015). Light deprivation

experiments in bees and ants support that this process is independent of visual stimuli (Fahrbach et al., 1998; Kühn–Bühlmann and Wehner, 2006). Besides the internal \`experience–expectant´ volume increase of the visual system, earlier studies further suggest that the sensory system is going to be prepared for future demands like the switch to outdoor foraging and is therefore described as \`experience–dependent´ plasticity (Fahrbach et al., 1998). Both independent components of neuronal plasticity have also been described in other ant species like *Camponotus floridanus* (Gronenberg et al., 1996), the desert ant *Cataglyphis bicolor* (Kühn–Bühlmann and Wehner, 2006) and the paper wasp *Polybia aequatorialis* (O'Donnell et al., 2004).

A lot of studies were further conducted to reveal the underlying neuronal mechanisms that accompany the well described volume increase of the MB calyces. As adult neurogenesis is absent in the honeybee brain, proliferation of KCs can be excluded (Fahrbach et al., 1995; Farris et al., 1999; Ganeshina et al., 2000). Previous studies in the honeybee revealed that the outgrowth of KC dendrites is a good candidate for the volumetric increase of the MB neuropil (Farris et al., 2001). New dendritic processes appear to grow out from the main neurite with the onset of foraging. This observation is in line with studies in mammals showing an increased dendritic branching in response to an enriched sensory environment (Volkmar and Greenough, 1972; Greenough and Volkmar, 1973; Uylings et al., 1978) or training experience (Greenough et al., 1985; Kolb and Whishaw, 1998). As synaptic complexes in the MB calyces, the MG, are composed of presynaptic PN boutons enwrapped by f–actin rich KC dendritic spines, they are of outstanding interest to study structural neuronal plasticity (Fig. 3; Farris et al., 2001; Ganeshina and Menzel, 2001; Groh et al., 2012). The components of the postsynaptic side, the KC dendritic spines, have been described to be highly plastic and flexible and therefore facilitate plastic changes that are accompanied with age (Coss et al., 1980) and sensory exposure (Brandon and Coss, 1982).

For the visually guided desert ant, *Cataglyphis fortis,* it was shown that the reorganization of neuronal circuits is mainly triggered by light input (Stieb et al., 2010, 2012). Light exposure for several days leads to a decrease in the synaptic density of MG in the visually innervated collar, but not in the lip (*Apis mellifera*: Scholl et al., 2014). The decrease of MG density in the collar may be caused by synaptic pruning of visual PNs and by a concurrent outgrowing of KC dendrites (Fig. 3; Stieb et al., 2010, 2012).

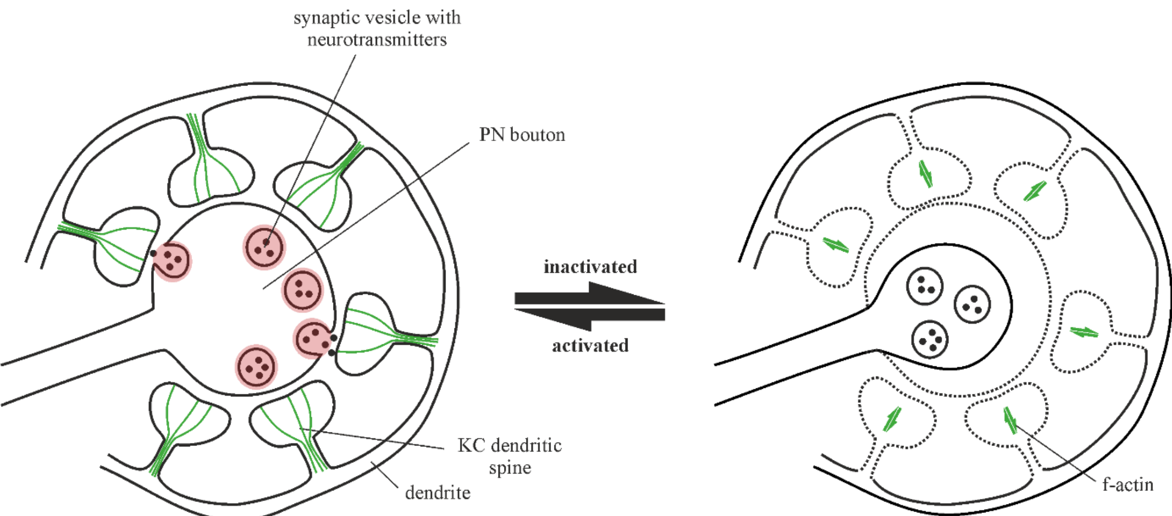

Figure 3: Schematic drawing of a microglomerulus of the mushroom body calyx that undergoes structural plasticity. One presynaptic bouton, built up by projection neuron (PN) terminals contains synaptic vesicles. This presynaptic terminal is surrounded by Kenyon cell (KC) dendritic spines. During pruning processes, postsynaptic KC spines (indicated in green) are removed and presynaptic PN boutons (indicated in red) are shrinking in their size. As this process is highly flexible, new dendritic spines can be built to PN boutons (adapted and modified from Groh et al., 2012).

This pruning is well described for the insect development, where axonal outgrowths of PNs are removed during maturation. This pruning provides neuronal complexity and wiring of the brain (Raff et al., 2002; Awasaki and Ito, 2004; Watts et al., 2004). It can be assumed, that axonal pruning potentially represents a generic process in adapting and modulating synaptic microcircuits during brain development and maturation (Truman and Reiss, 1976; Technau and Heisenberg, 1982; Levine and Truman, 1985; Weeks and Truman, 1986; Lee et al., 1999; Raff et al., 2002; Watts et al., 2003). In contrast to synaptic pruning caused by light exposure (Seid and Wehner, 2009; Stieb et al., 2010, 2012; Groh et al., 2012; Scholl et al., 2014), increases in synaptic density were found in the MB calyx lip due to formation of transcription–dependent long–term memories in the honeybee (Hourcade et al., 2010) and leaf–cutting ants (Falibene et al., 2015).

Hence, these magnificent structures are superb to study in *C. rufipes*, since this ant species shows a high potential for behavioral plasticity which results in seasonal dependent changes of behavior.

Thesis Outline

Individual ant workers perform different tasks like tending and caring queen and brood, go outside and forage for food or defend the nest. This highly flexible task allocation can be related to body size and worker age, or both at the same time. The individual transition from nursing to foraging activities is accompanied with enormous changes in the sensory environment and workers are forced to perform a new repertoire of behavioral tasks resulting in the reorganization of their neuronal architecture. Furthermore, ants must cope with periodical environmental changes at a daily and/ or seasonal basis. Activity patterns, like foraging or locomotor activity, are therefore based on time measuring mechanisms by an endogenous clock, running with a period length of approximately 24 hours.

In my thesis, I raised the following questions based on different aspects of division of labor and the underlying behavioral and neuronal changes in the ant species *Camponotus rufipes*:

- **At which worker age does the behavioral transition from nurse to forager occur?**

To date, nothing is known about the behavioral transition from nursing to foraging in *C. rufipes*. The first part of my thesis starts to investigate the age at the onset of foraging (**Chapter I**). To do so, I established a new behavioral setup by using camera systems to facilitate 24h video recordings of individually marked ants for an observation period of about 11 weeks. This experiment revealed that the interior–exterior transition occurs at a worker age of 14–20 days and is mainly performed by media–sized workers. Furthermore, the highest number of individual foragers was actively engaged in nocturnal foraging. Variations in proportion and the age of workers switching indicates how highly flexible and plastic the age–related division of labor occurs in this ant species. This study provides first insights into the complex and flexible behavioral maturation of *C. rufipes* workers. As all experiments were conducted under laboratory conditions they are completely lacking the natural environment of these ant species. In their natural habitat, *C. rufipes* workers are exposed to different daily and seasonal thermal fluctuations and need to perform longer and

more dangerous foraging trips as they need to do in the laboratory. Therefore, the second part of my thesis started to investigate the impact of temperature as abiotic factor on daily foraging and locomotor activity in *Camponotus rufipes* and the closely related *C. mus*.

- **How does temperature limit daily foraging and locomotor activity in two sympatrically living *Camponotus* species?**

As ants behave differently in their natural habitat than under laboratory conditions, the second part of my thesis provides first insights into natural foraging activity of *C. mus* and *C. rufipes* in La Coronilla, Uruguay (**Chapter II**). I therefore recorded daily foraging activity, ambient temperature, humidity and light intensity in different field nests of *C. mus* and *C. rufipes* for 24 hours. While *C. mus* workers were exclusively diurnal, workers of *C. rufipes* colonies foraged predominantly during the night. As some *C. rufipes* also showed elevated foraging activity levels during daytime, this species was active under a broader temperature spectrum than *C. mus*. To further reveal temperature as abiotic factor influencing daily activity cycles, I measured locomotor activity under winter and summer conditions in the laboratory. Experiments revealed diurnal activity rhythms under both temperature regimes in *C. mus*, whereas *C. rufipes* workers shifted their locomotor activity from nocturnal under summer to diurnal under winter conditions. With this study, I could highlight the thermal specialization of workers in coexisting *Camponotus* species in the field and the laboratory.

The first two parts of my thesis revealed, that *Camponotus* ants change their behavioral repertoire in accordance to changing environmental stimuli, mainly in ambient temperature cycles. These changes of sensory cues the workers receive e.g. during foraging trips need to be processed by higher order visual neuropils in the ant brain. I therefore raised the following question in the third part of my PhD thesis:

- **Is the prominent interior–exterior switch accompanied by adaptions in the peripheral and central visual system?**

In this chapter, I linked cellular as well as subcellular measurements of the primary and secondary visual brain neuropils, the optic lobes and the mushroom bodies, in light–deprived and –exposed workers of *C. rufipes* ants (**Chapter III**). Using synapsin immunolabeling to reveal volumetric changes as well as changes of synaptic complexes in

the brain, I could demonstrate, that the volumes of the optic lobes (lamina, medulla and lobula) and mushroom body calyces of *C. rufipes* workers of all tested ages (3–42 days) increased significantly within the first two weeks of adulthood. Light exposure for 1, 4 and 14 days induced a vast volume increase of the optic lobes but not of the mushroom bodies, demonstrating that visual information is processed first at the primary visual neuropil. Furthermore, exposure to light for four days caused a decrease of synaptic profiles, followed by an increase after extended exposure to light for 14 days. Also, the total number of microglomeruli within the mushroom body collar decreased significantly after four days of light exposure. I therefore conclude, that the structural plasticity of primary and secondary visual brain centers is mainly driven by `experience–independent´ and `experience–dependent´ elements. In this chapter of my thesis I could reveal synaptic changes of single microglomeruli in the mushroom body calyces at the structural level. Until now, less is known about the ultrastructural changes at individual synapses that accommodate the described plasticity of complex synaptic structures. I therefore started to implement a new high resolution microscopic technique and raised the following question:

- **How are synaptic architectures arranged in projection neurons of *C. rufipes* workers?**

So far, studies conducted with honeybee nurses and foragers revealed, that the interior–exterior transition is accompanied with vast structural changes in synaptic complexes of the mushroom body calyces at the pre– and postsynaptic site (Groh et al., 2012). The remarkable structural changes of these synaptic complexes suggest an active involvement of the mushroom body neuropil in lifetime plasticity that promotes the interior–exterior transition of eusocial insects. In this study, the visualization of single vesicles and the clear architecture of actives zones of projection neuron boutons were below a satisfying resolution. Therefore, I implement electron tomography to increase the synaptic resolution to gain a better understanding in architectural changes of neuronal processes (**Chapter IV**). By acquiring double tilt series (–65° to 65°) of synaptic complexes and consecutive analysis of the gained tomograms, I could resolve clear– and dense–core single vesicles in the cytoplasm of the axonal boutons. The abundance of the large dense–core vesicles (~70 nm) suggests that the projection neuron boutons are involved in neuromodulatory processes of visual and olfactory stimuli within the microglomeruli. I could further demonstrate, that single projection neuron boutons are surrounded by a high amount (~62) of postsynaptic Kenyon cell dendrites, where

most of them are in contact with two to three active zones. The results of this part of my thesis provide first insights into the complex presynaptic architecture of single projection neuron boutons in *C. rufipes* workers. This technique could further be used to examine differences of the synaptic composition in the mushroom bodies in light–deprived or light–exposed nurses and foragers of *Camponotus* workers. That would provide a better understanding of neuronal synaptic processes that occur during the lifetime of single ants.

Chapter I

Age–related Division of Labor of *Camponotus rufipes* Ant Workers

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Introduction

Eusocial Hymenoptera like ants, bees, and wasps represent 75% of the insect biomass of the world (Hölldobler and Wilson, 1990). Their enormous ecological success is mainly caused by division of labor (Oster and Wilson, 1978; Hölldobler and Wilson, 1990; Robinson, 1992; Schwander et al., 2005). Division of labor within the worker caste can be related to age (age or temporal polyethism) and/ or body size (size polymorphism) of single individuals (Hölldobler and Wilson, 2009). Age–related polyethism is defined as a phenomenon, where young individuals stay in the nest interior, by e.g. taking care for queen and brood and then switch to exterior tasks to collect food or defend the nest (Wilson, 1971). This form of division of labor is well known and has been described in honeybees (Lindauer and Watkin, 1953), paper wasps (Dew and Michener, 1981) termites (Hinze and Leuthold, 1999) and in many ant species (*Cataglyphis bicolor*: Wehner et al., 1972; Wehner and Rössler, 2013; *Camponotus floridanus:* Gronenberg et al., 1996; *Messor* and *Myrmica*: Ehrhardt, 1931; *Pheidole dentata*: Muscedere et al., 2009, 2013; *Platythyrea*: Bernadou et al. 2015; *Pogonomyrmex*: Gordon, 1989; Holbrook et al., 2013; *Solenopsis invicta*: Mirenda and Vinson, 1981; *Temnothorax albipennis*: Dornhaus, 2008). Hence, earlier studies conducted in *Camponotus rufipes* ants compared young (<45 days) and old (>45 days) workers, but revealed no differences in the frequency of workers engaged in nursing and foraging tasks (Soares et al., 2008). As the age–related division of labor typically occurs within the first 2–3 weeks of an ants´ life (Hölldobler and Wilson, 1990), Soares and colleagues (2008) might have missed the age–related transition from nurses to foragers, due to their broader age determination. Also the peripheral and central visual system of *C. rufipes* workers mature during the first two weeks of adulthood (Yilmaz et al., 2016). This suggests an age–related transition within the age range of two weeks post–eclosion.

Besides temporal polyethism, division of labor among workers can also be related to their body sizes (size polymorphism), but is only present in less than 15% of the ant genera

(Kaspari and Byrne, 1995; Anderson and McShea, 2001). For leaf–cutting ants is shown that worker polymorphism provide some advantages for the colonies: the larger workers (majors) are more specialized in cutting leaves or have an increased walking speed compared to smaller workers (minors; Kay and Rissing, 2005; Wilson, 1980). Additionally, major workers of fire ants are less effective in nursing the brood due to their body size (Porter and Tschinkel, 1985). Hence, also workers of the nectar–feeding ant species *C. rufipes* are known to exhibit a size–related division of labor, based on four different morphs of the worker caste: minor, media and major ants, and soldiers (Jáffe and Sanchez, 1984). Whereas major workers and soldiers were engaged in tasks like defending the nest, media and minor workers showed a higher occurrence frequency in tasks like nursing and foraging (Jáffe and Sanchez, 1984).

The duration of the interior period of nurses and therefore the timing of the interior–exterior transition is still unknown for *C. rufipes*. We hypothesize that the worker age at the onset of foraging is a flexible adaptation to the actual biological needs of the ant colony. In this study, we are focusing on the following question: 1) do different sized *C. rufipes* workers perform different tasks in relation to their age? 2) at what age does the interior–exterior transition occur? And 3) if they show an age–related division of labor, what is the duration of the different tasks as a nurse or forager? For that reason, *C. rufipes* subcolonies are reared in artificial nest and foraging areas to continuously video–track the single workers´ task affiliations (nursing or foraging). Our results indicate, that single workers start nursing tasks 1–2 days after eclosion until they leave the nest for foraging with an age up to 14 to 20 days, whereas some workers never switch to foraging tasks. We could further demonstrate, that nurses possess a broader body size range than foragers, indicating a higher morphological based specialization of foragers. This study provides a first, detailed analysis of the rich behavioral repertoire and the highly flexible task allocation single *C. rufipes* workers perform during their lifetimes.

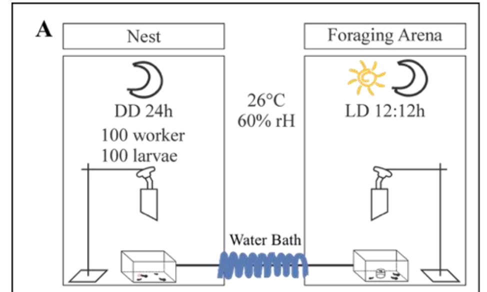
Materials & Methods

Study animals

To analyze the time point of the prominent interior–exterior transition in *Camponotus rufipes* workers, we set up five different subcolonies (named H–1, H–2, 14–1, 14–2 and A). To correct for colony effects, the five subcolonies were formed from three main colonies (named H, 14 and A). The main colonies were reared in climate chambers of the Biozentrum, University of Würzburg, under constant 25°C and 55%rH, with a light–dark cycle (12:12h LD, 300 lux during the light phase). Both, main and subcolonies were fed three times a week with honey diluted in water, frozen cockroaches and fresh water. Initially, queenless subcolonies consisted of about 100 uniformly marked workers and 100 larvae and were kept in a nest and foraging arena (for details see part `behavioral setup´). Workers and larvae were kept in the nest chamber under constant darkness (24h DD) and under a light–dark regime in the foraging arena (LD 12:12h). Both areas were reared under constant 26°C and 60%rH.

Behavioral Setup

Nest and foraging arena consisted of artificial plastic boxes (9.5 x 9.5 x 5 cm), placed in two different incubators (I–30BLL, CLF PlantClimatics GmbH), and were connected via a light impermeable tube (diameter: 0.5 cm, length: 100 cm) which allowed unrestricted access to both areas (Fig. 1, A). Food was provided at a platform (diameter: 5.5 cm, height: 2.3 cm) in the foraging arena, which allowed differentiation between foragers (workers on top of the platform collecting food) or other exterior workers (e.g. waste management or guarding; Fig. 1, B right). Ants were supplied with fresh food (ad libitum honey solution, cockroaches and fresh water) three times a week at randomized time points.



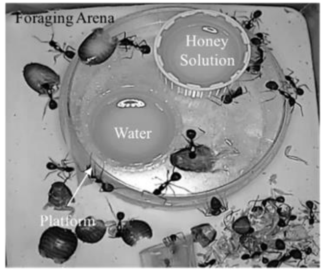


Figure 1: Experimental set up in two incubators. (A) The nest incubator (left; 24h DD) was connected to the foraging arena (right; LD 12:12h) via a light impermeable tube. One subcolony consisted of 100 uniquely marked workers and 100 larvae and were reared under 26°C and 60%rH. Nests and foraging arenas of the subcolonies were video–monitored 24 hours a day. The blue helix between the two incubators indicates the

▲ cooling water bath that prevent the nurses from moving into the connecting tube. (B) Exemplary pictures for nest area (left) and foraging arena (right). In the upper part of the nest area, nurses are taking care for the brood pile, consisting of pupae and larvae. In the foraging arena, the platform with two feeding devices for fresh water and honey solution is visible.

While the nest chamber was kept under constant darkness, a 12:12h light–dark cycle (4000 lux during light phase) was provided in the foraging arena. 100 initial workers were marked with a white dot (Edding) at their thoraces to easily distinguish them from freshly emerged ants. Newly emerged ants were marked individually every day by fixing small printed pieces of paper on their abdomen. Ants that died or lost their labelings during the experiment were excluded from further evaluations. During the first experimental series (lasting seven weeks), ants were disturbed heavily by daily handling (e.g. by collection of newborn ants) within the brood chamber and moved their brood pile from the nest chamber to the inside of the connecting tunnel. Consequently, the first experimental series was terminated after seven weeks so that video tracking of newly hatched workers was possible for at least three weeks (n=4 subcolonies H–1, H–2, 14–1 and 14–2). In the second experimental series, we installed tiny gates within the brood chamber to close the connecting tunnel to the foraging arena during handling procedures inside the nest. Furthermore, the temperature of the connecting tunnel was cooled down to 20°C by using a water bath (Fig. 1, A), as this temperature is not preferred by the ants for nesting (Roces and Núñez, 1995). In this experimental series, video tracking lasted for 11 weeks (n=1; subcolony A), so that video analysis of newly emerged workers was possible for at least 7 weeks. In order to maintain the brood–worker ratio constant, we added the same amount of fresh larvae every week as workers emerged in each subcolony.

Division of Labor Among the Worker Caste

To later identify the ants´ task affiliation, we continuously video monitored the nest and the foraging areas in each subcolony for several weeks (NVR video recorder with IPC cameras, Alomna GmbH). During the dark–phase, videos were recorded using infrared light. Workers were classified as nurses when they were taking care of the brood (licking, feeding, grooming, carrying etc.) at least once per day. Furthermore, workers were classified as foragers, when they were actively collecting food at the platform in the foraging arena either at day or night. Workers that were present in the foraging arena but did not pursue foraging

duties were classified as exterior workers. We did not further distinguish any other tasks besides the three mentioned above. Video material of the nest and foraging arena of each subcolony was evaluated by eye. Moreover, we counted the amount of workers present in the foraging arena every half an hour on a daily basis in order to measure foraging activity on a group–level. As *C. rufipes* workers are known to be polymorphic (Jáffe and Sanchez, 1984; Yilmaz et al., 2016), we further examined if the age at on– and offset of the different tasks is affected by the body size. Body size was measured as precisely described in Yilmaz et al. (2016) and defined as the distance between the head and the petiole of the respective workers.

Statistical Analyses

All datasets were tested for normal distribution using the Shapiro–Wilkinson test. To test for differences between the subcolonies at the on– and offset of the different tasks, Kruskal–Wallis analysis was conducted. To compare the age at on– and offset of the three defined tasks (nursing, exterior activity and foraging) per subcolony, a one–way ANOVA with posthoc Bonferroni analysis was performed. For differences between the subcolonies on an individual foraging level, χ^2 analysis was used. Datasets for foraging on subcolony level and body size measurement were analyzed using the Kruskal–Wallis analysis to check for intercolonial differences. The measured thorax length of nurses and foragers were compared by using Mann–Whitney *U*-analysis. In all statistical analyses, the level for significance was set to α=0.05. All statistical analyses were performed using STATISTICA (StatSoft, Inc., Version 13.0) software and graphs and figures were edited using COREL DRAW 8 (Corel Corporation Ltd., Ottawa, Canada).

Results

The behavioral transition from interior to exterior duties was related to age in all subcolonies (Fig. 2). As the time points of transitions and ages varied significantly between the tested subcolonies (H, 14 and A), datasets were not pooled (Kruskal–Wallis test; nursing onset: $H(4,320)=40.451$; $p<0.001$; nursing offset: $H(4,242)=66.772$; $p<0.001$; exterior onset: $H(4,137)=37.147$; $p<0.001$; exterior offset: $H(4,125)=22.549$; $p<0.001$; foraging onset: $H(4,91)=20.618$; $p<0.001$; foraging offset: $H(4,91)=12.273$; $p=0.015$).

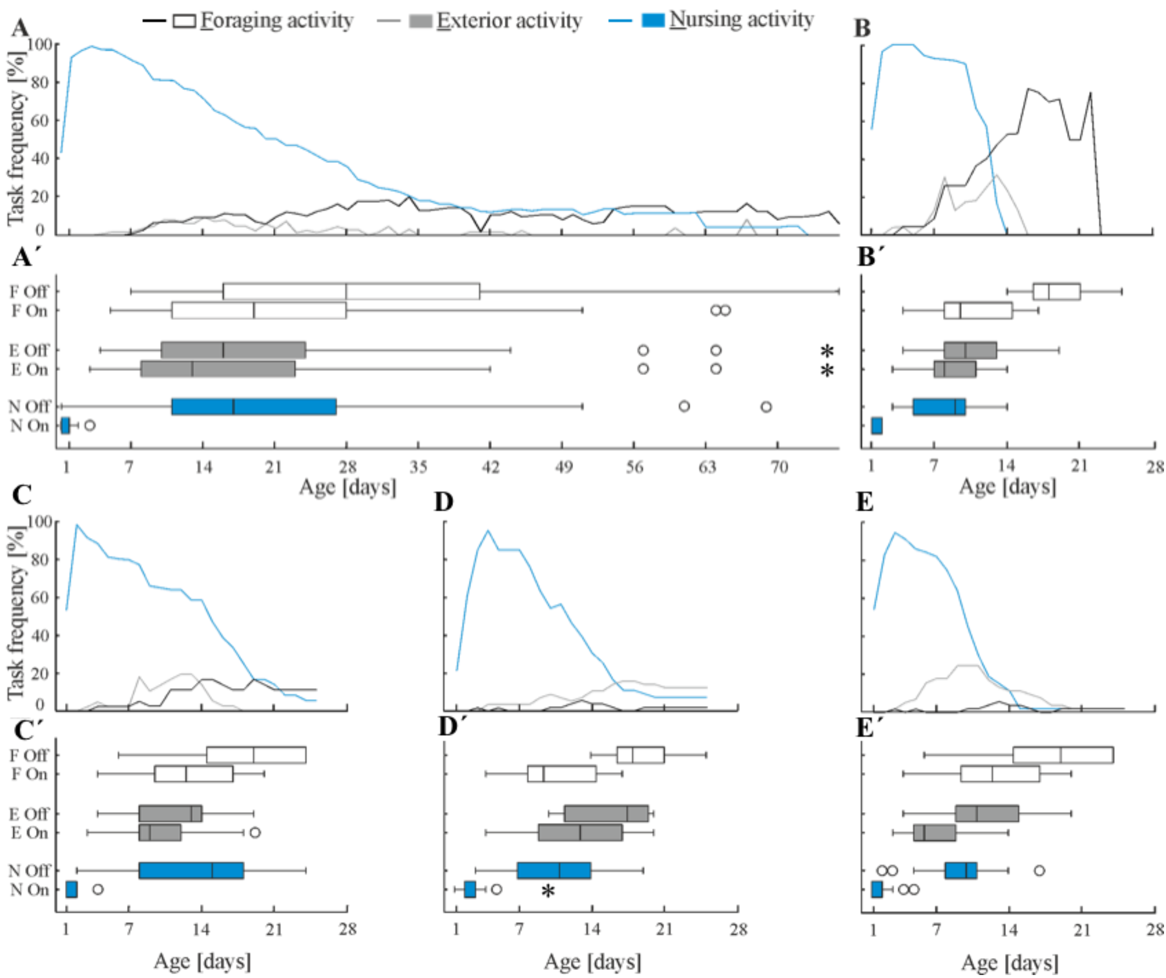


Figure 2: Age–related transition from interior to exterior work. (A–E) Proportion of workers from subcolony A (A), subcolonies H (B+C) and subcolonies 14 (D+E) engaged in nursing, exterior activity or foraging at different ages (days). (A´–E´) Average worker age (median±IQR) at both onset (ON) and offset (OFF) of nursing, exterior activity and foraging of subcolony A (A´), subcolonies H (B´+C´) and 14 (D´+E´). Blue: Nursing (N), gray: Exterior activity (E), white: Foraging (F).

Nearly all freshly emerged ants (one day old at onset) were directly engaged in nursing duties (Fig. 2), except for workers of subcolony 14–1 (Table 1; one–way ANOVA: df=334.00; 14–1 vs H–1: $p<0.001$; 14–1 vs H–2: $p<0.001$; 14–1 vs. 14–2: $p<0.001$; 14–1 vs A: $p<0.001$).

The age at nursing offset differed significantly between workers from subcolonies H+14 (9–11 days) and workers of subcolony A (18 days) (Table 1, one–way ANOVA: df=237.00; A vs H–1: p<0.001; A vs H–2: p<0.001; A vs 14–1: p<0.001; A vs 14–2: p<0.001). Workers of the different subcolonies were between 6–14 days old when first entering the foraging arena (exterior activities), differing significantly between workers of subcolony A and subcolonies H–1, H–2 and 14–2 (Table 1, one–way ANOVA; df=134.00; A vs H–1: p=0.003; A vs H–2: p=0.015; A vs 14–1: p=0.241; A vs 14–2: p<0.001). Worker age at the offset of exterior activities (10–17 days) again differed between workers of subcolony A and subcolonies H–1, H–2 and 14–2 (Table 1, one–way ANOVA: df=127.00; A vs H–1: p=0.003; A vs H–2: p=0.038; A vs 14–1: p=0.296; A vs 14–2: p=0.008). After spending time in the foraging arena, some exterior workers became foragers at an age of up to 9.5 to 20 days. Age at onset of foraging was different between workers of subcolony A and subcolony H–1 (Table 1, one–way ANOVA: df=76.00; A vs H–1: p=0.013; A vs H–2: p=0.079; A vs 14–1: p=0.314; A vs 14–2: p=0.130). The age at the offset of foraging was significantly higher in workers of subcolony A compared with subcolonies H–2 and 14–2 (Table 1, one–way ANOVA: df=74.00; A vs H–1: p=0.072; A vs H–2: p=0.041; A vs 14–1: p=0.095; A vs 14–2: p=0.022). The offset of foraging was defined when workers were engaged in other tasks than the three observed (nursing, exterior work or foraging), if the workers remained inactive inside the nest or if the respective foragers died.

Table 1: Worker age at on and offset of different tasks sequences.

Task sequence	Nursing [mdn±IQR]		Exterior activity [mdn±IQR]		Foraging [mdn±IQR]	
	Onset	Offset	Onset	Offset	Onset	Offset
H–1	1.0±1.0[a] (n=36)	9.0±5.0[a] (n=23)	8.0±4.0[a] (n=19)	10.0±5.0[a] (n=19)	9.5±6.5[b] (n=12)	18.0±4.5[ab] (n=12)
H–2	1.0±1.0[a] (n=49)	15.0±10.0[a] (n=33)	9.0±4.0[a] (n=18)	13.0±6.0[a] (n=17)	12.5±7.5[a] (n=12)	19.0±9.5[a] (n=12)
14–1	2.0±1.0[b] (n=61)	11.0±7.0[a] (n=51)	13.0±6.0[ab] (n=17)	13.0±9.0[ab] (n=15)	13.5±3.5[a] (n=8)	14.5±4.0[ab] (n=6)
14–2	1.0±1.0[a] (n=59)	10.0±3.0[a] (n=46)	6.0±4.0[a] (n=23)	11.0±6.0[a] (n=23)	11.0±8.0[a] (n=6)	14.0±11.0[a] (n=6)
A	2.0±1.0[a] (n=134)	18.0±16.0[b] (n=89)	14.0±15.0[b] (n=62)	17.0±14.0[b] (n=58)	20.0±17.0[a] (n=43)	29.0±25.0[b] (n=43)

Worker age (median ± inter quartile range (IQR)) at on– and offset of different task sequences (nursing, exterior and foraging activity) for the five different subcolonies (H–1, H–2, 14–1, 14–2, A). Different letters show significant differences between types of task sequences (one–way ANOVA under Bonferroni correction; $\alpha=0.05$).

55.73% of all emerged workers underwent the behavioral transition from interior to exterior tasks. We could further discriminate between four different types of workers that perform an age–related division of labor (Fig. 3). 44.27% (n=116) of all workers never switched from interior to exterior tasks during the observation time (Fig. 3, A). Some workers (19.47%; n=51) performed interior and exterior tasks at the same time (Fig. 3, B), whereas 35.11% (n=92) of workers switched completely from nursing to exterior work first and became foragers later on (Fig. 3, C). Due to longer recording periods (11 weeks) of subcolony A, we could observe three workers (1.15%; n=3) that switched back from exterior tasks to nursing duties (Fig. 3, D). Datasets over the five subcolonies were pooled, as the above mentioned different types of age–related labor division just display four exemplary task sequences.

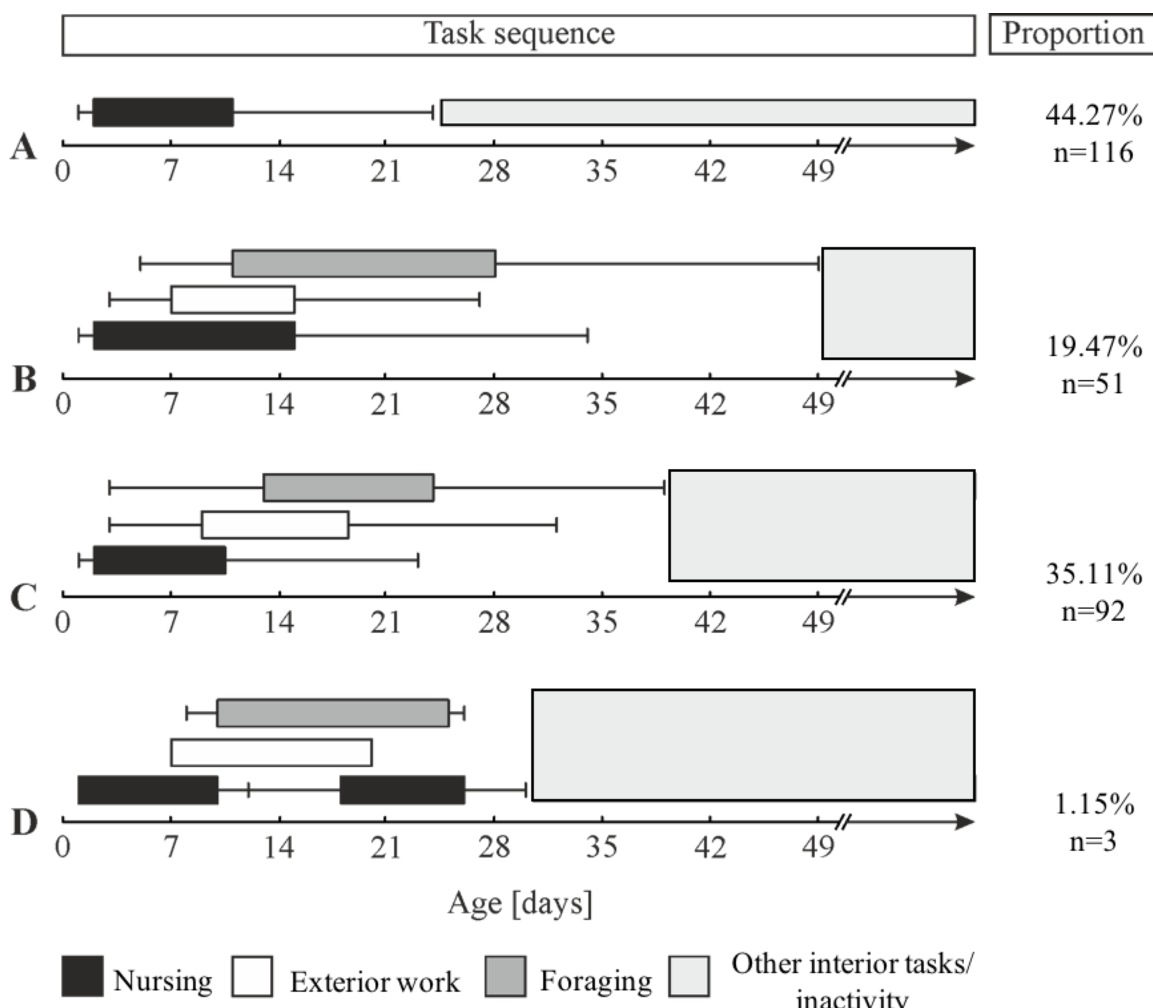


Figure 3: Proportion of age–related task sequences in *Camponotus rufipes* workers. (A) Workers remain nurses for several weeks (n=116). (B) Workers switching to exterior work while continuing nursing activity (n=51). (C) Workers with complete interior–exterior transition (n=92). (D) Workers with an interior–exterior transition that switched back to interior activity (n=3). Black: Nursing. White: Exterior activity. Dark Grey: Foraging. Light grey: other interior tasks (e.g. waste management or inactivity). Boxes indicate mean age at onset and offset of each task, whiskers indicate standard deviation error (SDE; left: SDE onset; right: SDE offset). Datasets were pooled over the five subcolonies.

Besides measuring foraging level on an individual level, we additionally measured foraging activity on a subcolony level. Subcolonies 14–1 and 14–2 differed significantly from each other, so foraging of the subcolony level was just pooled for subcolonies H–1 and H–2 (Kruskal–Wallis test, Subcolony H: $H(1,96)=0.174$; $p=0.895$; Subcolony 14: $H(1,96)=7.878$; $p=0.005$). We could demonstrate, that foragers preferably forage during nighttimes (Fig. 4), but with a low proportion of workers being present in the foraging arena during the day.

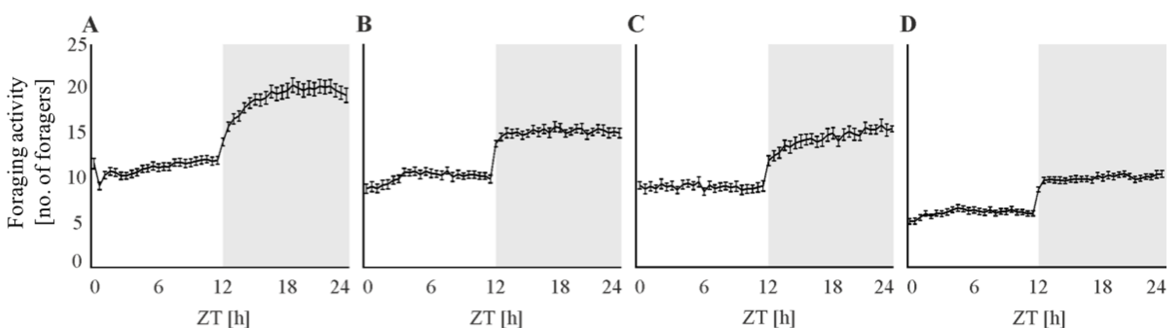


Figure 4: Foraging activity on a group level over 24 hours. (A) Subcolony A (observation period: 77 days). (B+C) Subcolony 14–1 (B) and 14–2 (C; observation period: 28 days). (D) Subcolonies H–1 and H–2 (observation period: 28 days); dataset was pooled over the two subcolonies. Black line indicates mean ±SDE. Activity was recorded by counting workers in the foraging arena every 30 minutes. The 12 hour dark phase is indicated in grey. ZT: zeitgeberzeit

On the individual level, no significant differences were found between all five subcolonies, so the datasets were pooled (χ^2 test, χ^2=4.012; p=0.404). Also at an individual level of foraging activity, most *C. rufipes* workers prefer foraging during the night (68.0%; Fig. 5). 30.7% of all tested individuals (n=46) showed foraging activity at both, day– and nighttimes. Only two workers in all five tested subcolonies were solely diurnal (1.3%).

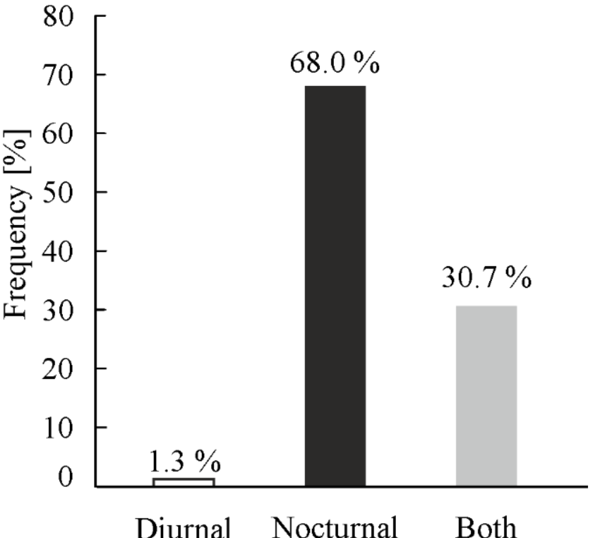


Figure 5: Temporal preferences in foraging activity. Proportion of individuals that foraged either strictly diurnally (white, n=2), strictly nocturnally (black, n=102) or were active during both light regimes (grey, n=46). Data sets were pooled over five subcolonies.

Not all workers underwent the transition from nurses to foragers in our experiments, thus not only the ongoing age can account for the prominent interior–exterior transition. As also polymorphic differences can cause division of labor, we measured the thorax length of all individuals for a size distribution (Fig. 6, A). The highest number of measured workers ranged in between 2.5 and 3.5 mm thorax lengths (Fig. 6, A). Datasets over the five

subcolonies were pooled, since we found no significant difference between the subcolonies (Kruskal–Wallis test, nurses: H(4,107)=6.734; p=0.151; foragers: H(4,57)=1.451; p=0.835). Our data mentioned above suggests that the switch from nurses to foragers occurs at an age of up to 14–20 days. Therefore, we compared the thorax lengths of workers older than 14 days between foragers and nurses. Nurses (2.21 mm to 4.45 mm; n=107) showed a higher variation in their body size than foragers (2.13 mm to 3.5 mm; n=56; Fig. 6, B). Nurses possess a significantly larger thorax lengths than foragers (Fig. 6, C; Mann–Whitney *U*–test: Z=-3.738; p<0.001; mean nurses: 3.11 mm; mean foragers: 2.83 mm).

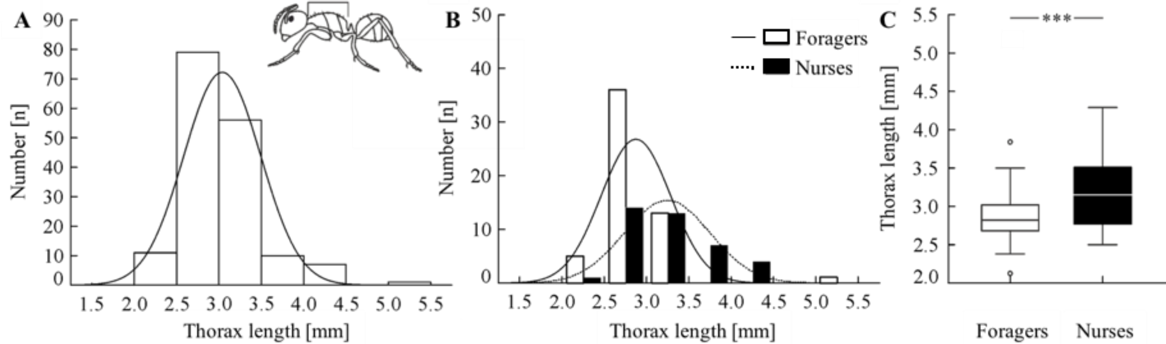


Figure 6: Body size distribution of individually nurses and foragers. Thorax lengths were compared between foragers and nurses of ants older than 14 days. Data sets were pooled over five subcolonies. (A) Frequency distribution of all measured individuals (n=163). Thorax length was defined as the distance between the head and the petiole. (B) Frequency distribution of foragers (white) and nurses (black). (C) Boxplots. Boxes show medians (center lines) and interquartile ranges (boxes) for foragers (white; n=56) and nurses (black; n=107). Whiskers indicate the minimum and maximum values**,** open circles show outliers. Asterisks indicate significant differences between groups (Mann–Whitney *U*–test; α=0.05). Insert in (A) adapted from Yilmaz et al., 2016.

Discussion

In all tested subcolonies of *Camponotus rufipes* ants, we could observe an age–related division of labor. After hatching, young ants are mainly immobile and nurtured by older workers within the first 48 hours after eclosion, before they start themselves performing nursing tasks. After a short transition rate of exploring the foraging arena, workers switch to active foraging tasks at an age of up to 14 to 20 days. This slight variation at the onset of foraging suggests, that not only the age can account for the interior–exterior transition of C. *rufipes* workers. We could demonstrate, that additionally different morphs of workers are engaged in different tasks, with foraging tasks mainly done by media–sized workers. In this study, we could show for the first time that division of labor is related to both, body size and age of *C. rufipes* worker ants.

Age–related Division of Labor in *C. rufipes* Workers

The observed age–range (2–3 weeks) of workers at the onset of foraging tasks in our study was comparable to that reported for various ant species (*Messor* and *Myrmica*: Ehrhardt, 1931; *Pheidole dentata*: Muscedere et al., 2009, 2013; *Platythyrea*: Bernadou et al., 2015; *Pogonomyrmex*: Gordon, 1989; Holbrook et al., 2013; *Solenopsis invicta*: Mirenda and Vinson, 1981; *Temnothorax albipennis*: Dornhaus, 2008). Nevertheless, some ant species were also reported for a later switch to exterior tasks: workers of *C. floridanus* switched to foraging duties at an age of 10 weeks (Gronenberg et al., 1996) and workers of the desert ant *Cataglyphis bicolor* started to perform foraging tasks after 28 days (Wehner et al., 1972; Wehner and Rössler, 2013). Division of labor related to age was also shown for other eusocial Hymenoptera, like in workers of the termite *Macrotermes* (13–32 days; Hinze and Leuthold, 1999) and the honeybee (28–49 days; reviewed in Robinson, 1992; Winston, 1991). These differences among the different taxa of eusocial Hymenoptera suggest, that the age–related division of labor shows a higher variety and flexibility in ant species. The observed temporal polyethism of *C. rufipes* workers in this study matched the age range of the maturation of the peripheral and central visual brain neuropils previously reported for this species (Yilmaz et al., 2016). We furthermore could confirm our hypothesis, that behavioral observations conducted in earlier studies on this species (Soares et al., 2008) chose a too broad age range (younger and older than 45 days) to detect age–related division of labor. However, the age at on– and offset of nursing and foraging duties of *C. rufipes*

workers varied between the observed subcolonies, indicating the high flexibility of task allocation in this species (Gordon, 1989; Robinson et al., 2009; Waddington and Hughes, 2010).

Beside age–related changes of brain neuropils, also task–related physiological changes occur among ant workers of different tasks. Hence, most studies investigated changes of the cuticular hydrocarbon profile (*Camponotus*: Bonavita–Cougourdan et al., 1993; Lavine et al., 1990; *Pogonomyrmex:* Wagner et al., 1998, 2000, 2001) and in juvenile hormone titers in the hemolymph (Brent et al., 2006; Dolezal et al., 2012) that might alter the individual thresholds to fulfill certain tasks and therefore might be accountable for age–related division of labor. As these physiological mechanisms are so far not studied for *C. rufipes* ants, our study provides an overall stable basis to examine further physiological task–related changes in differently aged workers.

Task Sequences of *C. rufipes* Workers

In our experiments, *C. rufipes* nurses started to explore the foraging arena (as exterior workers) before they started to forage actively at an age of two to three weeks. Hence, the same individuals were observed performing nursing and exterior tasks at the same time. Also for *Cataglyphis fortis* ants it was shown, that interior workers intensively explore the close vicinity of their nest entrance (Stieb et al., 2012; Fleischmann et al., 2016), before they leave the nest to become foragers. This exploration runs are hypothesized to serve as learning and orientation walks (Wehner et al., 2004; Fleischmann et al., 2017). As *C. rufipes* workers were reared in small artificial plastic chambers in our study, their need to perform long foraging runs or to recruit other foragers to establish a new food source is reduced. This results in an extended lifespan and high survival of foragers under laboratory conditions, as foraging under natural conditions is normally associated with the death of the respective worker after some time (Porter and Jorgensen, 1981; Gordon and Hölldobler, 1987; Oettler and Johnson, 2009). Most common extrinsic factors influencing forager mortality are extreme soil temperatures, desiccation, navigational errors, and predators (Kwapich and Tschinkel, 2015). In the subcolonies reared in our experiments, solely the few initially old foragers died and were barely replaced by younger ones. These overaged foragers prevent the transition of younger workers into the forager population and young workers accumulate inside the nest and survive beyond their typical lifespan (Kwapich and Tschinkel, 2015).

This could be a possible explanation, why not all marked *C. rufipes* workers switched from nursing to foraging tasks, which was also shown in the laboratory for *Pogonomyrmex* workers (Gordon et al., 2005). Even though we did control for the worker–brood ratio by frequently adding new larvae, we did not control for the final nurse–forager ratio. As young ants emerged faster than the interior–exterior transition occurred, this may explain the high amount of nursing activity in all subcolonies as well as the low proportion of active foragers.

In addition to the permanent nurses in our study, we could also observe some *C. rufipes* workers switching back to nursing tasks after performing foraging duties. The different task processes an individual can undergo were further described in several ponerine ant species: workers that can shift from interior to exterior work, workers that remain inactive inside the nest in the course of their life, workers that remain nursing all their life and some workers that became foragers without caring the brood (*Odontomachus*: Dejean and Lachaud, 1991; *Amblypone*: Masuko, 1996; *Diacamma*: Nakata, 1995). Besides, workers being actively engaged in nursing, exterior and foraging activities, we could observe a high number of inactive workers inside the nests. Also more than 50% of workers in an insect colony remain inactive (bees: Lindauer, 1952; Jandt et al., 2009; wasps: Gadagkar and Joshi, 1984; ants: Mirenda and Vinson, 1981; Herbers, 1983; Cole, 1986; Retana and Cerdá, 1990; Schmid–Hempel, 1990; Dornhaus, 2008; Dornhaus et al., 2009; termites: Rosengaus and Traniello, 1991), inactivity within social insects is so far hardly examined (Charbonneau and Dornhaus, 2015). Inactive workers may deal as \`reserves´ that become active when the workload is increasing, or they play a fundamental role in communication (O'Donnell and Bulova, 2007) or act as food reserves (Sendova–Franks et al., 2010). Indeed, all workers may spend some time inactive because of their physiological need for sleep or rest (Klein et al., 2003, 2010). As we only classified and assigned three tasks but did not further classified workers that remained inactive, we cannot prove one of the above mentioned explanations for *C. rufipes* workers. Therefore, ants otherwise engaging in these duties might have remained inactive in the present study. In *C. rufipes* workers, we could observe a certain degree of variation in the proportion and the amount of workers undergoing the behavioral transition. These variations existed within a single subcolony and in between different subcolonies, indicating a high degree of flexibility and plasticity of age–related division of labor.

Moreover, workers of *C. rufipes* were mainly described as nightactive foragers on a colony level (Del–Claro and Oliveira, 1999; Fagundes et al., 2005; Jáffe and Sanchez, 1984;

Lindenberg et al., *in prep*). In this study, we could as well confirm nocturnal foraging activity on a colony level but additionally demonstrated nocturnal foraging at an individually level by continuous video–tracking of single workers. Besides, a couple of workers foraged also actively during daytimes, but were relatively rare and almost always nocturnally at the same time. Further field studies in this species could proof an increased diurnal foraging activity (Del–Claro and Oliveira, 1999; Fagundes et al., 2005; Lindenberg et al., *in prep*), which is maybe an adaption to seasonally thermal fluctuations in the environment. The simultaneous presence of nocturnal and diurnal foragers in one colony may support the flexible shift between different foraging periods.

Size–related Division of Labor in *C. rufipes* Workers

Our results indicate, that some individuals switch to exterior tasks as they matured, whereas others kept performing interior work. Same–aged ants were shown to differ in their thresholds to perform nursing (Weidenmüller et al., 2009) and foraging duties (Detrain and Pasteels, 1991), resulting in differences of the task allocation. As therefore not only the age can account for the division of labor within the worker caste, we further compared the body sizes of >14 day old *C. rufipes* nurses and foragers. Nurses showed an overall broader body size than foragers, whereas predominantly media–sized workers became foragers. Indeed, previous studies could confirm media–sized workers of *C. rufipes* engaged in foraging and major workers serving as repletes (Jáffe and Sanchez, 1984; Soares et al., 2008). Interestingly, media–sized workers seem to constitute the largest proportion within the *C. rufipes* colonies (Yilmaz et al., 2016; media–sized workers in this study were described as workers with a thorax length of 2.5 to 4.0 mm). This is also in accordance with our study, where the highest number of measured workers was exactly within this size range (2.5 to 3.5 mm). Worker polymorphism is also well described for other ant species: minor ants are engaged in nursing tasks, media workers are important for foraging activity and major workers are reported as guards of the nest entrance due to their disproportional large heads (*Camponotus sericeiventris*: Busher et al., 1985; *Orectoganthus versicolor:* Carlin, 1981; *Solenopsis invicta* Wilson, 1978). An explanation for mainly minors and medias being engaged in foraging tasks is given in *Pheidole pallidula* ants: 1) They are numerous and therefore are able to precisely control mass recruitment and 2) loss of minors and medias is less affecting the ant colony than the loss of the more rarely produced major workers (Detrain

and Pasteels, 1991). This may explain the higher amount of media–sized ants engaged in foraging tasks we could observe in our study. Indeed, also major ants can perform nursing behavior if the number of majors is artificially increased (Detrain and Pasteels, 1991).

In this study we could, for the first time, demonstrate a simultaneous age– and size–related division of labor in *C. rufipes* worker ants. Freshly hatched ants start performing nursing tasks in the nest interior within 48 hours after emergence and switch to external foraging tasks with an age of up to 14–20 days. Additional to the temporal polyethism, we could further confirm a size–related division of labor, as mainly media–sized workers switched to foraging activity. The simultaneous existence of both, temporal and size–related division of labor among the worker caste of *C. rufipes*, indicates how highly flexible and plastic task allocation occurs in this ant species.

Chapter II

Daily Foraging Activity Patterns of *Camponotus mus* and *C. rufipes* Workers

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Daily Foraging Activity Patterns of *Camponotus mus* and *C. rufipes* Workers

Introduction

Ants are among the most successful insects on earth. They are thought to make up 15–25% of the terrestrial animal biomass (Schultz, 2000). They count as principal predators in most habitats and adapted themselves perfectly to almost every geographical and climatic condition. Among ants, the genus *Camponotus* comprises about 1,000 species worldwide (Bolton, 1995). Different *Camponotus* species often coexist in the same habitat like the here investigated C. *mus* and *C. rufipes*, which live sympatrically in parts of Uruguay, South America (Goni et al., 1983). Even though workers of both ant species are found to forage on extrafloral nectaries and honeydew producers, they show a different nesting biology. Whereas nests of *C. mus* are mainly found stone walls, dead wood and roof trusses, workers of *C. rufipes* build nests in open grasslands, using plants or digging channels (own observation). Many *Camponotus* ants show characteristic foraging activity rhythms, which can be documented on both, a daily and a seasonal basis (Briese and Macauley, 1980; Cros et al., 1997; Cerdá et al., 1998; Santini et al., 2007). Workers of *C. rufipes* forage actively during the night (Jáffe and Sanchez, 1984) with some occasional daytime foraging activities (Del–Claro and Oliveira, 1999; Fagundes et al., 2005), while the sympatric existing species *C. mus* is strictly diurnal (Falibene and Josens, 2014). To date, less is known about the underlying mechanisms, accountable for the switch from day– to nighttime activity and vice versa in *C. rufipes.* The highly flexible adaption of an ants´ activity period to certain times of the day or the season might be correlated to food availability (*C. rufipes*: Mildner and Roces, 2017) or environmental factors, like temperature and light, but can also result from interspecies interactions within an ant community. In this study, daily activity patterns will be investigated under the consideration of the abiotic factors temperature and light in the coexisting species *C. mus* and *C. rufipes*.

As all ants are ectothermic animals, their activity is limited to certain times of the day when environmental conditions are physiologically tolerable (Marsh, 1988; Cerdá et al., 1998).

Besides humidity (Whitford and Ettershank, 1975; Azcárate et al., 2007) and light intensity (Christian and Morton, 1992), temperature is one dominant abiotic factor constraining activity periods (Porter and Tschinkel, 1987; Drees et al., 2007; Ashikin and Hashim, 2015). Temperature directly influences walking speed (Shapley, 1920; Barnes and Kohn, 1932; Drees et al., 2007), respiration (Jensen and Nielsen, 1975) and desiccation (Lighton and Feener, 1989) in ants, therefore foraging activity is limited to a certain temperature range. To deal with high temperatures, *Cataglyphis bombycina* desert ants are able to produce heat–stable proteins frequently (Gehring and Wehner, 1995), have long legs that allow them to keep their body as far away from the surface as possible (Cros et al., 1997; Clémencet et al., 2010) and evolved special hair structures in order to increase sun reflection and decrease body temperature (*Cataglyphis bombycina*: Shi et al., 2015). The selection of particular thermal environments on a daily and seasonal basis is a behavioral strategy providing certain advantages for ants, i.e. avoiding of competition in coexisting ant species, where subordinate species (species at the bottom of hierarchy) are active during daytimes to avoid competition with dominant species (species at the top of hierarchy), that are active during nighttime (Cerdá et al., 1998). In a recent study of two coexisting leaf–cutting desert ants, the ambient temperature was the most dominant factor restricting foraging activity. While the dominant *Acromyrmex lobicornis* switched from dayactivity under moderate temperatures in autumn to nocturnal foraging activity in summer and spring, workers of the subordinate *A. striatus* were exclusively dayactive irrespective of the season (Nobua Behrmann et al., 2017). Also the foraging activity in two coexisting and sympatric living *Myrmecia* species is affected by both, thermal tolerances and competition (Jayatilaka et al., 2011). As the dominant species *M. pyriformis* prefers foraging at cooler temperatures during the entire year, the subordinate *M. croslandi* is forced to forage under higher temperatures during daytimes. In case of overlapping dietary niches, foraging interests and similar thermal tolerances, species abundance and dominance play a major role in temporal separation (Blüthgen and Fiedler, 2004; Jayatilaka et al., 2011; Barbieri et al., 2015; Houadria et al., 2015; Anjos et al., 2017).

Different seasonal and daily timing of an animals´ activity are based on time measuring mechanisms that allow living organisms to face periodical environmental changes and adapt their own mode of life (Sharma, 2003). Time measuring is accomplished by endogenous oscillators that are temperature compensated, meaning their periodicity is stable over a broad

range of temperatures (Pittendrigh, 1954; Hastings and Sweeney, 1957). However, coping with daily changes in the environment, referred to as ‘zeitgebers’, the oscillator is generating rhythms with a period length around 24 hours. In absence of external zeitgebers, the oscillator is free–running with a periodicity close to 24 hours (Dunlap et al., 2003). Potential zeitgebers can be either biotic, like food availability or social interactions or abiotic, like light–dark cycles and temperature (Saunders, 2002; Bloch, 2009; Mildner and Roces, 2017). Field studies in different ant species revealed, that temperature influences foraging activity (Holt, 1955; Sheata and Kaschef, 1971; Nielsen, 1981; Porter and Tschinkel, 1987), by controlling metabolic efficiency (Peakin and Josens, 1978) and transit rates (Holt, 1955; Marsh, 1985). Various studies already demonstrated the presence of an endogenous clock in several ant species (e.g. Hölldobler, 1961; Kipyatkov, 1995; Kumar Sharma et al., 2004; Mildner and Roces, 2017). Under laboratory conditions, workers of the wood ant species *Formica* showed a circadian rhythm under cycling temperature ranges in light–dark (LD) conditions as well as constant darkness (DD) in both foraging activity and locomotor activity (North, 1993), indicating that temperature is a sufficient zeitgeber for daily activities.

In this study, we combined field and laboratory experiments to test if temperature and light could be potential restricting abiotic factors that shape daily activity patterns of the two sympatric *Camponotus* species, *C. mus* and *C. rufipes*. Therefore, field experiments were conducted under natural conditions in La Coronilla, Uruguay, to examine foraging activity rhythms of both *Camponotus* species in correlation to temperature, humidity and light intensity. In two laboratory experiments, we further examined whether daily cycles in temperature could be one main environmental factor restraining the locomotor activity rhythms of these two species. Therefore, we first determined critical thermal limits of individual workers, and then simulated different temperature regimes to investigate how daily locomotor activity patterns of both species are affected by changing thermal conditions.

Material & Methods

Study Site in the Field and Experimental Animals in the Laboratory

We comparatively investigated the daily foraging activity of *C. mus* and *C. rufipes* colonies in the field in La Coronilla, Uruguay (33°53'25.2"S, 53°31'27.6"W) at local spring time (November – December 2015) to identify potential abiotic factors that limit foraging activity. Nests of *C. mus* were located in stone walls and *C. rufipes* in grasslands near small trees and shrubberies and can be found in close proximity. As single colonies can consist of several satellite nests, we therefore tested every selected nest for its independency by translocating and observing marked individuals between the adjacent satellite nests to test for aggressive behavior between individual workers.

All laboratory colonies of *C. mus* and *C. rufipes* used in this study were founded by a single queen (collected during their mating flights in December 2011 and 2015 in La Coronilla) and maintained in a climate chamber at the Biozentrum, University of Würzburg, at 25°C and 50% rH under a light–dark (LD) cycle of 12:12h (300 lux during the light phase). The colonies as well as subcolonies (see below) were fed *ad libitum* with water, honey diluted in water and twice a week with frozen cockroaches. To determine the temperature tolerance window of both species in the laboratory, four different *C. mus* colonies (n=32) and three different *C. rufipes* colonies (n=35) were used. To clearly identify temperature as zeitgeber for the endogenous clock of the ants, locomotor activity rhythms of *C. mus* and *C. rufipes* ants were individually monitored in the laboratory under controlled thermal regimes, using two main *C. mus* and *C. rufipes* colonies. Commissions to export both ant species from Uruguay are officially declared by the Dirección General de Recursos Naturales Renovables, Ministerio de Ganadería, Agricultura y Pesca. Furthermore, the authors declare that both specimen are not protected under the Convention on International Trade in Endangered Species (CITES), the European or the German regulations.

Field Experiment: Monitoring Daily Foraging Activity Rhythms in the Field

Daily foraging activity periods of three independent *C. mus* and six independent *C. rufipes* nests was visually monitored, by first identifying the main foraging direction of every nest. In the case of *C. mus*, counts of in– and outgoing ants were determined on a reference circle of 5 cm diameter around each nest entrance (Fig. 1, A). In the case of *C. rufipes*, all plants in a radius of 30 cm around each nest were removed to facilitate behavioral observations and plastic barriers covered with paraffin oil were installed in a radius of 20 cm around each nest to prevent ants from leaving the nest in all directions (Fig. 1, B+C). Two to three cardboard bridges were installed in the main foraging pathways of the colony for two days, and were then reduced to one bridge on the next day to channel foraging activity of the whole colony (Fig. 1, D).

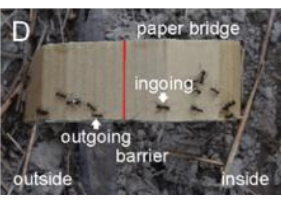


Figure 1: Monitoring foraging activity of two sympatric *Camponotus* species in the field. (A) Nest of *C. mus* located in stonewall. A virtual circle (diameter 5 cm) was drawn around the nest entrance to simplify counting of in– and outgoing ants. (B+C) Thatch nests of *C. rufipes* are located in open grasslands and usually covered by vegetation, which was removed in a 30 cm radius around the nest to facilitate observations. (C) A plastic barrier was installed around the nest to prevent uncontrolled foraging bouts and enabled precise counting of foragers. (D) Foraging bouts were only possible by crossing a cardboard bridge placed over the plastic barrier. Foraging activity was recorded as the number of in– and outgoing workers crossing a virtual line in the center of the cardboard bridge (indicated by red line) over 24h.

At each *C. mus* and *C. rufipes* nest, in– and outgoing ants were counted for 5 min every 30 min on a 24h basis. During the night, countings were done under low intensity red light. Simultaneously, we measured air temperature and ambient humidity (EL–USB–2–LCD+, Lascar Electronics) as well as light intensity (light meter LM–100, Amprobe) every 30 min in close vicinity of the nest entrance.

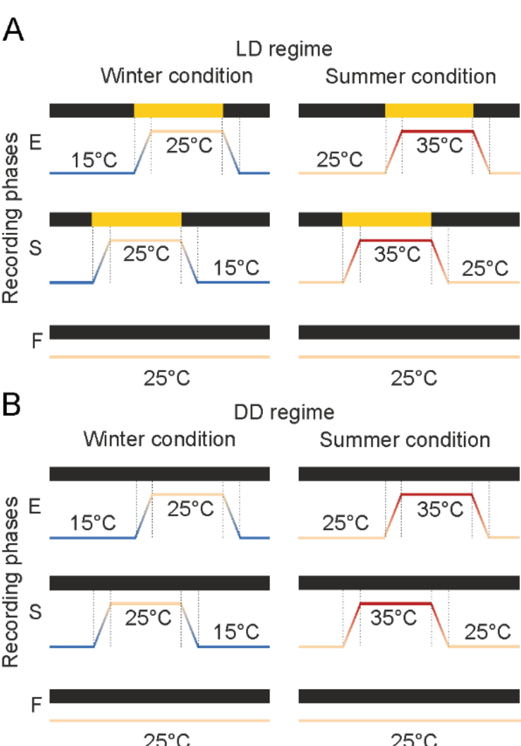
Laboratory Experiment I: Temperature Tolerance Values Shaping Daily Activity Patterns of *Camponotus* Ants

We analyzed critical temperature limits of workers from both *Camponotus* species in the laboratory to test if the two species vary in their temperature tolerances, which might account for their different foraging activities that occur in the field. Furthermore, the obtained data were used to adjust winter and summer temperature conditions used in the laboratory (see methods below).

Prior to the testing of the temperature tolerance values of both species, the body weight of every individual was determined with a micro scale (ABS–N, Kern & Sohn GmbH, Ballingen, Germany), to serve as a measure of the body size of the ants (Josens, 2002). Individual ants were placed inside a small plastic container (height: 5 cm, diameter: 3 cm), which was then placed on top of a water bath to manipulate the floor temperatures. For both, critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}), the ants were kept at an initial floor temperature of 25°C for 10 min prior to testing. We chose this temperature as main colonies of both species were maintained in climate chambers at 25°C before (see above). The floor temperature was increased or decreased, respectively, from 25°C at a rate of 1.0°C min^{-1} to determine the CT_{max} and CT_{min} for every ant individually. Similar rates close to 1.0°C min^{-1} have previously been used in studies with ectotherms (e.g.: Hu and Appel, 2004; Jayatilaka et al., 2011; Esch et al., 2017). At every temperature step, the movement abilities of the ants were tested by tapping them with forceps. A temperature value was considered as critical as soon as ants could neither move anymore nor return to their upright position.

Laboratory Experiment II: Temperature and Locomotor Activity Rhythms of *C. mus* and *C. rufipes* Workers

To clearly identify temperature as zeitgeber to entrain the endogenous clock, locomotor activity rhythms of *C. mus* and *C. rufipes* ants were individually monitored in the laboratory under controlled thermal regimes. Therefore, we established queenless subcolonies consisting of approximately 50 individual workers of unknown age and task, taken from two main *C. mus* and *C. rufipes* colonies (see above). Subcolonies were transferred into incubators (Memmert GmbH + Co. KG, Schwabach, Germany) and reared under two different thermal conditions (see below) with constant humidity (55% rH). Different temperature and light regimes were used to identify the potential entraining effect of these two zeitgebers (Fig. 2). In the first experimental series, we recorded daily locomotor rhythms under both, temperature and 12:12h light–dark regime (LD) to evaluate how these two zeitgebers interact (Fig. 2, A). In the second experimental series, daily locomotor rhythms were recorded under both, the respective thermal regime and constant darkness (24h DD) to test if temperature alone can act as zeitgeber for these ants (Fig. 2, B).



▲ **Figure 2: Light and thermal regimes to monitor locomotor activity rhythms in the laboratory.** (A+B) Thermal regimes for winter and summer conditions under light–dark cycle (LD; A) and constant darkness (DD; B) for all recording phases. Winter conditions consisted of constant 15°C during the respective nighttime and constant 25°C during the respective daytime, summer conditions of constant 25°C during the respective nighttime and constant 35°C during the respective daytime. Dotted lines indicate one hour temperature ramp between the constant temperatures. Bars: black (dark phase), yellow (light phase); E: entrainment, F: free–run, Lines: beige (25°C), blue (15°C), red (35°C); S: phase–shift.

Based on weather data from the collecting site in La Coronilla and on evaluations of our thermal tolerance values, we chose a thermal gradient from 35°C at daytimes to 25°C at nighttimes (from now on termed summer thermal regime) and from 25°C at daytimes and 15°C at nighttimes (from now on termed winter thermal regime) both to stimulate natural relevant thermal conditions (average temperatures in La Coronilla, Uruguay in January 2015: 22.5°C and June 2015: 12.0°C; https://de.climate–data.org/location/632814/). The temperature ramp of 10°C difference between high and low temperature values started at each light on– and offset and took one hour to complete, followed by 11 h of constant temperature.

After one week of entrainment of each treatment (either thermal regime + LD or thermal regime + DD) in subcolonies, ants were placed individually in glass tubes of locomotor activity monitors established for observation on *Drosophila* (LAM, TriKinetics Inc, Waltham, MA USA) and further modified as described precisely in Mildner and Roces (2017) to test the effect of light and temperature on individual daily locomotor rhythms. Locomotor activity was recorded over 20 days under three different thermal and light regimes, each lasting one week (entrainment: 7 days; phase–shift: 6 days; free–run: 7 days). After one day of acclimatization, daily activity of ants was recorded under the same thermal regime and 12:12h LD cycle or 24h DD used for the subcolonies to evaluate how the ants synchronized their locomotor activity with these two zeitgebers (entrainment: E). In the second week of recordings, we shifted the zeitgeber cycles (LD treatment: thermal + light regime, DD treatment: thermal regime) six hours in advance to test if individuals could resynchronize their locomotor activity (phase–shift: S). Finally, individual locomotor rhythms were recorded under DD and 25°C for one week to quantify endogenous, free–running rhythms (free–run: F). Locomotor rhythms recorded over the three recording periods (20 days) were displayed in form of actograms and raw data were evaluated using the Fiji

Plug–in ActogramJ (Schmid et al., 2011). We calculated average activity patterns of all surviving ants for the recording periods under the used zeitgeber cycles (LD treatment: thermal + light regime, DD treatment: thermal regime; week 1), respectively.

Statistical Analysis

Relations between outgoing foraging activity rhythms in the field and the ambient temperature were analyzed using a general linearized mixed model (GLMM) with R v3 (R Foundation for Statistical Computing, Vienna, Austria). As the data revealed no normal distribution (Shapiro–Wilk test) and no homogeneity of variances (Levene test), we performed a squareroot transformation for better approximation. The outgoing foraging activity was used as response variable and tested against the fixed factors `species´, `ambient temperature´, `day– or nighttime´ (daytime was identified if the light intensity was higher than 10.000 lux) and the interaction between these factors. As every colony was repeatedly measured throughout the day, `colony´ and `measurement´ were included as random factor. For post–hoc analysis of the GLMM, a Spearman–rank test under Bonferroni correction (α=0.008) was used to compensate for multiple testing.

CT_{max} and CT_{min} were compared between the species afterwards in relation to the ants´ body mass (ANCOVA, GraphPadPrism Version 5.00). Effect of the ants´ mass on critical temperature limits was determined via Spearman´s correlation. As the mass had no influence on the CT_{max}, values were compared between species via an unpaired t–test. The statistical analysis concerning CT_{max} and CT_{min} were done using STATISTICA (StatSoft, Inc., Version 13.0). For each actogram, the survival rate was determined per eye and tested for differences between thermal regimes within each recording phases (E, S and F) and experimental series (LD and DD regime; χ^2 tests or Fisher´s exact tests, α=0.05). Furthermore, we determined the proportion of rhythmic individuals for each recording phase, separated for each experimental series (Lomb–Scargle–method, significance level: α=0.05, ActogramJ) and tested for differences within recording phases (χ^2 tests or Fisher´s exact tests, α=0.05). Total activity levels as well as relative activity during the respective night phase on the second day of each recording period were calculated to avoid pseudoreplicates and to quantitatively compare activity patterns between the temperature regimes within each recording period and experimental series (Mann–Whitney *U*–tests, α=0.05). Using periodogram analysis (Lomb–

Scargle–method, significance level: α=0.05, ActogramJ) we calculated the period and power of each individual ant and tested for significant differences between temperature regimes within each recording phase and experimental series (Mann–Whitney *U*–tests, α=0.05). To determine day or nightactivity, levels of respective nightactivity were tested against random activity in both, the respective light and dark phase, using Wilcoxon signed–rank tests, and tested for differences between winter and summer conditions within each recording phase and light regime under Bonferroni correction (α=0.025). The statistical analysis concerning locomotor activity rhythms in the laboratory were performed using SPSS 23 (IBM, Chicago, IL, USA) software after testing datasets for normal distribution (Shapiro–Wilkinson test). Graphs and figures were edited using COREL DRAW X8 (Corel Corporation Ltd., Ottawa, Canada).

Results

Field Experiment: Monitoring Daily Foraging Activity Rhythms in the Field

Workers of the two sympatric living *Camponotus* species, *C. mus* and *C. rufipes* foraged at acacia trees, where they collected acacia seeds and nectar at extrafloral nectaries and honeydew from treehoppers and small insect prey, like aphids. Different foraging activity patterns of both species in the field could be observed. *C. mus* foragers were exclusively active during daytimes with an onset of foraging around 7:00 am and offset around 8:00 pm (Fig. 3, A–C). In *C. rufipes* all nests display a predominant nocturnal foraging activity, but showed different foraging activity levels during daytimes: whereas some nests display a low number of foraging bouts (Fig. 3, D–F), the other nests showed higher foraging activity during daytimes (Fig. 3, G–I), but still less than the half of the nocturnal foraging activity. Most *C. rufipes* nests showed a decrease in foraging activity between 11:00 am and 13:00 pm.

Whereas the species and day– or nighttime significantly influenced the outgoing foraging activity (GLMM, Species: $F=11.107$, $p<0.001$; DayNight: $F=32.362$, $p<0.001$) temperature did not (with both species included; GLMM, $F=0.251$, $p=0.616$). Indeed, temperature had a strong impact on the species (GLMM, $F=239.099$, $p<0.001$).

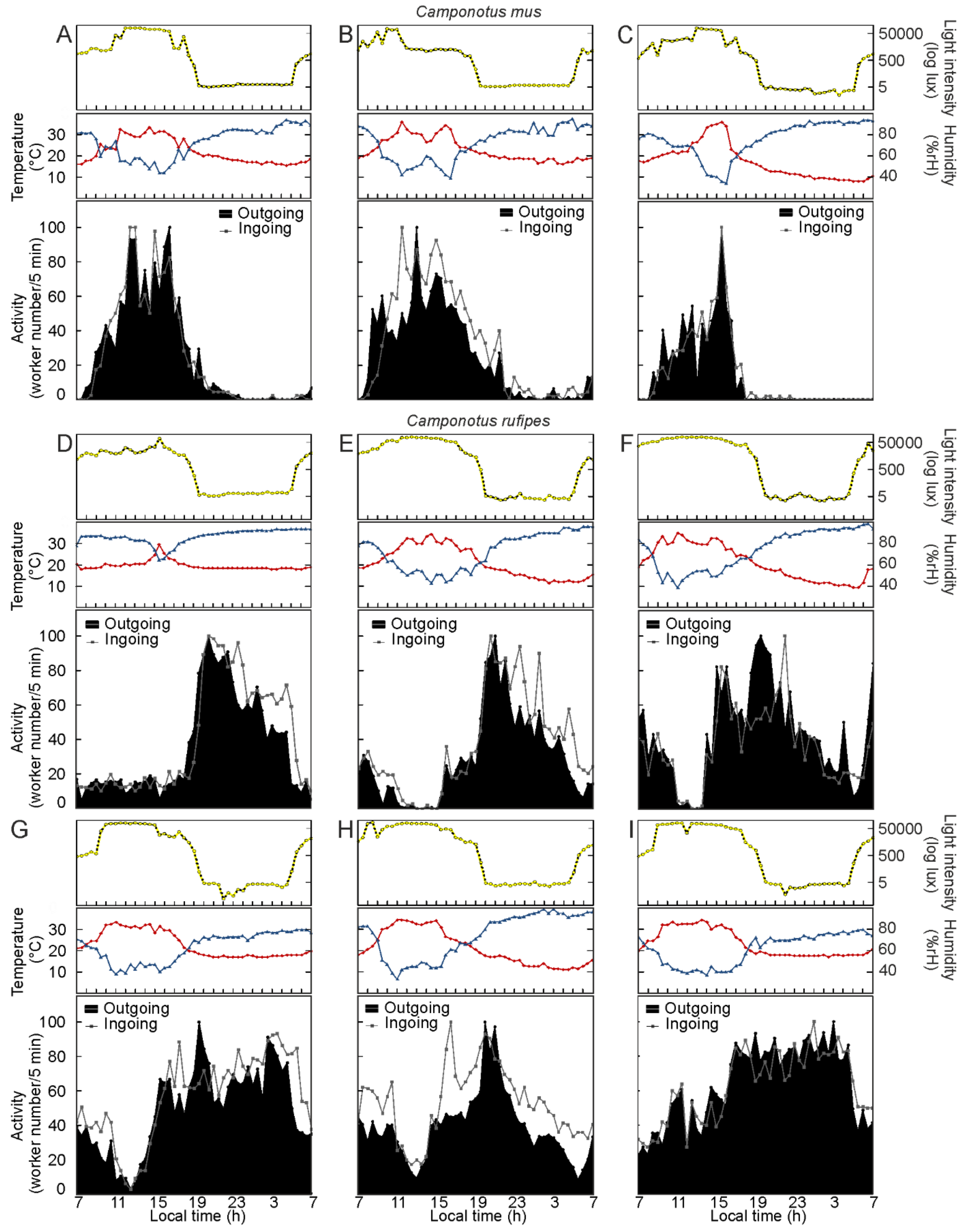
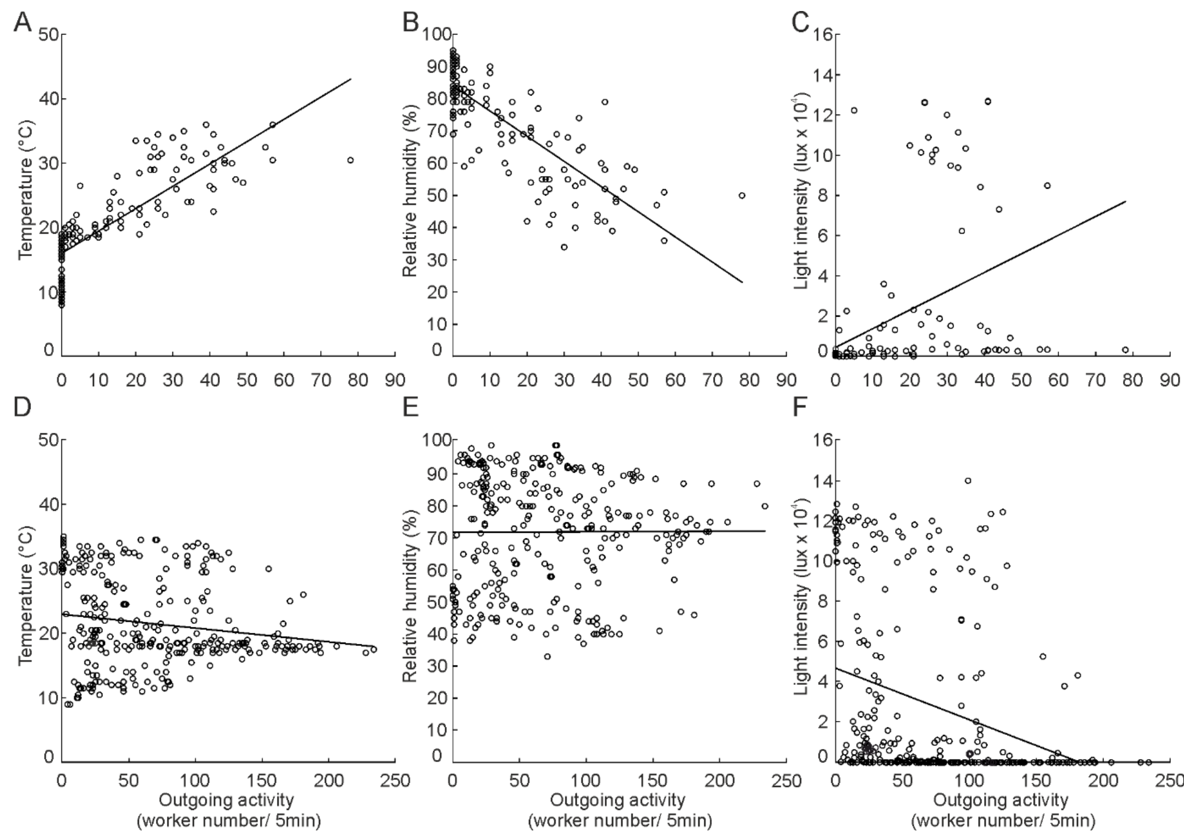


Figure 3: Daily foraging activity in the field. Counts of outgoing (black) and ingoing (grey) foragers were performed for 5 min every 30 min on a 24h basis at three *Camponotus mus* (A–C) and six *C. rufipes* nests (D–I). (A–C) Workers from all *C. mus* nests displayed diurnal foraging activity. (D–I) All *C. rufipes* nests were

▲ predominantly nightactive with different diurnal foraging activities: nests with low diurnal foraging activity (D–F) and elevated diurnal activity in some nests (G–I), but still less than the half of the nocturnal foraging activity. Blue: humidity (% rH); red: ambient temperature (°C); yellow: light intensity (lux).

Post hoc analysis revealed, that outgoing foraging activity of *C. mus* workers was positively correlated with temperature (Fig. 4, A; Spearman´s rank correlation: p<0.001; ρ=0.923), whereas negatively correlated in *C. rufipes* foragers (Fig. 4, D; Spearman´s rank correlation: p=0.002; ρ=-0.179). Humidity correlated negatively with outgoing foraging activity in *C. mus* foragers (Fig. 4, B; Spearman´s rank correlation: p<0.001; ρ=-0.824) and did not affect outgoing activity in *C. rufipes* (Fig. 4, E; Spearman´s rank correlation: p=0.456; ρ=-0.044). Foraging activity of *C. mus* workers was positively correlated to light intensity (Fig. 4, C; Spearman´s rank correlation: p<0.001; ρ=0.684) and negatively in *C. rufipes* (Fig. 4, F; Spearman´s rank correlation: p<0.001; ρ=-0.446).



▲ **Figure 4: Correlation of abiotic factors in the field and foraging activity.** Correlation between ambient temperature (°C; A+D), relative humidity (%; B+E) and light intensity (lux; C+F) of all three tested *C. mus* nests (A–C) and all six *C. rufipes* nests (D–F) and outgoing worker activity. Regression lines are indicated in black, single data points as circles.

Laboratory Experiment I: Temperature Tolerance Values Coincides with Daily Activity Patterns of *Camponotus* Ants

In the laboratory, the CT_{max} and CT_{min} were evaluated for both *Camponotus* species in relation to the worker body mass (Fig. 5). Larger ants of both species were more prone to tolerate colder temperatures than smaller ants (*C. mus*: linear regression: y=8.3–0.187x, p=0.012, R^2=0.4; Spearman´s correlation: r_s=-0.75, p<0.001; *C. rufipes*: linear regression: y=11.9–0.173x, p=0.008, R^2=0.4). In general, workers of *C. mus* exhibited lower minimal temperature limits than *C. rufipes* workers (ANCOVA; slopes of regression lines: $F_{1,29}$=0.02, p=0.891; intercepts of regression lines: $F_{1,30}$=9.2, p=0.004).

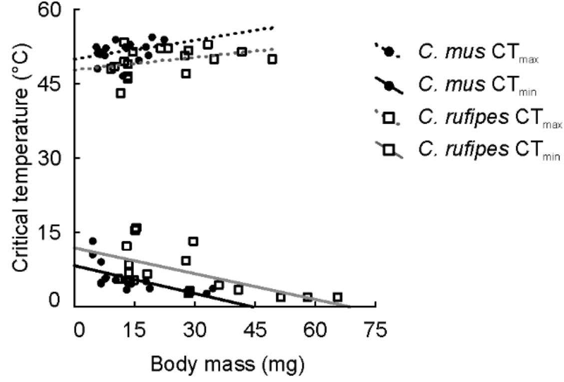


Figure 5: Temperature tolerance values of workers in the laboratory. Critical maximal (CT_{max}) and minimal temperatures (CT_{min}) of *C. mus* (black circles; n=32) and *C. rufipes* (white squares; n=35) as a function of their body mass (mg).

In contrast to the minimal temperature limit, body size had no influence on the maximal temperature limits of both species (*C. mus*: linear regression: y=49.9+0.126x, p=0.199, R^2=0.1; Spearman´s correlation: r_s=0.33, p=0.212; *C. rufipes*: linear regression: y=47.7+0.086x, p=0.121, R^2=0.1; Spearman´s correlation: r_s=0.42, p=0.084). Independent of the body size, *C. mus* ants tolerated higher temperature values than *C. rufipes* ants (*C. mus*: 51.8±2.1°C, *C. rufipes*: 49.4±3.6°C, median±IQR; unpaired t–test: t(32)=-2.16, p=0.038).

Laboratory Experiment II: Temperature and Locomotor Activity Rhythms of *C. mus* and *C. rufipes* Workers

Temperature and Locomotor Activity Rhythms of Camponotus mus:

Exemplary actograms for rhythmic *C. mus* workers during the three recording phases (E, S and F) under the thermal regimes are shown in Fig. 6, A–D. Comparing the survival rate of *C. mus* workers reared under winter and summer regimes, respectively, no significant differences were found in all three recording phases under the LD regime (Table 1; E: n_{winter}=61, n_{summer}=10, χ^2=3.10, p=0.078; S: n_{winter}=9, n_{summer}=39, Fisher´s exact test: p=0.098; F: n_{winter}=4, n_{summer}=29, Fisher´s exact test: p=0.092) and DD, except for the freerunning period (Table 1; E: n_{winter}=31, n_{summer}=31, Fisher´s exact test: p=0.754; S: n_{winter}=30, n_{summer}=25, Fisher´s exact test: p=0.052; F: n_{winter}=27, n_{summer}=17, Fisher´s exact test: p=0.045). No significant differences between winter and summer reared workers in rhythmicity were found within the LD regime (Table 1; E: n_{winter}=10, n_{summer}=44, Fisher´s exact test: p=0.102; S: n_{winter}=9, n_{summer}=29, Fisher´s exact test: p=0.158; F: n_{winter}=3, n_{summer}=25, Fisher´s exact test: p=0.527) and under DD during free–running conditions (Table 1; E: n_{winter}=31, n_{summer}=22, Fisher´s exact test: p<0.001; S: n_{winter}=25, n_{summer}=18, Fisher´s exact test: p=0.002; F: n_{winter}=11, n_{summer}=11, χ^2=0.83, p=0.361). Total activity levels were calculated to quantitatively compare the ants´ locomotor activity rhythms. *C. mus* ants reared under winter conditions showed lower total activity levels than the summer reared workers in the phase–shift under the LD regime (Table 1; Mann–Whitney *U*–tests; E: Z=-1.521, p=0.128; S: Z=-2.245, p=0.025; F LD: Z=-1.600, p=0.110) and in the entrainment and phase–shift under DD (Table 1: E: Z=-4.717, p<0.001; S: Z=-4.868, p<0.001; F: Z=-1.398, p=0.162).

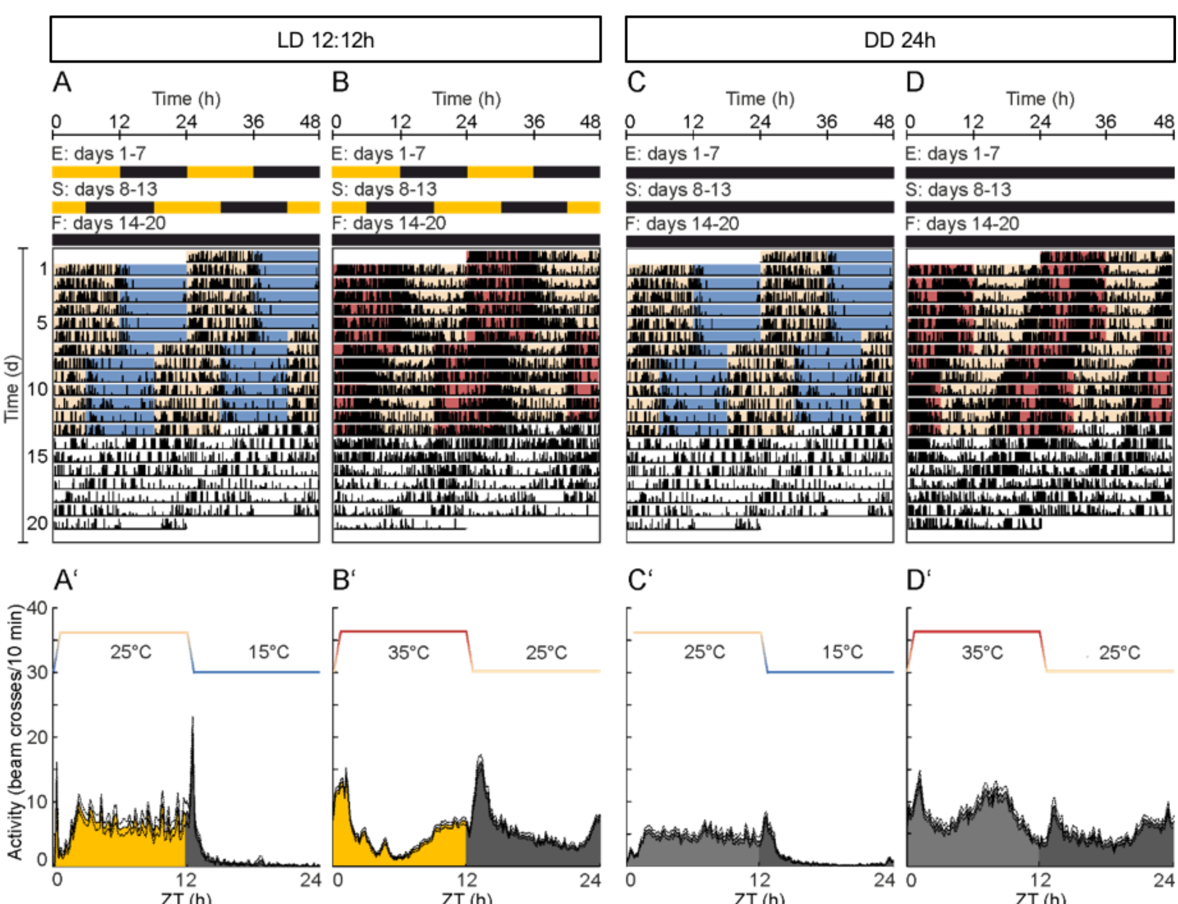


Figure 6: Locomotor activity rhythms of *Camponotus mus* exposed to different thermal regimes under different light regimes in the laboratory. (A–D) Exemplary actograms of single rhythmic workers. Locomotor activity (indicated as black ticks) is shown as double plot under 12:12h LD cycle (A+B) and 24h DD (C+D) cycle and thermal (winter/summer) regime (day 1–7), after a six hour phase advance of the LD– and thermal (winter/summer) regime (day 8–13) and constant darkness at 25°C (day 14–20). (A+C) Exemplary actogram under thermal winter conditions under LD (A) and under DD (C). (B+D) Exemplary actogram under thermal summer conditions under LD (B) and under DD (D). (A'–D') Average activity (mean: solid lines; mean±SE: dashed lines) calculated over 7 days under the LD (A´+B´) and DD (C´+D´) and the respective thermal regime (day 1–7). (A´+C') Average activity patterns under winter regimes under LD (A'; n=10) and under DD (C'; n=31). (B´+D') Average activity patterns under thermal summer regimes under LD (B'; n=61) and under DD (D´; n=31). Bars: beige: 25°C, black: dark phase, blue: 15°C, red: 35°C, yellow bars: light phase; E: entrainment; F: free–run; Lines: beige lines: 25°C, blue lines: 15°C, red lines: 35°C, S: phase–shift; ZT: zeitgeber time.

Furthermore, we tested if all workers were either nocturnal or diurnal during the E and S recording phases. Under the LD regime, ants reared under the winter thermal regime were diurnal under both, E and S recording phases, under the summer thermal regime workers only showed significant higher activity levels during daytime under the S (Table 1; Wilcoxon signed–rank tests; summer thermal regime: E: Z=-0.201, p=0.841; S: Z=-2.337, p=0.019; winter thermal regime: E: Z=-3.180, p=0.001; S: Z=-2.666, p=0.008). Under DD, workers

were significantly more active during the warm phase, i.e. the respective daytime (Table 1; Wilcoxon signed–rank tests; summer thermal regime: E: Z=-3.508, p<0.001; S: Z=-2.704, p=0.007; winter thermal regime: E: Z=-2.666, p=0.008; S: Z=-4.595, p<0.001). Consequently, *C. mus* workers under the winter thermal regime showed lower nightactivity levels than summer reared workers under both, the LD regime (Table 1; Mann–Whitney *U*–tests: E: Z=-4.562, p<0.001; S: Z=-2.245, p=0.025) and DD (Table 1; Mann–Whitney *U*–tests; E: Z=-5.399, p<0.001; S: Z=-3.989, p<0.001).

Table 1: Tested locomotor activity parameters of *Camponotus mus* workers reared under different thermal and light regimes.

Treatment and Rearing Conditions		Survival (%)	Rhythmicity (%)	Period (h)	Power (rU)	Total Activity (beam crosses/day)	Respective Nightactivity (%)
E	Winter	43.5 (n=10)^{a}	100.0 (n=10)^{a}	24.0±0.2^{a} (n=10)	65.2±33.5^{a} (n=10)	502.5±408.0^{a} (n=10)	15.2±9.7^{a} (n=10)
	Summer	63.5 (n=61)^{a}	73.8 (n=45)^{a}	23.8±1.2^{a} (n=45)	15.0±14.7^{b} (n=61)	763.0±608.0^{a} (n=61)	48.2±25.3^{b} (n=61)
S	Winter	90.0 (n=9)^{a}	100.0 (n=9)^{a}	24.0±0.3^{a} (n=9)	58.5±24.2^{a} (n=9)	614.0±293.0^{a} (n=9)	18.3±6.4^{a} (n=9)
	Summer	63.9 (n=39)^{a}	74.4 (n=29)^{a}	24.2±0.7^{a} (n=29)	16.7±24.2^{b} (n=39)	942.0±620.0^{b} (n=39)	45.9±10.8^{b} (n=39)
F	Winter	44.4 (n=4)^{a}	60.0 (n=3)^{a}	22.7±5.3^{a} (n=3)	12.7±5.2^{a} (n=4)	477.0±274.0^{a} (n=4)	____
	Summer	74.4 (n=29)^{a}	72.4 (n=21)^{a}	21.0±2.7^{a} (n=21)	14.1±12.3^{a} (n=29)	859.0±671.0^{a} (n=29)	____
E	Winter	96.9 (n=31)^{a}	100.0 (n=31)^{a}	24.2±0.5^{a} (n=31)	56.9±31.5^{a} (n=31)	412.0±366.0^{a} (n=31)	14.1±12.0^{a} (n=31)
	Summer	96.9 (n=31)^{a}	71.0 (n=22)^{b}	23.8±0.7^{b} (n=22)	17.2±30.1^{b} (n=31)	891.0±588.0^{b} (n=31)	39.9±20.9^{b} (n=31)
S	Winter	80.7 (n=25)^{a}	100.0 (n=25)^{a}	23.8±0.3^{a} (n=25)	36.0±18.1^{a} (n=25)	337.0±389.0^{a} (n=25)	21.4±11.8^{a} (n=25)
	Summer	96.8 (n=30)^{a}	60.0 (n=18)^{b}	23.9±1.0^{a} (n=18)	13.8±15.6^{b} (n=30)	856.0±590.0^{b} (n=30)	40.7±11.6^{b} (n=30)
F	Winter	68.0 (n=17)^{a}	64.7 (n=11)^{a}	23.2±2.3^{a} (n=11)	17.2±21.0^{a} (n=17)	478.0±389.0^{a} (n=17)	____
	Summer	90.0 (n=27)^{b}	33.3 (n=11)^{a}	23.8±2.5^{a} (n=11)	8.1±6.6^{b} (n=27)	576.0±535.0^{a} (n=27)	____

Different letters indicate significant differences (p<0.05) between *C. mus* workers exposed to thermal winter and summer regimes within the three recording phases, entrainment (E), 6h phase–shift (S), and free–run (F). Single values of period, power, total and nightactivity are shown as median± interquartile range and were statistically analyzed using Mann–Whitney *U*–tests; χ^2 analysis and Fisher´s exact test were used for statistical comparison of survival rate and rhythmicity. Symbols indicate different light treatments and rearing conditions.

Average activity patterns revealed higher activity levels during the warmer phase under the thermal winter regime under both light conditions (Fig. 6, A´+C´) and activity all–around the clock under the thermal summer regime (Fig. 6, B´+D´) in *C. mus* workers. Here, ants showed a period length of approximately 24h under both temperature and light regimes, differing significantly between winter and summer reared ants under DD (Table 1, E; Mann–

Whitney *U*–tests; LD: Z=-0.638, p=0.524; DD: Z=-3.103, p=0.002). Ants reared under the winter thermal regime showed a higher power of rhythms when compared with summer reared workers under both light regimes (Table 1, E; Mann–Whitney *U*–test; LD: Z=-4.413, p<0.001; DD: Z=-4.737, p<0.001). By shifting both, the temperature (Fig. 6, A–D) and light regime (Fig. 6, A+B) six hours' forwards, workers were immediately able to resynchronize their activity patterns. Like during the E recording phase, period length was approximately 24h during both light regimes and not statistically different comparing winter and summer kept workers (Table 1, S; Mann–Whitney *U*–tests; LD: Z=-0.017, p=0.986; DD: Z=-0.421, p=0.673). Here, too, the calculated power was higher in winter than in summer reared ants under both light regimes (Table 1, S; Mann–Whitney *U*–tests; LD: Z=-3.526, p<0.001; DD: Z=-4.108, p<0.001). With period lengths shorter than 24 hours, winter and summer reared workers did not differ in their period values under constant conditions (Table 1, F; Mann–Whitney *U*–tests; LD: Z=-1.604, p=0.109; DD: Z=-1.381, p=0.167). Winter reared workers showed a lower power of rhythms than summer reared ants under DD (Table 1, F; Mann–Whitney *U*–test; LD: Z=-0.772, p=0.440; DD: Z=-2.061, p=0.039).

Temperature and Locomotor Activity Rhythms of Camponotus rufipes:

Exemplary actograms for rhythmic *C. rufipes* workers during the three recording phases (E, S and F) under winter and summer thermal regimes are shown in Fig. 7, A–D. In *C. rufipes***,** survival rates of workers in the E phase were higher in winter reared workers under the LD regime (Table 2; χ^2 tests; E: n_{winter}=18, n_{summer}=6, χ^2=9.09, p=0.003; S: n_{winter}=17, n_{summer}=5, Fisher´s exact test: p=0.394; F: n_{winter}=13, n_{summer}=4, Fisher´s exact test: p=0.687). Survival rates were not significantly different under DD throughout the recording phases, except for the E phase (Table 2; χ^2 tests; E: n_{winter}=15, n_{summer}=18, χ^2=6.70, p=0.010; S: n_{winter}=11, n_{summer}=16, Fisher´s exact test: p=0.242; F: n_{winter}=10, n_{summer}=13, Fisher´s exact test: p=0.455).

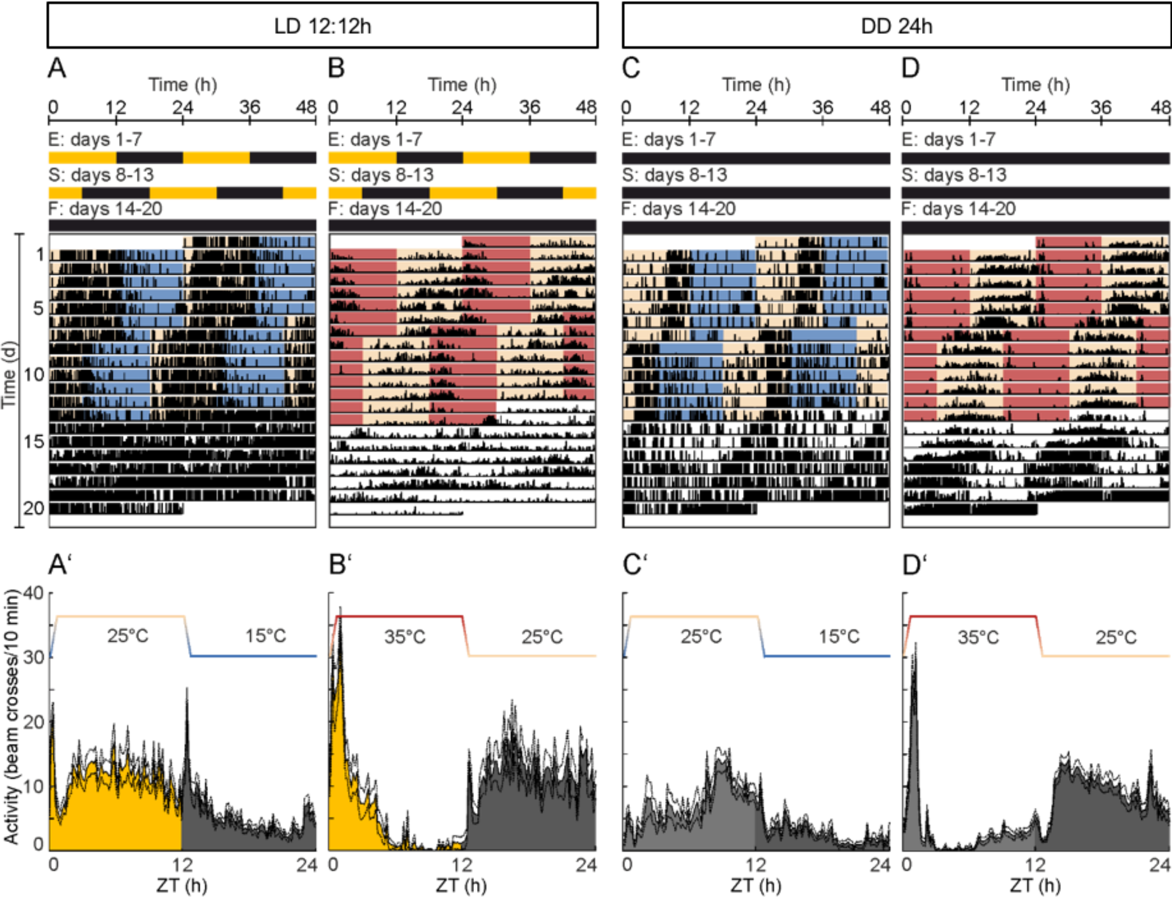


Figure 7: Locomotor activity rhythms of *Camponotus rufipes* exposed to different thermal regimes under different light regimes in the laboratory. (A–D) Exemplary actograms of single rhythmic workers. Locomotor activity (indicated as black ticks) is shown as double plot under 12:12h LD cycle (A+B) and 24h DD (C+D) cycle and thermal (winter/summer) regime (day 1–7), after a six hour phase advance of the LD– and thermal (winter/summer) regime (day 8–13) and constant darkness at 25°C (day 14–20). (A+C) Exemplary actograms under thermal winter conditions under LD (A) and under DD (C). (B+D) Exemplary actograms

▲under thermal summer conditions under LD (B) and under DD (D). (A'–D') Average activity (mean: solid lines; mean±SE: dashed lines) calculated over 7 days under the LD (A´+B´) and DD (C´+D´) and the respective thermal regime (day 1–7). (A´+C') Average activity patterns under winter regimes under LD (A'; n=18) and under DD (C'; n=15). (B´+D') Average activity patterns under thermal summer regimes under LD (B'; n=6) and under DD (D´; n=18). Bars: beige: 25°C, black: dark phase, blue: 15°C, red: 35°C, yellow bars: light phase; E: entrainment; F: free–run; Lines: beige lines: 25°C, blue lines: 15°C, red lines: 35°C, S: phase–shift; ZT: zeitgeber time.

In all three recording phases under the LD and DD regime, no significant differences between winter and summer reared workers were found in rhythmicity levels (Table 2; LD regime: E: n_{winter}=15, n_{summer}=6, Fisher´s exact test: p=0.455; S: n_{winter}=13, n_{summer}=5, Fisher´s exact test: p=0.400; F: n_{winter}=10, n_{summer}=4, Fisher´s exact test: p=0.491; DD: E: n_{winter}=14, n_{summer}=17, Fisher´s exact test: p=0.713; S: n_{winter}=11, n_{summer}=16, χ^2=0.00, p=1.000; F: n_{winter}=9, n_{summer}=12, Fisher´s exact test: p=0.697). Total activity was calculated to quantitatively compare the ants´ locomotor activity. Workers did not differ in total activity levels during all three recording phases under the LD regime (Table 2; Mann–Whitney *U*–tests; E: Z=-1.200, p=0.230; S: Z=-1.606, p=0.108; F: Z=-0.453, p=0.651) and DD regime (Table 2; Mann–Whitney *U*–tests; E: Z=-1.048, p=0.294; S: Z=-0.839, p=0.402; F: Z=-0.434, p=0.664). As for *C. mus* workers, we tested if workers were significantly more active during the respective night– or daytime.

Under the LD regime, workers showed significantly higher activity levels during daytime under the winter regime (Table 2; Wilcoxon signed–rank test; E: Z=-3.462, p<0.001; S: Z=-3.195, p=0.001) and higher activity levels during nighttime under the thermal summer regime, even though this effect was not significant in the E and S (Table 2; Wilcoxon signed–rank test under Bonferroni correction; E: Z=-2.201, p=0.028; S: Z=-1.483, p=0.138). Under the DD regime, workers were significantly more active during the warm phase (respective daytime) under the thermal winter regime (Table 2; Wilcoxon signed–rank test; E: Z=-3.408, p=0.001; S: Z=-2.934, p=0.003) and significantly more active during the cold phase (respective nighttime) under the thermal summer regime (Table 2; Wilcoxon signed–rank test; E: Z=-3.114, p=0.002; S: Z=-3.516, p<0.001). Consequently, respective nocturnal activity was significantly different when comparing winter and summer reared ants during the E and S under the LD and DD regimes (Table 2; Mann–Whitney *U*–tests; LD: E: Z=-3.400, p=0.001; S: Z=-3.016, p=0.003; DD: E: Z=-4.592, p<0.001; S: Z=-4.342, p<0.001).

Table 2: Tested locomotor activity parameters of *Camponotus rufipes* workers reared under different thermal and light regimes.

Treatment and Rearing Conditions		Survival (%)	Rhythmicity (%)	Period (h)	Power (rU)	Total Activity (beam crosses/day)	Respective Nightactivity (%)
E	Winter	56.3 (n=18)^{a}	83.3 (n=15)^{a}	24.0±0.7^{a} (n=15)	19.6±28.3^{a} (n=18)	855.5±1065.0^{a} (n=18)	29.5±15.5^{a} (n=18)
	Summer	19.4 (n=6)^{b}	100.0 (n=6)^{a}	23.8±0.0^{a} (n=6)	72.7±36.3^{b} (n=6)	950.5±918.0^{a} (n=6)	66.8±20.0^{b} (n=6)
S	Winter	94.4 (n=17)^{a}	76.5 (n=13)^{a}	23.5±0.7^{a} (n=13)	21.9±51.0^{a} (n=17)	1351.0±1084.0^{a} (n=17)	23.4±18.8^{a} (n=17)
	Summer	83.3 (n=5)^{a}	100.0 (n=5)^{a}	23.5±0.5^{a} (n=5)	27.0±84.5^{a} (n=5)	1010.0±657.0^{a} (n=5)	65.9±12.1^{b} (n=5)
F	Winter	76.5 (n=13)^{a}	76.9 (n=10)^{a}	24.3±2.3^{a} (n=10)	14.7±13.4^{a} (n=13)	764.0±684.0^{a} (n=13)	——
	Summer	80.0 (n=4)^{a}	100.0 (n=4)^{a}	23.4±7.1^{a} (n=4)	24.1±8.3^{a} (n=4)	1204.0±1515.5^{a} (n=4)	——
E	Winter	46.9 (n=15)^{a}	93.3 (n=14)^{a}	23.9±0.7^{a} (n=14)	30.6±25.0^{a} (n=15)	425.0±587.0^{a} (n=15)	24.2±16.4^{a} (n=15)
	Summer	81.8 (n=18)^{b}	94.4 (n=17)^{a}	24.0±0.8^{a} (n=17)	53.1±57.2^{b} (n=18)	661.0±431.0^{a} (n=18)	73.1±23.9^{b} (n=18)
S	Winter	73.3 (n=11)^{a}	100.0 (n=11)^{a}	24.7±0.5^{a} (n=11)	51.7±20.9^{a} (n=11)	737.0±802.0^{a} (n=11)	28.3±15.0^{a} (n=11)
	Summer	88.9 (n=16)^{a}	100.0 (n=16)^{a}	23.7±0.2^{b} (n=16)	58.8±99.6^{a} (n=16)	751.0±717.0^{a} (n=16)	75.1±12.5^{b} (n=16)
F	Winter	90.9 (n=10)^{a}	90.0 (n=9)^{a}	21.3±1.5^{a} (n=9)	37.5±48.9^{a} (n=10)	1203.5±831.0^{a} (n=10)	——
	Summer	81.3 (n=13)^{a}	92.3 (n=12)^{a}	22.4±0.8^{a} (n=12)	42.8±42.8^{a} (n=13)	1190.0±994.0^{a} (n=13)	——

Different letters indicate significant differences (p<0.05) between *C. rufipes* workers exposed to thermal winter and summer regimes within the three recording phases, entrainment (E), 6h phase–shift (S), and free–run (F). Single values of period, power, total and nightactivity are shown as median± interquartile range and were statistically analyzed using Mann–Whitney *U*–tests; χ^2 analysis and Fisher´s exact test were used for statistical comparison of survival rate and rhythmicity. Symbols indicate different light treatments and rearing conditions.

Average activity patterns revealed higher activity levels during the warmer phase under thermal winter regimes under both light regimes (Fig. 7, A´+C´) and in the cooler phase under the thermal summer regime (Fig. 7, B´+D´) during the E phase. Here, winter and summer reared workers showed a similar period length of approximately 24 h under both thermal and light regimes (Table 2; Mann–Whitney *U*–tests; LD: Z=-0.198, p=0.843; DD: Z=-0.838, p=0.402). Winter and summer reared workers differed in the power of their rhythms under the LD and DD regime (Table 2; Mann–Whitney *U*–test; LD: Z=-2.667, p=0.008; DD: Z=-2.097, p=0.036). By shifting the temperature (Fig. 7, A–D) and light cycle (Fig. 7, A+B) six hours forwards, workers were able to immediately resynchronize their activity patterns. Here, the period length was again around 24h and differed only significantly between winter and summer reared workers under the DD regime (Table 2; Mann–Whitney *U*–test; LD: Z=-0.248, p=0.804; DD: Z=-3.651, p<0.001). The power of rhythms was not significantly different in winter and summer reared workers under both

light regimes (Table 2; Mann–Whitney *U*–tests; LD: Z=-0.979, p=0.327; DD: Z=-0.691, p=0.490). Under constant conditions (F), workers entrained under the former LD regime exhibited a period length around 24h (Table 2; Mann–Whitney *U*–test; Z=-0.709, p=0.479). When comparing winter and summer reared workers under free–running conditions of the DD regime, they showed a period length shorter than 24h and were not significantly different from each other (Table 2; Mann–Whitney *U*–test, F: Z=-1.462, p=0.144) with equal power of rhythms in workers of both temperature regimes (Table 2; Mann–Whitney *U*–tests, LD: Z=-1.585, p=0.113; DD: Z=-0.248, p=0.804).

Discussion

Monitoring of the foraging activity of two sympatric species in the field in La Coronilla, Uruguay revealed that workers of *C. mus* were exclusively dayactive, whereas nests of *C. rufipes* were predominantly nightactive with elevated diurnal activity in some nests. Furthermore, we could demonstrate that temperature and light as abiotic factors mainly shape foraging activity of those species. Studies conducted under controllable laboratory conditions indicated, that *C. mus* and *C. rufipes* indeed exhibited the same temperature tolerance values, although they showed different locomotor activity patterns under winter and summer thermal regimes. Here again, *C. mus* workers were exclusively dayactive while *C. rufipes* workers linked their locomotor activity to 25°C independent of the offered thermal regime.

Monitoring Daily Foraging Activity Rhythms in the Field

Workers of both species foraged actively at acacia trees, where they collected acacia seeds, nectar at extrafloral nectaries, honeydew from treehoppers or aphids and small insect prey. Although they share the same food source, they were found to prefer foraging partly at different environmental conditions. Two promising key players investigated in this study were the ambient temperature and the daytime. Foragers of *C. mus* were exclusively active during daytimes and did not show activity below 15°C, which is within the critical temperature range for foraging activity that has been described before for this species (Falibene and Josens, 2014), whereas *C. rufipes* workers foraged predominantly nocturnal. Herewith we could confirm the activity pattern of *C. rufipes* seen in the laboratory under controlled conditions (Jaffé and Sánchez, 1984; Mildner and Roces, 2017), which probably reflects the endogenous foraging rhythm in this species. Indeed, several field studies demonstrated occasional diurnal activity in *C. rufipes* (Oliveira et al., 1995; Del–Claro and Oliveira, 1999; Fagundes et al., 2005). As this field study took place during local spring time under moderate night temperatures, two different foraging activity types of *C. rufipes* nests could be observed: Some nests showed an almost exclusive nocturnal foraging activity which is expected for local summer time, whereas other nests displayed a pronounced diurnal foraging activity which is maybe typical for local winter times. To date, no biotic or abiotic factors shaping the foraging activity of *C. rufipes* are known so far.

We hypothesize, that this switch of foraging activity might be a behavioral adaptation to more moderate temperatures of the respective season, as it was described in several other *Camponotus* species (Briese and Macauley, 1980; Cros et al., 1997). It was earlier suggested, that exposure of ectothermic animals to too extreme environmental conditions (if the ambient temperature is too high or too low) is reducing their foraging efficiency. Therefore, workers need to adapt themselves physiologically to extreme environmental temperatures which has led to a high degree of thermoregulatory adjustments (Angilletta, 2009). Hence, social insects are described as \`thermal warriors´ as their ecological success mostly relies on their maintenance of optimal temperatures (Heinrich, 1996). As ants are in general thermophilic, their foraging activity patterns are constricted by the environmental temperature and the species´ physiological tolerances (Hölldobler and Wilson, 1990). Ant workers are described to change their foraging activity in a daily and seasonal manner which is mostly linked to changing temperature values (Andersen, 1983; Fellers, 1989; Cerdá et al., 1997). Species that cope with extreme temperatures are described as heat tolerant (*C. mus* in this study) and are reported for diurnal foraging activity, independent of the season (Cerdá et al., 1998). Contrary, ants that avoid extreme temperature values (heat averse, *C. rufipes* in this study) switch their foraging activity period throughout the time of the year (Cerdá et al., 1998) to the more tolerable temperature value. Thus, these heat averse species were nocturnal during summer to avoid unfavorable heat at daytimes, and switched to diurnal foraging activity in the winter, as the ambient temperature at nighttimes drops below their CT_{min}. Heat averse species are hypothesized to be the more dominant species and therefore restrict foraging activity of heat tolerant species (referred to as non–dominant species) to more severe environmental conditions at daytimes (Anjos et al., 2017). Non–dominant species are generally active under wider temperature ranges (Bestelmeyer, 1997; Cerdá et al., 1998; Lessard et al., 2009) and therefore possess higher maximal thermal limits (Jayatilaka et al., 2011). Workers of *C. rufipes* are considered as dominant visitors of extrafloral nectaries (Del–Claro and Oliveira, 1999, 2000) and may therefore favor nocturnal foraging, because of reduced thermal stress and predation risk. Due to the behavioral observation conducted in our field study, we speculate that workers of *C. mus* are the non–dominant species as they strictly forage during daytimes.

Additionally, we assume that the non–dominant species *C. mus* owns some physiological adaptions to heat that allow them foraging under higher temperatures. *Cataglyphis bombycina* desert ants, for example, are known to forage at extremely high soil temperatures of 53.6°C with bodies of foragers covered by metallic shimmering hairs that were found to help keeping the body temperature below the CT_{max} by enhancing optical reflection and heat dissipation (Shi et al., 2015). Similar to *C. bombycina*, gasters of *Camponotus mus* workers are covered with golden hairs that may serve as a thermoregulatory solution to deal with high temperatures. Thus, workers of *C. mus* might can maintain low body temperatures when exposed to light and/ or high temperatures during local summer time. Contrary to the body morphology of *C. mus*, bodies of *C. rufipes* ants are covered with distinct less hairs and are of brownish to black color. Therefore, they might favor less extreme temperature values under natural conditions as they are more prone for desiccation and for overheating. Another physiological adaptation to diurnal foraging of *C. mus* workers might be structure of their compound eyes. The cathemeral *C. aethiops* ant species possess less ommatidia, a greater facet diameter and greater interommatidial angles than the sympatric living diurnal *Formica cunicularia* (Yilmaz et al., 2014), which reflects their different specializations to foraging activity and visual niches. Also in sympatric existing *Myrmecia* species, workers of solely dayactive species possess the smallest facet lens, whereas the lens diameter was largest in crepuscular/nocturnal species (Greiner et al., 2007). To deal with dim light conditions and low levels of illumination, compound eyes of nocturnal species contain special adaptations to capture more light to ensure an optimal nighttime vision (Warrant and McIntyre, 1990; Land, 1997). We therefore hypothesize, that the physiological composition of the compound eyes of *C. mus* and *C. rufipes* workers reflect the interspecific different foraging activities observed in our field study.

Temperature Tolerance Values Coincides with Daily Activity Patterns of *Camponotus* Ants

In this study, we additionally measured the thermal tolerance values of both *Camponotus* species in the laboratory, as these values might account for the different foraging activities that occur under natural conditions. The thermal tolerance of single individuals is described as the range of temperatures an animal can exist and is a common method to measure the physiological tolerable thermal values (Addo–Bediako et al., 2000). The CT_{max} is defined as the maximal tolerable temperature above which the animals´ locomotion ceases (Oberg et al., 2012), whereas the CT_{min} is determined as the minimal temperature value an animal can physiological tolerate. Within the same body mass range, the diurnal species *C. mus* exhibited both, lower and higher critical temperature values than *C. rufipes*, but differences in their thermal range were rather small. In general, thermal limits increased with body mass (e.g. Ribeiro et al., 2012; Baudier et al., 2015), whereas *C. rufipes* workers exhibited higher body masses. Consequently, *C. rufipes* reaches the same CT_{max} and CT_{min} as *C. mus* and could therefore be active within the same thermal ranges in the field. The interspecific differences of physiological tolerable temperatures documented in this study provide an excellent opportunity to explore individual performances of single workers performed under natural conditions in the field. The foraging activity of *C. mus* workers under natural conditions did not fall below 15°C, even though *C. mus* reached a CT_{min} of less than 10°C in the laboratory. Critical temperature ranges for worker activity in the laboratory are usually wider than the actual critical temperature ranges of foraging activity in the field (Cerdá et al., 1998), because long–term effects of exposure on physiology and foraging efficiency become not evident. Although survival of ants is not affected by this temperature values, foraging efficiency is reduced to decreasing nectar intake rates (Falibene and Josens, 2014). As *C. rufipes* proceeds foraging under this low temperature values, nocturnal foraging activity might be rather caused by the competition between the species than because of physiological constraints. Costs and benefits of foraging determine the lower temperature limit of activity range (Dreisig, 1985) whereas the upper limit is considered to be set by the impairment of physiological functions. Thus, thermal stress limits foraging activity and locomotion to certain times of the day (Cerdá et al., 1998; Bishop et al., 2017). In 2011,

Jayatilaka et al. investigated the preferred thermal range for the diurnal *Myrmecia croslandi* and nocturnal *M. pyriformis* and demonstrated the diurnal species having a higher CT_{max} than the nocturnal species. Discrepancies between critical temperature and natural foraging temperatures have been reported in the honey ant, *Myrmecocystus* and in *Myrmecia* ants which stop foraging activity at temperatures below their thermal maximum at which movement is impaired (Kay and Whitford, 1978; Jayatilaka et al., 2011). Walking speed is suggested as an ecologically relevant measure of thermal tolerance since in *Myrmecia* ants temperatures at which thermal discomfort is displayed by increasing walking speed are closer to temperatures at which foraging activity ceases in the field (Jayatilaka et al., 2011).

Temperature and Locomotor Activity Rhythms of *C. mus* and *C. rufipes* Workers

To evaluate how temperature as environmental factor limits locomotor activity rhythms, the daily locomotor rhythms of both species were monitored under controlled conditions in the laboratory, after determining the critical temperature values of the workers. Differences in thermal preferences of single workers may be a consequence of a physiological tolerance to environmental conditions (Cerdá et al., 1998). In the laboratory under controlled conditions, *C. mus* workers were strongly dayactive independent of the offered temperature regime, whereas *C. rufipes* ants switched their locomotion activity from diurnal under winter conditions to nocturnal under summer conditions. This is in line with the observations made under natural conditions in the field for *C. mus*, where we could observe strictly diurnal foraging patterns and for *C. rufipes*, were two different activity types occur: the more \`diurnal´ winter and more \`nocturnal´ summer activity type.

As individuals of both ant species could shift their locomotion activity pattern due to six hours advanced temperature and light regimes, they were truly entrained by both, the LD and temperature cycle. Strong increasing activity peaks in *C. mus* and *C. rufipes* after light onset could be explained by stress reactions of the individuals and therefore may represent masking effects (Schlichting and Helfrich–Förster, 2015; Mildner and Roces, 2017). In a previous study, solely LD cycles were shown to act as zeitgebers in *C. rufipes* workers (Mildner and Roces, 2017). Here, we entrained two sympatric *Camponotus* ants to temperature regimes under DD to show that temperature alone can act as zeitgeber, too. Early studies in *Drosophila melanogaster* proved that temperature cycles similarly to light can entrain locomotor rhythms (Konopka et al., 1989; Sawyer et al., 1997). Also honeybee workers could be entrained to temperature cycles alone with a 10°C amplitude under DD, whereas temperature cycles with a lower amplitude were not sufficient to entrain their locomotor activity (Moore and Rankin, 1993). Also workers of *Formica* woodland ants were entrained by thermal cyclical changes with increasing locomotor activity before the onset of the warm phase (North, 1993). These previous studies promote the reported results that temperature can act as zeitgeber without an additional light–dark cycle.

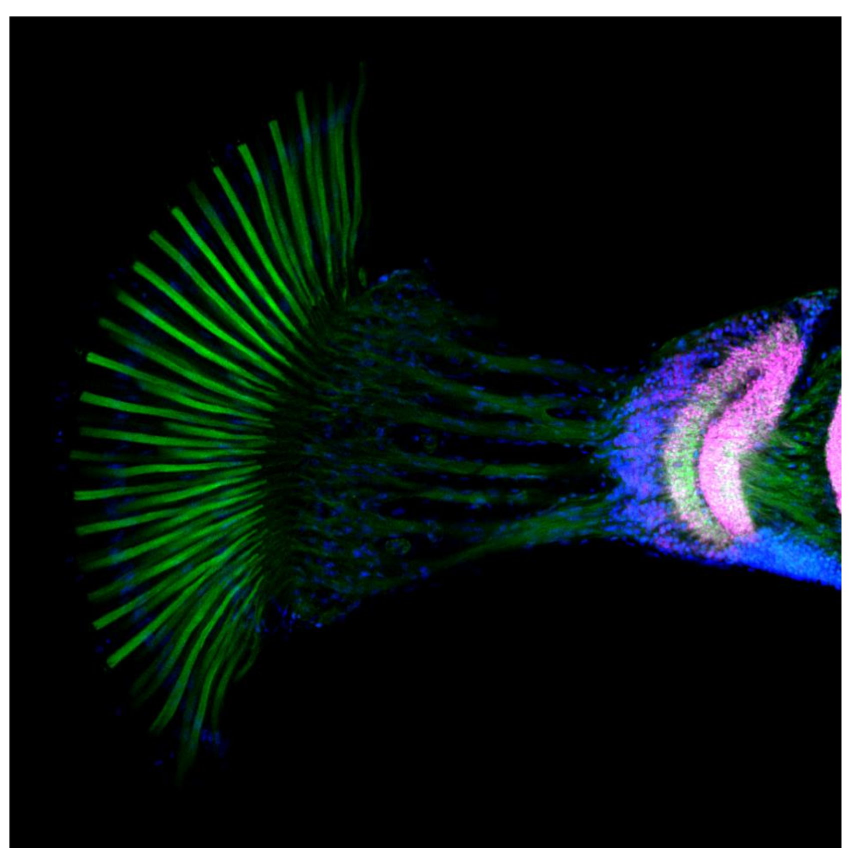
Measurements of total activity patterns of both species revealed higher activity patterns in *C. rufipes* than in *C. mus* workers during all recording phases and light regimes. Interspecific differences of the total activity levels between *Camponotus mus* and *C. rufipes* workers could be explained by species–specific activity patterns. Comparing locomotor activity patterns of both species with the foraging activity observed in the field, *C. mus* foragers showed lower foraging activity throughout the day as they remain nearly inactive during night times. The locomotor activity patterns of *C. rufipes* in the LD and temperature regime differ from a previous study done by Mildner and Roces (2017). They showed an overall higher activity in individually recorded nurses and foragers reared under constant 25°C. Higher locomotion activity under constant conditions could be explained by the different temperature regimes used in both studies. As this study used temperature ramps instead of constant temperatures, workers need to adapt their locomotor activity to the fluctuating temperature. As we hypothesized that *C. rufipes* is a dominant species, they are less forced to deal with higher temperatures than *C. mus*, even though they shared the same critical temperature limits measured in the laboratory. Furthermore, foraging activity of *C. rufipes* workers strongly decreased in the field when surface temperature ranged above 32°C. Also evaluation of single rhythmic workers in the laboratory under the summer regime showed lowered locomotor activity during the warm phase (LD and DD regime). Supporting the hypothesis, that high temperatures lowers total locomotor activity in *C. rufipes*, their total activity increased under both thermal regimes under constant conditions (25°C and DD). Lower activity under winter regimes could be explained by slower worker movement at cooler air temperatures *(Messor*: Azcárate et al., 2007; *Myrmecia*: Jayatilaka et al., 2011). As *C. rufipes* is mainly described as nocturnal species (Jáffe and Sanchez, 1984; Oliveira et al., 1995; Del–Claro and Oliveira, 1999; own data), the worker survival rate significantly decreased under LD conditions in summer reared workers, due to stressful light exposure and heat–intolerance of workers. This effect was absent in *C. mus*, as they are strictly diurnal (Falibene and Josens, 2014; own data) and sustain higher temperatures than *C. rufipes* workers. Earlier studies could show that subordinate species (in this study *C. mus*) forage at temperatures closer to their critical thermal limits than dominant species (Cerdá et al., 1998; in this study *C. rufipes*). As nearly no differences were found between rhythmicity comparing winter and summer reared workers in both species, we could demonstrate the

proper function of an endogenous clock. Arrhythmicity under both, thermal and light regimes, could be explained by isolation of workers from the social context, which is known to be a major stress factor that affects behavior and survival rates in ants (Grassé and Chauvin, 1946; Boulay et al., 1999; Koto et al., 2015). Exposed to DD, workers of both species displayed free–running locomotor activity rhythms, indicating an endogenous character of these rhythms. For both species, the period length under freerunning conditions was approximately 24 hours. From honeybees is known, that locomotor rhythms freeran with a periodicity lower than 24 hours under DD and constant temperature (Moore and Rankin, 1983). *Camponotus compressus* ants that are nocturnal under LD cycles attained a freerunning rhythm with periods shorter than 24 hours, whereas diurnal workers possess a periodicity longer than 24 hours (Sharma et al., 2004).

Our study demonstrates that *C. mus* and *C. rufipes* occupy the same dietary niche in the field within different timing of foraging activities. Whereas the activity peak of *C. mus* workers occurred always during the day phase with higher temperatures, *C. rufipes* workers display two different activity types, the more diurnal \`winter´ activity type and the more nocturnal \`summer´ activity type. The coexistence of both species at the field side might be shaped rather by the dominance of *C. rufipes* rather than by physiological constraints, as laboratory experiments indicate comparable critical temperature ranges in the species. Nevertheless, as both species synchronize their activity with different thermal regimes in the laboratory, temperature serves as a sufficient zeitgeber for locomotor activity. We therefore conclude that environmental temperature cycles may not only limit daily activity of both *Camponotus* species, but may be used as non–photic zeitgeber to schedule foraging activity. Examining the interplay of abiotic, like competition and biotic factors, like temperature and light that may restrict activity patterns of ant workers to certain times of the day is one further step to understand the highly flexible organization of daily and seasonally occurring activity cycles in *Camponotus* ants.

Chapter III

Age–related and Light–induced Plasticity in Primary and Secondary Visual Centers of the Nectar–feeding Ant *Camponotus rufipes*



The cover image by Ayse Yilmaz et al., is based on the research article *Age–related and light–induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar–feeding ant Camponotus rufipes.* Developmental Neurobiology 76: 1041–1057

Chapter III
Age–related and Light–induced Plasticity in Primary and Secondary Visual Centers of the Nectar–feeding Ant *Camponotus rufipes*

This chapter is largely adapted from the published manuscript:

Yilmaz A*, **Lindenberg A***, Albert S, Grübel K, Späthe J, Rössler W and Groh C (2016) Age–related and light–induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar–feeding ant *Camponotus rufipes.* Developmental Neurobiology 76: 1041–1057 (* both authors contributed equally to this work)

Introduction

Division of labor is a characteristic feature in eusocial insects and to a great extent accounts for their ecological and evolutionary success (Hölldobler and Wilson, 1990, 2009). In most eusocial insect species, individuals within the worker caste perform a variety of tasks that range from nursing to nest defense and foraging. This division of labor among workers can be associated with variations in body size. In the nectar–feeding ant *Camponotus rufipes*, large workers and soldiers mainly act in colony defense, while minor and media workers participate in a variety of tasks including nursing, exploring, foraging and recruitment (Jàffe and Sanchez, 1984; Soares et al., 2008). In addition, *C. rufipes* minor and media workers exhibit an age–related polyethism with young ants first performing tasks inside the nest before starting to forage outside. Foragers are mostly nightactive and forage at low light intensities, but are also able to forage during the day (Del–Claro and Oliveira, 1999). This prominent behavioral transition is associated with major changes in sensory input and motor activities. The peripheral and central neuronal system must cope with new challenges like visual orientation, long distance navigation, and learning and memory of the location of rich food sources (Kühn–Bühlmann and Wehner, 2006; Stieb et al., 2010). In this context, vision constitutes one of the sensory domains in which most drastic changes are experienced. Besides olfaction, vision was shown to be crucial for orientation and navigation in a variety of central–place foraging ants (Rosengren, 1971; Hölldobler, 1980; Collett et al., 1992; Wehner, 2003). Therefore, the question of how changes in the visual environment affect the

peripheral and central nervous system is crucial for identifying general mechanisms underlying adaptation of visual perception associated with division of labor. We used *C. rufipes* ants to address this question.

One prominent candidate for adaptive adjustments in the neuronal circuitry to changes in the visual environment during the behavioral transition are primary and secondary visual centers like the optic lobes (OLs) and the mushroom bodies (MBs). Visual information received by the photoreceptors in the retina is processed in retinotopic OL neuropils, the lamina (LA), medulla (ME), and lobula (LO). Visual processing in the LA underlies adaptation in response to changing light intensities, summation, enhancement of signal–to–noise–ratio, and lateral inhibition (Strausfeld, 1989; reviewed in Gronenberg, 2008). LA neurons proceed to the ME, the largest neuropil of the OL (Strausfeld, 1976). The ME processes color information and probably extracts motion information from the visual input (Gronenberg, 2008), and the LO probably represents both, color and motion information (Strausfeld, 1989; Paulk et al., 2008). In ants, a prominent tract, the anterior superior tract (asot), named after a homologous tract described for honeybees (Kenyon, 1896; Mobbs, 1984), connects the ME with the MBs (e.g. Ehmer and Gronenberg, 2004), centers for higher–order sensory integration, learning and memory (Strausfeld et al., 1998; Heisenberg, 2003; Giurfa, 2007; Devaud et al., 2015; Falibene et al., 2015). Within the MB–calyx, asot afferents innervate a distinct subregion, the visual collar. Neuronal circuits within the visual collar, the neighboring lip (olfactory input), and the basal ring (visual and olfactory input) are organized in synaptic complexes (microglomeruli, MGs) (Groh et al., 2004, 2006; Seid et al., 2005; Seid and Wehner, 2008; Stieb et al., 2010, 2012; Groh and Rössler, 2011; Groh et al. 2014). In Hymenoptera, MGs have been shown to undergo structural reorganization in response to experience, age, and changes in environmental conditions (ants: Seid et al., 2005; Stieb et al., 2010, 2012; Falibene et al., 2015; honeybees: Groh et al., 2004, 2006; Hourcade et al., 2010; Scholl et al., 2014; Muenz et al., 2015).

As the visual input received by photo receptors is further processed in the OLs and in the MBs, we aimed to investigate adaptive changes in the central visual system of *C. rufipes* workers during their behavioral transition. We used age cohorts and tested how age and light exposure affect neuronal plasticity in the OLs and MBs. In detail, we analyzed 1) volumetric

plasticity in the OLs and 2) volumetric and synaptic plasticity in the MB–calyx collar with respect to segregated visual input from the ME as shown via tracer applications into the OLs.

Materials & Methods

Study Animals and Rearing Conditions

To exclude inter–colonial differences, we used *C. rufipes* workers from one main laboratory colony (collected by O. Geissler in La Coronilla, Uruguay, 2010) reared at the Biozentrum, University of Würzburg, at 25°C and 50% rH under a light–dark (LD) cycle of 12:12h. The colony as well as subcolonies (see below) were fed *ad libitum* with honey water and twice a week with frozen cockroaches.

To control the age of individual workers, ~30 larvae were transferred from the main colony into a small plastic box and reared in an incubator (Percival, I–30BLL, Germany) in constant darkness of 24h (DD) under constant conditions (25°C, 55% rH; Fig. 1, A). 20 adult workers from the same main colony were paint–labeled on the abdomen and added to the larvae to provide them care during growth and help during eclosion. To keep the conditions stable during the experiments, a sufficient amount of larvae was subsequently added to the subcolonies. Freshly emerged workers were individually marked under red light (>670 nm). 28 days post eclosion, a subgroup of workers was transferred to a second incubator (Percival) and exposed to a LD cycle of 12:12h (light–on: 07 am, light–off: 07 pm; 1100 lx) for 1, 4 and 14 days, respectively. Two fluorescent tubes (10.0 desert and 2.0 full spectrum, Repti Glo, 15W, 45 cm/18; Exo Terra, Holm, Germany) were used as artificial light sources. The emission spectrum of both light sources ranged from 300 – 630 nm and comprised both UVB as well as light in the visible range (Fig. 1).

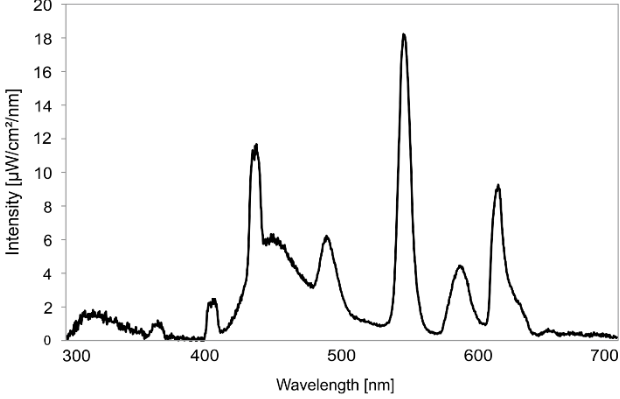


Figure 1: The emission spectrum of two fluorescent tubes. During the LD treatment artificial light was provided by two fluorescent tubes (Repti Glo 2.0 full spectrum –with visible output– and Repti Glo 10.0 full

▲ spectrum –with UVB output–, 15W, 45 cm/18", Exo Terra, Holm, Germany): The emission spectrum of both tubes were measured with a calibrated JAZ–Combo spectrometer (Ocean Optics, Dunedin, Florida, USA).

At defined ages (for details see Fig. 2, A), workers were collected between 1 and 2 pm for opsin gene quantification and neuroanatomical procedures. To minimize inter–individual variation, only media–sized workers with a thorax length (measured after Weber, 1946) between 2.3–4.0 mm were used for further analyses (Fig. 2, B).

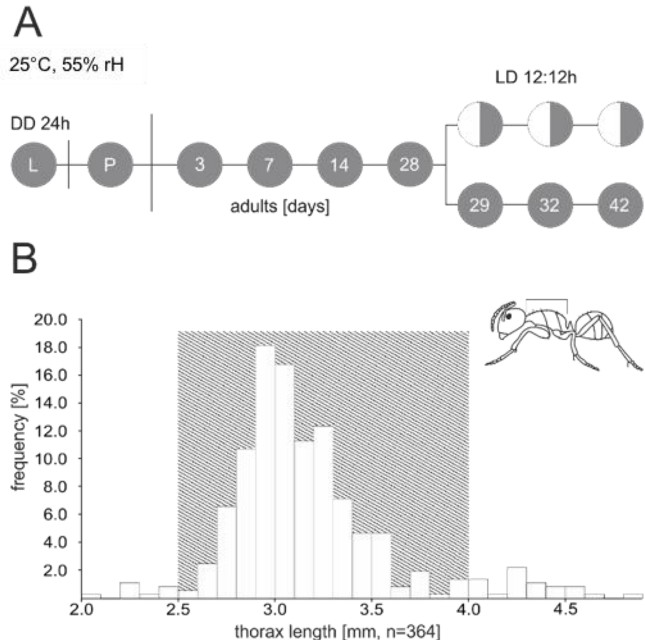


Figure 2: Rearing conditions and morphometric variation among *C. rufipes* workers. (A) Study animals were reared under controlled conditions in subcolonies from larval stage (L) onward during the whole pupal phase (P) and collected for neuroanatomical analyses at defined ages post eclosion. First data analyses post eclosion was 3 days. (B) Thorax length frequency distribution of individuals emerged in subcolonies. Thorax length was defined as the distance between anterior edge of the pronotum and the petiole. The range of workers evaluated is indicated by the grey–dashed box. DD: constant darkness of 24h, h: hour, LD: light–dark cycle of 12:12h, rH: relative humidity.

Neuroanatomical Procedures

Neuronal Tract Tracing

To trace the connections from the OL to the MB calyx a modified protocol from Kirschner et al. (2006) was used. Ants were mounted and a window was cut into the frontal head capsule. Rhodamine dextran with biotin (3,000 MW, lysine fixable; Microruby, D–7162, Molecular Probes, Eugene, OR, USA) or Alexa Fluor 488 dextran (10.000 MW, lysine fixable, D–22910, Molecular Probes) were delivered into the brain using glass electrodes with broken tips applying weak pressure. To identify all visual output tracts from the OL to their target regions within the protocerebrum, Microruby was applied into the OL neuropils (n=15). To distinguish visual PNs emerging from the ME and the LO, we used selective labeling with Alexa Fluor 488 dextran and Microruby in the same preparation (see arrow heads 1 and 2 in Fig. 3, A; n=3). To distinguish the borders of the visual and olfactory subregions of the MB calyx, Microruby and Alexa Fluor 488 dextran were inserted into the OL and the AL, respectively (arrow heads 1 and 3 in Fig. 3, A; n=5). To analyze projection patterns from the anterior and posterior ME within the MB–calyx collar, the anterior part of the ME was labeled using Microruby and the posterior part with Alexa Fluor 488 dextran (arrow heads 1 and 4 in Fig. 3, A; n=1). After tracer application, the head capsule was resealed and animals were kept alive in a moist dark chamber for 2 h to let the dye diffuse. Afterwards, brains were dissected, fixed in 4% formaldehyde in phosphate–buffered saline (PBS; pH 7.2), dehydrated in an ascending ethanol series, and cleared in methyl salicylate (MS).

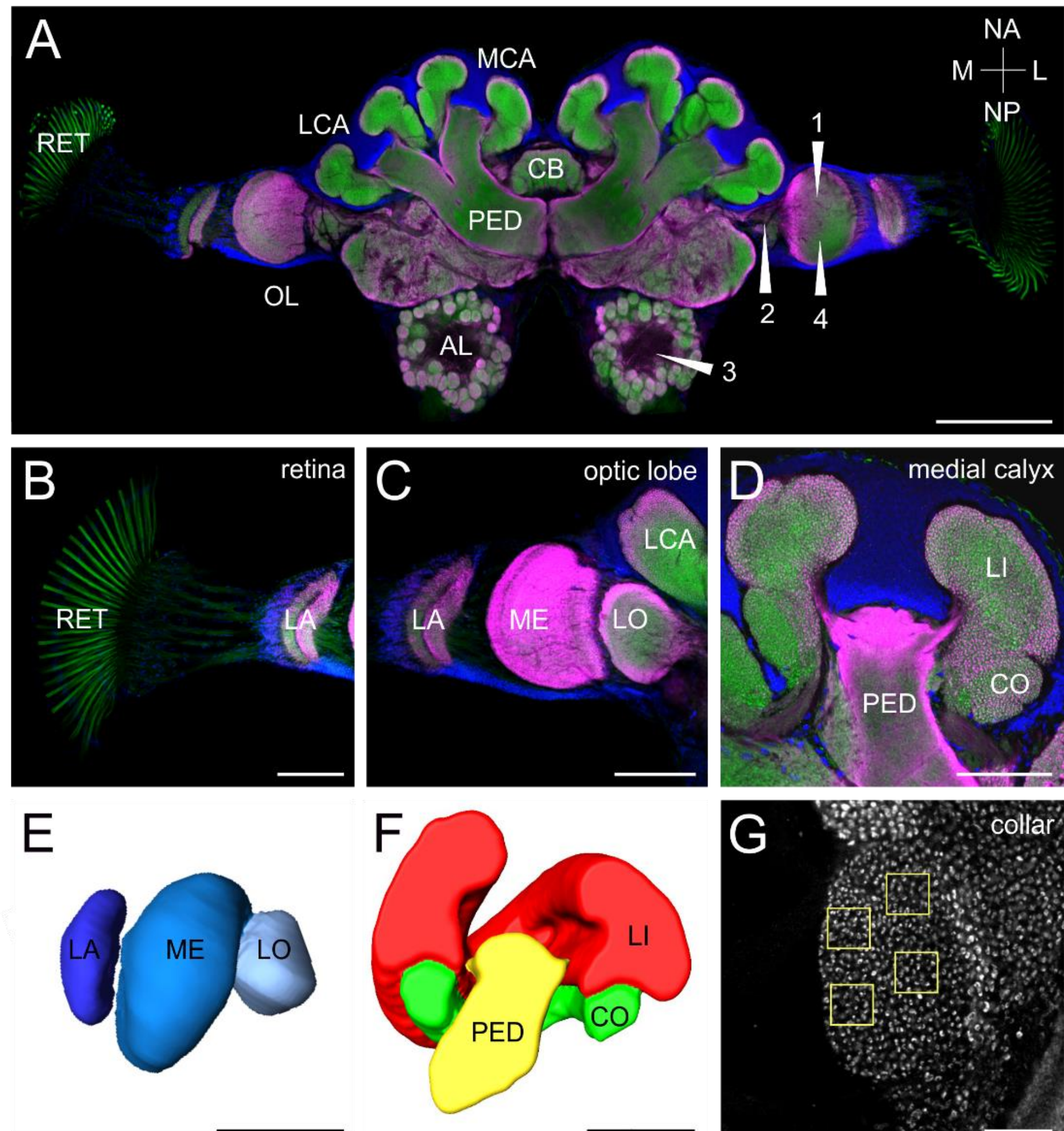


Figure 3: Immunofluorescence labeling and 3D reconstructions of a *C. rufipes* worker brain. (A–D) Frontal views of a central plane of the brain triple–labeled with an antibody to synapsin (red), f–actin phalloidin (green; CF 488 phalloidin, Biotium) and Hoechst nuclear marker (blue; Hoechst 34580, Invitrogen). (A) Brain overview with indication of prominent neuropils. The numbers 1–4 indicate locations for tracer application. Neuraxes (according to Ito et al., 2014): lateral (L), medial (M), anterior (NA), posterior (NP). (B+C) Higher magnification of the retina (RET; B) and of the optic lobe (OL), consisting of lamina (LA), medulla (ME) and lobula (LO), forming the primary optic neuropil (C). (D) Enlarged view of one medial MB calyx (MCA) of the left hemisphere showing the lip (LI) and the collar (CO). (E+F) 3D–reconstruction of the LA, ME and LO (E) and one MCA cut in a sagittal plane (F). (G) Regions of interest used to quantify synapsin labeled boutons in the CO are indicated by yellow squares. AL: antennal lobe, CB: central body, LCA: lateral calyx, PED: peduncle. Scale bars: A: 500 µm; B–F: 100 µm; G: 10 µm.

Synapsin Immunolabeling in Whole Mount Brains

We used a well–established protocol (Groh et al., 2012, 2014) to immunolabel presynaptic terminals in whole mount preparations of age–controlled ants. As synapsin was not exclusively concentrated within the PN axonal boutons and still distributed along its axon in 1–day old ants, we used 3–day old ants as youngest observation group for neuroanatomical analyses. Fixed whole mount brains were rinsed in PBS and permeabilized in PBS with Triton X, before being incubated in a mouse monoclonal antibody to the *Drosophila* synaptic vesicle associated protein synapsin (1:50; SYNORF1, kindly provided by E. Buchner, University of Würzburg, Germany). For fluorescence labeling, brains were incubated in CF488A goat anti–mouse secondary antibody (1:250; Biotium), before they were dehydrated using increasing ethanol concentrations and cleared in MS.

Confocal Laser Scanning Microscopy, Image Processing and Data Analysis

Brains were analyzed using a laser scanning confocal microscope (Leica TCS SP2 AOBS, Leica Microsystems AG, Wetzlar, Germany). Optical sections were taken at a resolution of 1,024 x 1,024 pixels. For neuronal tract tracing and for volume measurements (OL subneuropils and MB–calyx collar and lip; Fig. 3, C+D), optical sections were taken at intervals of 5 µm (10x / 0.4 NA imm or 20x / 0.7 NA with additional digital zoom). To quantify synapsin positive boutons in the visual collar and in the olfactory lip as an estimate of MG numbers, the innermost part of one medial calyx was scanned at high resolution through a depth of 10 µm at 0.5 µm intervals (63x / 1.4 NA imm, digital zoom 2.0, Figure 3, D). The 3D–analyses software AMIRA 5.6 (FEI Visualization Sciences Group, Düsseldorf, Germany) was used to analyze digital image stacks. For volumetric measurements, the outer borders of both neuropils of interest were encircled on every 3rd section, interpolated, 3D reconstructed, and the volumes were calculated (Fig. 3, E+F). To estimate the density of synapsin positive PN boutons in the collar, we counted synapsin–IR puncta within four 1,000 μm^3 cuboids (Fig. 3, G). To estimate the density of synapsin positive PN boutons in the lip, we counted synapsin–IR punctae within two cuboids (each 486.6 μm^3) in the outermost layer of the lip where synapsin positive boutons were more densely arranged (dense region, D) and within two cuboids (each 1,000 μm^3) in the central layer, where synapsin positive boutons were less densely arranged (non–dense region, ND).

A mean–value was calculated for the D and ND region in each age and treatment group, and bouton density was calculated as number of boutons per μm^3. Bouton numbers were averaged for each individual, and a mean–value was calculated for each age and treatment group. To estimate the total bouton number per collar and lip, respectively, the mean bouton density was extrapolated to the respective reconstructed collar or lip volume (for more details see Groh et al., 2012).

Statistical Analysis and Graphical Editing

All statistical calculations were performed with SPSS 22 (IBM, Chicago, IL, USA). Paired sample *t*–tests were performed to test differences in mean bouton numbers between the outer rim and inner core of the collar. One–way analysis of covariance (ANCOVA) followed by post–hoc SIDAK test was applied for all experiments with the body size (thorax length) as a covariant. The significance level for all analyses was set to $p < 0.05$. Graphs and figures were edited using COREL DRAW X7 (Corel Corporation Ltd., Ottawa, Canada).

Results

Neuronal Plasticity in Primary and Secondary Visual Centers

Age–Related and Light–Induced Neuronal Plasticity in the Optic Lobes

Using synapsin immunolabeling of whole–mount preparations in *C. rufipes* workers, the LA, ME and LO were 3D–reconstructed and quantified (Fig. 4). After controlling for body size, a significant age–related effect on the LA, ME and LO was determined under DD (LA, $F_{6,58}$=5.053, p=0.000; ME, $F_{6,58}$=6.276, p=0.000; LO, $F_{6,758}$ = 6.214, p=0.000, Fig. 4). Post–hoc SIDAK pairwise analysis revealed a significant increase of neuropil volume for all compartments within the first week of adult life (3 vs. 7 days: LA, p=0.011; ME, p=0.000; LO, p=0.000). Afterwards, the volume of the LA and LO decreased between days 7 and 32 (7 vs. 32 days: LA, p=0.016; LO, p=0.014, Fig. 4, A+C). To investigate a possible effect of light exposure on volume plasticity, 28–day old workers were exposed to a LD cycle of 12:12h for 1, 4 or 14 days and compared with workers of the same age kept in DD. While there was no significant difference in the volume of all three OL subneuropils between ants that were exposed to light for 1 day and the corresponding dark group (29 days: LA, $F_{1,14}$=0.612, p=0.447; ME, $F_{1,14}$=0.219, p=0.647; LO, $F_{1,14}$=1.319, p=0.270), light exposure for 4 and 14 days caused a significant volume increase (32 days: LA, $F_{1,16}$=14.356, p=0.002; ME, $F_{1,16}$ =18.998, p=0.000; LO, $F_{1,16}$=20.601, p=0.000; 42 days: LA, $F_{1,14}$ =22.267, p=0.000; ME, $F_{1,14}$ =17.768, p=0.001; LO, $F_{1,14}$=8.215, p=0.012). When comparing ants that were exposed to light for 1, 4 or 14 days, we found that subneuropil volumes increased with extended light exposure. This increase was significant in the ME within 4 days (1 vs 4 days LD: LA, p=0.246; ME, p=0.023; LO, p=0.182) and in the LA and LO within 14 days of LD (1 vs 14 days LD: LA, p=0.010; ME, p=0.000; LO, p=0.016; 4 vs 14 days LD: LA, p=0.177; ME, p=0.016; LO, p=0.339).

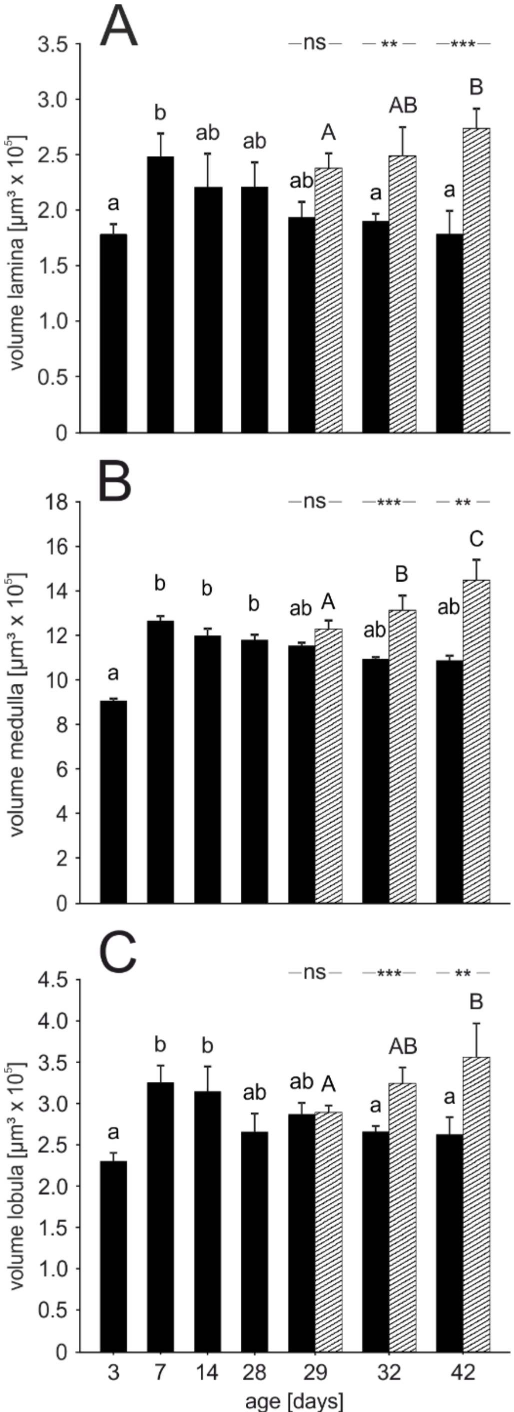


Figure 4: Age–related and light–induced neuronal volume plasticity of the lamina (A), medulla (B) and lobula (C). DD and LD treated animals of same age group are denoted by black and stripe pattern bars, respectively. Significant differences in neuropil volume within the DD treatment are marked with lowercase letters, within the LD treatment with capital letters and between DD/LD treatments with asterisks. DD treatment: 3 days old: n=9, 7 days old: n=8–10, 14 days old: n=8, 28 days old: n=11, 29 days old: n=8, 32 days old: n=11, 42 days old: n=9; LD treatment: 29 days old: n=10, 32 days old: n=8, 42 days old: n=8.

Central Projections of Visual Tracts from the Medulla and the Lobula

To determine the connection between the OLs and the MB calyx and other parts of the protocerebrum, we used tracer injections into the OL (Fig. 5, A). We showed that the asot is the only tract emerging from the OL innervating the MBs. This tract projects to the collar of both the lateral and medial calyces of the ipsilateral MBs, crosses the brain midline and innervates the contralateral calyces. The OL mass fill also revealed two distinct commissures that connect both OLs, the inferior and posterior optic commissure (IOC, POC; Hertel et al., 1987) as well as one distinct tract connecting the OL and the anterior optic tubercle (AOTU), the anterior optic tract (AOT; Strausfeld and Blest, 1970). Selective labeling of the ME and LO neurons with two tracers (Fig. 5, B) revealed that the asot as well as the POC exclusively emerge from the ME, whereas the IOC conveys information only from the LO. Furthermore, ME and LO fibers bundle together in the AOT innervating the AOTU. Within the AOTU two compartments can be distinguished according to their innervation profile by different visual PNs (Fig. 5, B). Labeling the OL and AL output tracts in the same preparation we further distinguish the visual and olfactory subdivisions of the MB calyx (Fig. 5, C). The collar was exclusively innervated by PNs from the OL (via the asot) and the lip only received olfactory input via the AL tracts (ALTs). Simultaneously injected tracers into the anterior and posterior halves of the ME revealed two layers in the collar, referred to as inner core (layer I) and outer rim (layer II, Fig. 5, D) receiving input from PNs from the anterior part and the posterior part of the ME, respectively. Their PN axonal boutons were evenly distributed within the collar.

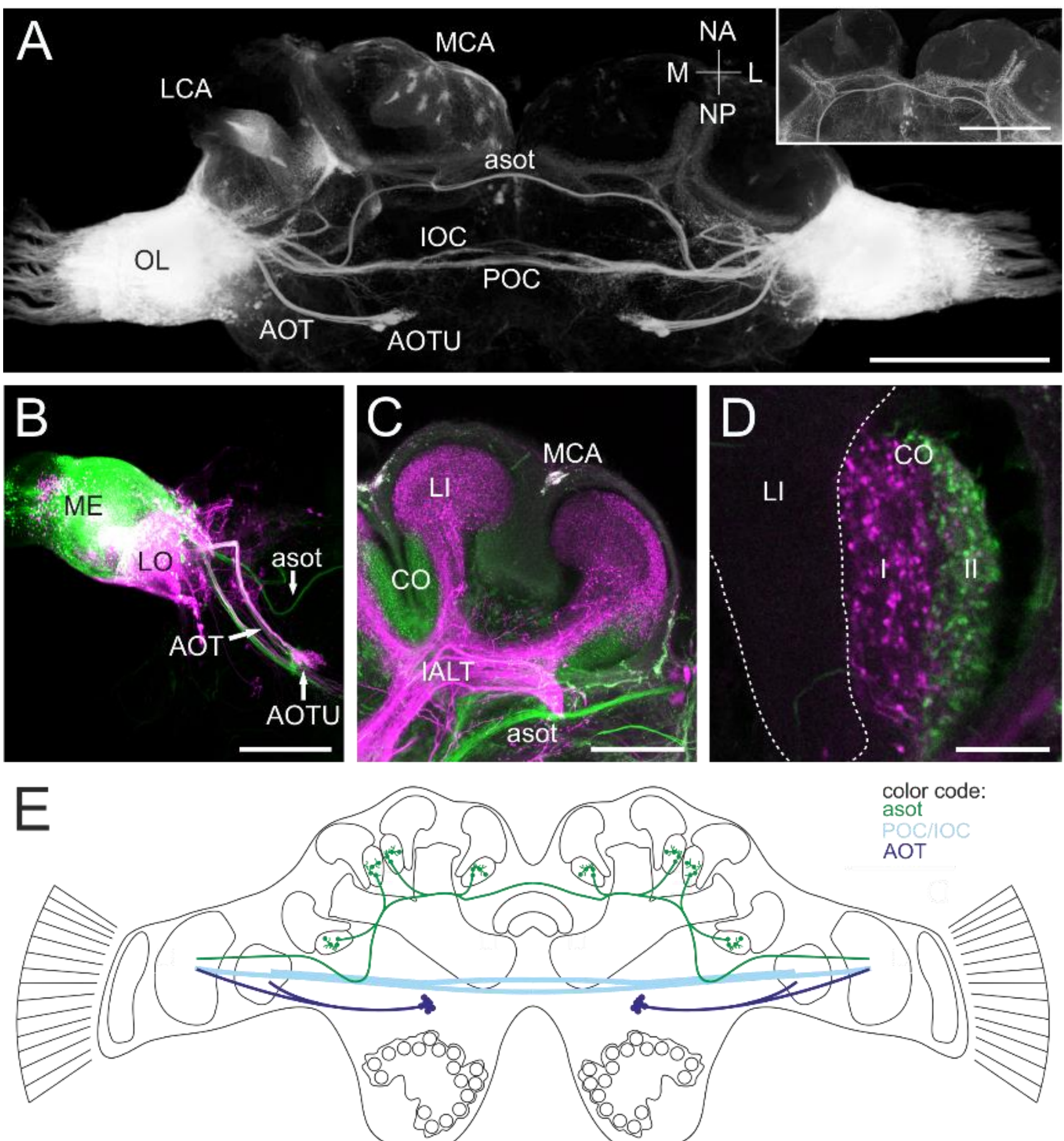


Figure 5: Projections of visual tracts from the optic lobes. (A) Composite of frontal sections of an anterograde mass fill (Microruby, grey) of the optic lobe (OL) output tracts and their projections to the protocerebrum. Projections from the OL to the MB calyx are provided by the anterior superior optic tract (asot, see also insert in A), to the contralateral OL by the inferior and posterior optic commissure (IOC and POC), and to the anterior optic tubercle (AOTU) by the anterior optic tract (AOT). Neuraxes (according to Ito et al., 2014): lateral (L), medial (M), anterior (NA), posterior (NP). (B) Composite of frontal sections after selective tracer injection into the ME (Alexa Fluor 488 dextran, green) and LO (Microruby, magenta) reveals that ME axons contribute to the asot and to the POC, LO axons to the IOC, and ME as well as LO axons to the AOT. C: Composite of frontal views of one medial calyx (MCA). Anterograde mass fills of OL (Alexa Fluor 488 dextran) and AL (Microruby) output tracts reveal that antennal input to the lip (LI) does not spread out into the visual collar (CO). (D) High magnification of the visual CO. Segregated visual input from different partitions of the ME to the CO: subregion I (magenta) with innervations from the posterior ME and subregion II (green)

▲ with innervations from the anterior ME. (E) Schematic drawing of a C. rufipes brain highlighting the visual tracts emerging from the ME and LO. lALT: lateral antennal lobe tract, LCA: lateral calyx. Scale bars: A (+ insert): 250 µm; B: 200 µm; C: 100 µm; D: 50 µm.

Age–Related and Light–Induced Neuronal Plasticity in the MB–Calyx Collar

To determine the absolute volume of the visual input region per MB calyx as determined via tract tracings, the collar of one medial calyx per brain was quantified based on low magnification scans. After controlling for body size, we found a significant increase of the MB–calyx collar volume under DD ($F_{6,75}$=3.193, p=0.008, Fig. 6, A). Post–hoc analysis revealed that the collar volume significantly increased within 14 days of DD (3 vs. 14 days: p=0.005) followed by a significant decrease between days 14 and 28 (14 vs. 28 days: p=0.042). In contrast to all OL subneuropils, exposure to light for 1, 4 or 14 days did not affect the volume of the collar when compared with the corresponding dark reared groups (29 days: $F_{1,20}$=0.094, p=0.762; 32 days: $F_{1,23}$=1.234, p=0.278; 42 days: $F_{1,21}$=0.235, p=0.633) and when comparing different times of exposure to light (1 vs. 4 days of LD: p=0.089; 4 vs. 14 days of LD: p=0.467; 1 vs. 14 days of LD: p=0.691).

We combined volume measurements with quantification of synapsin–positive boutons (Fig. 6, B). As differential labeling of the anterior and posterior ME revealed two layers in the collar (Fig. 5, D), we first determined the density of synapsin positive profiles by separately quantifying bouton profiles within the outer rim and inner core of the collar (Fig. 5, D). We found no significant difference in the density of presynaptic boutons between the outer rim and inner core (t–test, t–value=1.452, p=0.147). Based on this result, a mean density of synaptic profiles per individual was calculated from all four defined cuboids per collar. In dark reared ants, the synaptic density of the collar was significantly affected by age ($F_{6,72}$=2.000, p=0.077; Fig. 6, B). Synaptic density increased within 28 days after emergence (3 vs. 28 days: p=0.034) and remained constant afterwards (28 vs. 29 days: p=0.706; 28 vs. 32 days: p=0.953; 28 vs. 42 days: p=1.000, Fig. 6, B). After 4 days of light exposure bouton densities significantly decreased compared to the corresponding dark reared group of the same age (32 days: $F_{1,20}$=7.597, p=0.012). No significant difference was found after 1 day and 14 days (29 days: $F_{1,18}$=0.182, p=0.675; 42 days: $F_{1,20}$=0.462, p=0.505). Within the LD group we found no effect of light exposure on bouton density within 4 days (1 vs. 4 days of

LD: p=0.491), but a significant increase after 14 days (4 vs. 14 days of LD: p=0.009; 1 vs. 14 days of LD: p=0.280).

To estimate the total bouton number per collar region, the mean bouton numbers per 1,000 μm^3 were extrapolated to the volume of the visual calyx subregion (Fig. 6, C). The total number of boutons per collar was affected by age ($F_{6,70}$=3.273, p=0.007). Bouton numbers increased within 14 days of DD (3 vs. 14 days: p=0.002) and, statistically, did not change with ongoing age in the observed time window (14 vs. 28 days: p=0.427; 14 vs. 29 days: p=0.626; 14 vs. 32 days: p=0.547; 14 vs. 42 days: p=0.999). Light and dark reared ants of the same age did not differ in the total amount of boutons (29 days: $F_{1,17}$=0.000, p=0.993; 32 days: $F_{1,19}$=3.293, p=0.085; 42 days: $F_{1,20}$=0.000, p=0.994). Comparison of ants exposed to light for 1, 4 or 14 days revealed a significant increase in total bouton numbers from day 4 to day 14 (4 vs. 14 days of LD: p=0.046).

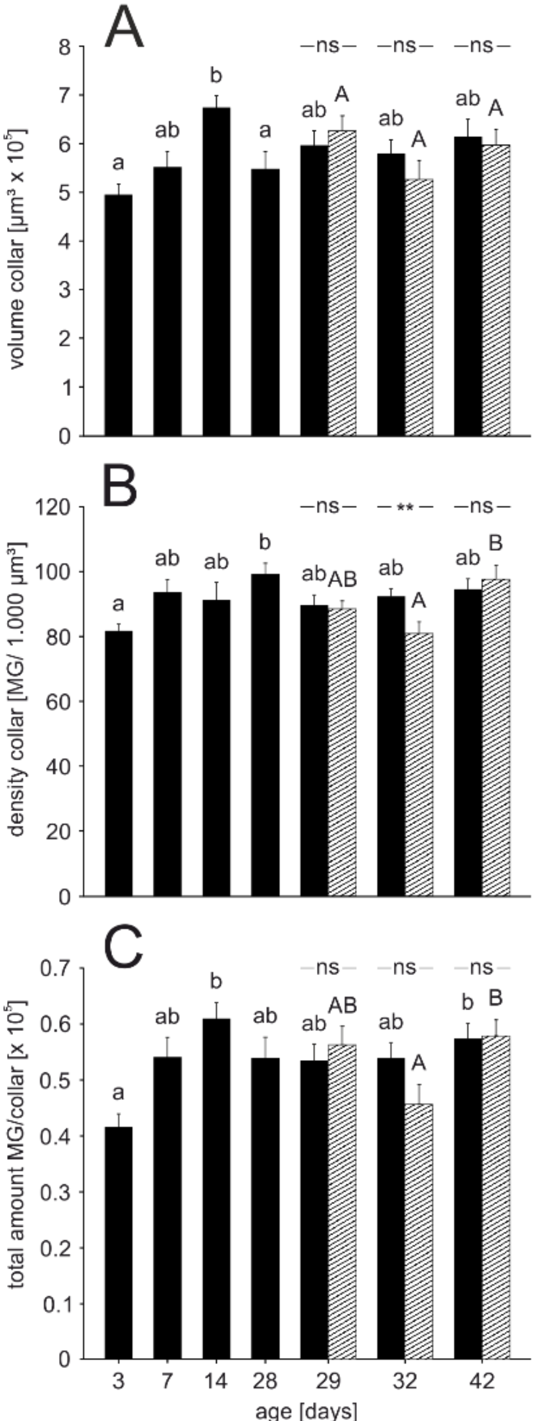


Figure 6: Age–related and light–induced changes in the volume of the calyx collar (A), bouton density (B) and total amount of boutons (C). DD and LD treated animals of same age group are denoted by black and stripe pattern bars, respectively. Significant differences in neuronal structures are marked with lowercase letters, within the LD treatment with capital letters and between DD/LD treatments with asterisks. DD treatment: 3 days old: n=8–9, 7 days old: n=11–12, 14 days old: n=10, 28 days old: n=14–15, 29 days old: n=10–12, 32 days old: n=13–14, 42 days old: n=12; LD treatment: 29 days old: n=10–11, 32 days old: n=9–12, 42 days old: n=11–12.

Age–Related and Light–Induced Neuronal Plasticity in the MB–Calyx Lip

To determine the absolute volume of the olfactory lip input region per MB calyx as determined via tract tracings, the lip of one medial calyx per brain was quantified based on low magnification scans. After controlling for body size, we found a significant increase of the MB–calyx lip volume under DD ($F_{6,60}$=4.512, p=0.001, Fig. 7, A). Post–hoc analysis revealed that the lip volume significantly increased within 14 days of DD (3 vs. 14 days: p=0.002). In contrast to the volume increase under DD, exposure to light for 1, 4 or 14 days did not affect the volume of the lip when compared with the corresponding dark reared groups (29 days: $F_{1,17}$=0.146, p=0.707; 32 days: $F_{1,17}$=0.696, p=0.416; 42 days: $F_{1,17}$=1.090, p=0.311) and when comparing different times of exposure to light (1 vs. 4 days of LD: p = 0.0896; 4 vs. 14 days of LD: p=0.66; 1 vs. 14 days of LD: p=0.285).

We combined volume measurements with quantification of synapsin positive boutons. Synapsin labeled boutons in the MB lip were more densely packed in the D (Fig. 7, B) than in the ND lip (Fig. 7, C). In dark reared ants, the synaptic density of the D and ND lip was significantly affected by age (D: $F_{6,58}$=3.004, p=0.013; ND: $F_{6,58}$=3.909, p=0.002). Synaptic density increased within 29 days after emergence in the D (3 vs. 29 days: p=0.015) and within 28 days in the ND lip region (3 vs. 28 days: p=0.025). Exposure to light for 1, 4 or 14 days did neither affect the synaptic density in the D nor in the ND lip region when compared with the corresponding dark reared groups (D: 29 days: $F_{1,17}$=4.045, p=0.06; 32 days: $F_{1,17}$=0.715, p=0.208; 42 days: $F_{1,17}$=1.604, p=0.222; ND: 29 days: $F_{1,17}$=0.177, p=0.679; 32 days: $F_{1,17}$=0.254, p=0.621; 42 days: $F_{1,17}$=0.94, p=0.763) and when comparing different times of exposure to light (D: 1 vs. 4 days of LD: p=0.155; 4 vs. 14 days of LD: p=0.999; 1 vs. 14 days of LD: p=0.13; ND: 1 vs. 4 days of LD: p=0.949; 4 vs. 14 days of LD: p=0.937; 1 vs. 14 days of LD: p=0.686). The total number of boutons per lip was affected by age ($F_{6,57}$=6.005, p=0.000; Fig. 7, D). Bouton numbers increased within 7 days of DD (3 vs. 7 days: p=0.023). Light and dark reared ants of the same age did not differ in the total amount of boutons (29 days: $F_{1,16}$=1.659, p=0.216; 32 days: $F_{1,17}$=0.131, p=0.722; 42 days: $F_{1,17}$=1.961, p=0.179). When comparing different times of exposure to light, light exposure did not affect the total amount of boutons (1 vs. 4 days of LD: p=0.417; 4 vs. 14 days of LD: p=0.729; 1 vs. 14 days of LD: p=0.084).

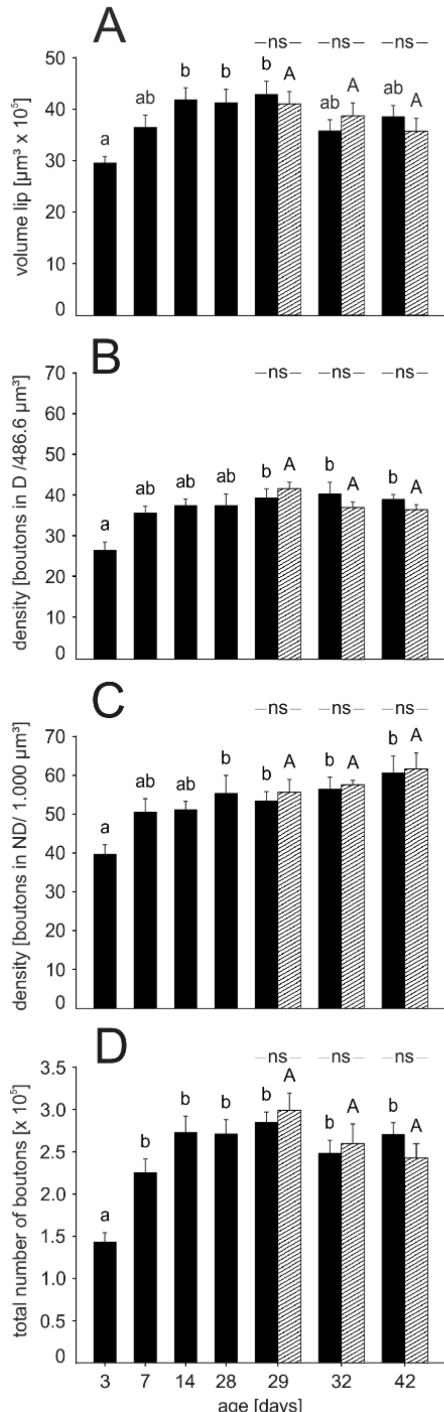


Figure 7: Age–related and light–induced changes in the volume of the MB calyx lip (A), bouton density in the dense (B) and in the non–dense (C) lip region, and total amount of boutons (D). DD and LD treated animals of same age group are denoted by black and stripe pattern bars, respectively. Significant differences in neuronal structures are marked with lowercase letters, within the LD treatment with capital letters and between DD/LD treatments with asterisks. DD treatment: 3 days: n=6–9, 7 days: n=10, 14 days: n=9–10, 28 days: n=10, 29 days: n=10, 32 days: n=10, 42 days: n=10; LD treatment: 29 days: n=9–10, 32 days: n=10, 42 days: n=10.

Discussion

Using tracer applications into the OL, we could show a segregation of visual input from the ME into the MB–calyx collar via the asot. This enabled us to determine age–related and light–induced synaptic changes in subcompartments of the collar. Most importantly, we show that the visual system of *C. rufipes* is highly plastic at the central brain. The volume of all OL neuropils increased and were accompanied by an increase in volume and PN axonal bouton densities in the collar. Exposure to light for four days induced a volume increase in the OLs and a decrease of PN axonal boutons in the MB–calyx collar. Extended light exposure for 14 days resulted in an increase in OL volume and PN axonal boutons in the collar. The results indicate that both experience–independent and experience–dependent components significantly shape the central visual pathway in *C. rufipes*. This plasticity of the visual system is likely important for optimal timing of the interior–forager transition, thus promoting flexibility of an age–related division of labor.

Neuronal Plasticity in Primary and Secondary Visual Centers

Projections of Visual Tracts from the Medulla and the Lobula

Our tracing study indicates that the ME has substantial connections via the asot to the MB–calyx collar. This pathway is bilateral and conveys visual input to both ipsi– and contralateral MBs as shown in other ant species and the honeybee (Gronenberg, 1999; Ehmer and Gronenberg, 2002, 2004). Contrary to honeybees (Ehmer and Gronenberg, 2002) and some other ant species (Ehmer and Gronenberg, 2004), our tracer applications did not reveal any evidence for the presence of other ME– and LO–MB connecting neurons. Although we cannot completely exclude the possibility of potential input from the LO to MB collar, these differences in tract innervation from the OL to the MB might correlate with the significance of vision in different Hymenoptera (Gronenberg, 2001; Ehmer and Gronenberg, 2004). Tracer injections in the dorsal and ventral part of the ME in *C. rufipes* revealed that neurons from the anterior ME predominantly terminate in the posterior collar, whereas neurons from the lower ME terminate in the inner part (dorso–ventral layering). This partitioning was also suggested for other ants (Gronenberg, 2001) and also found in bumblebees (Paulk and Gronenberg, 2008; Paulk et al., 2009). ME and LO neurons also convey information to the contralateral OL via the POC and IOC, as shown earlier in honeybees (Hertel and Maronde,

1987; Hertel et al., 1987). The AOT which was recently described in other ants (Schmitt et al., 2015) connects the ME and LO with the AOTU (e.g. in honeybees Mota et al., 2011). In *C. rufipes* ME and LO neurons innervate two separate areas in the AOTU. This rich diversity of visual projections indicates a prominent function of vision in *C. rufipes*.

Age–Related and Light–Induced Neuronal Plasticity in the Optic Lobes and the Calyx Collar

Our findings on plasticity in the optic centers suggest two independent processes – an age–related and light–induced neuronal plasticity. A remarkable volume increase in all OL compartments (LA, ME and LO) during the first week of adult life and, one week later, in the MB collar supports the idea that volume plasticity of different brain neuropils is not affected uniformly or synchronously by age as suggested for *C. floridanus* (Gronenberg et al., 1996). A neuronal volume increase during adult maturation in DD likely reflects intrinsic developmental processes, independent from external input. During early weeks of adult life, ant workers perform nursing tasks and are mainly restricted to the dark nest in the absence of visual input (Hölldobler and Wilson, 1990). Initial volume increases in the MB–calyx collar during this early phase of adult maturation was suggested to be caused by an experience–independent internal program in natural honeybee colonies (Farris et al., 2001; Ismail et al., 2006; Muenz et al., 2015), in bees prevented from foraging (Withers et al., 1995), and in bees and ants deprived from visual experience (Fahrbach et al., 1998; Kühn–Bühlmann and Wehner, 2006). This initial experience–independent maturation likely represents a preparation of the central visual system for future demands associated with the transition to outdoor foraging, previously termed as ‘experience–expectant’ plasticity (Fahrbach et al., 1998).

In this study, the exposure to light revealed pronounced effects on volume plasticity of the OL, but not in the MB–calyx collar. Light–induced volume increase in all three OL subneuropils might be explained by changes in size and number of terminal branches of PR neurons (Heisenberg et al., 1995; Barth et al., 1997). In contrast to the OL, MB–calyx collar volumes remained unchanged after constant daylight exposure and may respond only to more complex tasks like visual orientation. In this line, experience dependent volume increases in the MB–calyx collar were associated with intense foraging experiences in both

ants and honeybees (Farris et al., 2001; Ismail et al., 2006; Kühn–Bühlmann and Wehner, 2006).

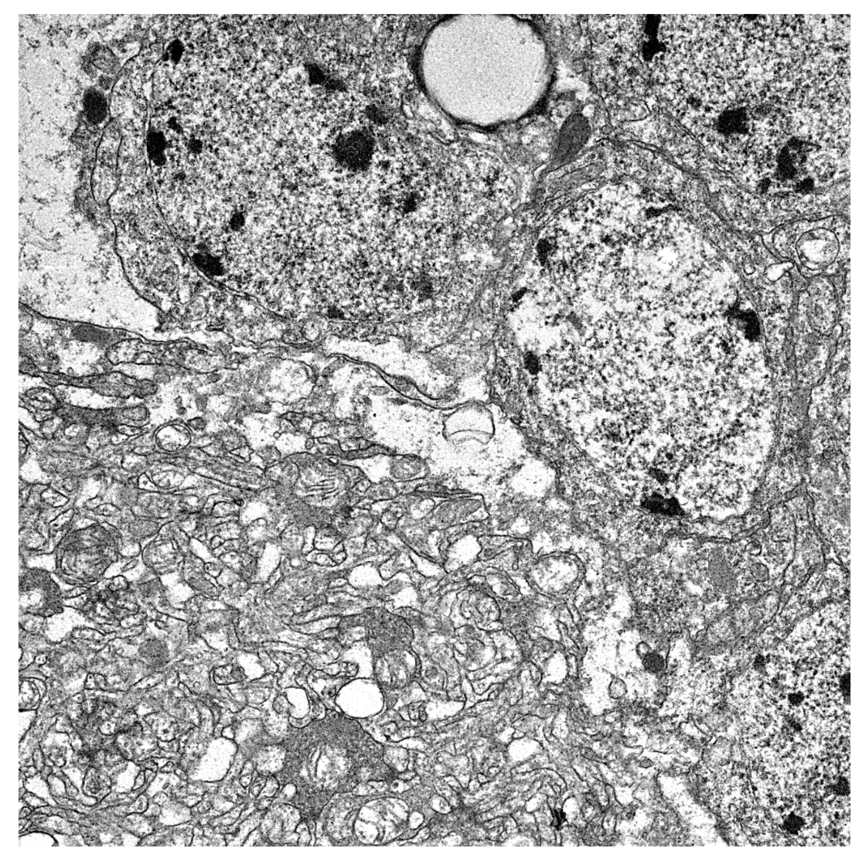
The volume increase in the MB–calyx collar during the first two weeks of adult life was accompanied by an increase in PN bouton density and total numbers of PN boutons. This most likely reflects the formation of new presynaptic boutons of sprouting PNs resulting from a general outgrowth of Kenyon cells (KCs) dendritic branches (Farris et al., 2001; Stieb et al., 2010; Muenz et al., 2015). Furthermore, we observed that short–term light exposure for 1 day did not induce structural synaptic changes in *C. rufipes* workers, whereas 4 days of light exposure induced a significant decrease in bouton densities in the MB–calyx collar. A similar synaptic pruning was also shown for desert ants (Stieb et al., 2010, 2012) and in honeybees (Scholl et al., 2014) in response to a light pulse program over several days. The increase in bouton density and numbers we observed in *C. rufipes* after extended light exposure for 14 days was independent of the MB–calyx collar volume. Interestingly, volume independent increases in bouton densities in the MB calyx olfactory lip was shown to be associated with transcription–dependent long–term memory formation in honeybees (Hourcade et al., 2010) and in leaf cutting ants (Falibene et al., 2015).

Little is known about the physiological mechanisms mediating age–related and light–induced neuroplasticity. Studies on honeybees have shown that juvenile hormone (JH) does not directly affect the growth of the MB neuropil (Fahrbach et al., 2003) and the reorganization of MG densities (Scholl et al., 2014). On the other hand, precocious light exposure triggers an increase in JH levels in the hemolymph of young bees suggesting a dual effect of light exposure (Scholl et al., 2014). Activation of muscarinic receptors in the honeybee caused an increase in MB volume and KC dendritic complexity (Ismail et al., 2006; Dobrin et al., 2011), and rho–GTPase activation mediates growth of KC dendrites (Dobrin and Fahrbach, 2012) giving first hints on potential molecular pathways involved in the remarkable activity dependent structural plasticity in the MBs. Regarding long–term memory (LTM) associated structural plasticity in the MB calyx, the calcium–dependent protein kinase II (CaMKII) was suggested to play a role in LTM induced KC dendritic plasticity (Pasch et al., 2011; Scholl et al., 2015).

Our findings show that *C. rufipes* workers possess a highly plastic visual system affected by both age and changing environmental conditions. This plasticity in neuronal structures comprises both `experience–independent´ and `experience–dependent´ components. Although the underlying mechanisms of this considerable plasticity in the visual system still need further investigation, it likely constitutes an important factor promoting the major behavioral transition.

Chapter IV

Ultrastructural Analysis of Synaptic Complexes in the Mushroom Body Calyces in *Camponotus rufipes*



Chapter IV
Ultrastructural Analysis of Synaptic Complexes in the Mushroom Body Calyces in *Camponotus rufipes*

Introduction

In eusocial Hymenoptera, the behavioral transition from nursing to foraging of single workers is accompanied by both, age– and experience–related plasticity of neuronal complexes (Farris et al., 2001). This results in a maturation of brain neuropils and an outgrowth of synaptic dendrites (Gronenberg et al., 1996). The allocation in different tasks in an ant colony can be related to age and/ or the body size of the workers (Hölldobler and Wilson, 1990). Certainly, the underlying neuronal mechanisms that are associated with the specialization for different tasks requires further investigation. To study these underlying neuronal mechanisms, ants constitute a rewarding model, as the behavioral repertoire of workers is already described, and their brains are easily accessible. Especially the species *Camponotus rufipes* provides a useful model system, as they are known for their simultaneous age– and size–related division of labor (Jáffe and Sanchez, 1984; Soares et al., 2008; see own data **chapter I**). Workers of *C. rufipes* have been described to undergo a smooth, age–related transition: newly eclosed ants are nurtured by older ones during the first 48 hours of their life before they themselves perform nursing tasks. After 2–3 weeks, around 53% switch to exterior tasks, like foraging, whereas the other half remains nurses (own data **chapter I**).

In the insect brain, the mushroom bodies (MB) are known to play a crucial role in the control of task allocation and information processing (Strausfeld et al., 1998; Ganeshina and Menzel, 2001). The MBs are paired neuropils in each brain hemisphere, and contain thousands of intrinsic projection neurons, the Kenyon cells (KC, after Kenyon (1896)). The dendritic network of the KCs shapes the MB calyces, whereas the parallel arranged axons of the KCs are shaping the pedunculus. The calyces are the main input region of the MBs, that are connected via the peduncle the MB main output region, the medial and vertical output lobes (Fahrbach, 2006; Gronenberg, 2008). The dendritic arborizations of the KCs innervate

different areas in the MB calyces that differ in their function: olfactory projection neurons (PN) are terminating in the lip region, whereas visual PNs are branching in the collar region (Mobbs, 1982; Strausfeld et al., 1998; Strausfeld, 2002). Within both regions, the PN terminals (so called `boutons´) are surrounded by dendritic spines of the KC dendrites and form synaptic complexes, termed microglomeruli (MG; Yasuyama et al., 2002; Frambach et al., 2004; Groh et al., 2004, 2006; Leiss et al., 2009; Stieb et al., 2010). In eusocial insect colonies, the performance of different tasks has been described to be accompanied by architectural changes of the MG. In the MB calyces of honeybees, for instance, the number of dendritic spines undergoes dramatic task–related changes (Fahrbach et al., 1998; Farris et al., 2001). Moreover, the densities of synaptic architectures (the MG) in the MB calyces in *C. rufipes* ants have been described to increase with ongoing age but to decrease after exposure to light (Yilmaz et al., 2016). This shows that the structural neuroplasticity in the MB calyces comprises both, `experience–independent´ and `experience–dependent´ elements (Yilmaz et al., 2016). The remarkable structural plasticity of these synaptic complexes suggests that the MG play an active role in life–long plasticity and adjustments of neuronal circuits that promote the behavioural transition in *C. rufipes* workers. To reveal how sensory information and how neuronal mechanisms that trigger task– and age–related plasticity are integrated in the MB calyces requires a deeper understanding of the connectivity and synaptic properties of the MG. So far, studies in the honeybee based on electron microscopic 3D–reconstructions in serial brain sections revealed that the interior–exterior transition is correlated with substantial structural changes in modular synaptic complexes in MB input regions at both, the pre– and postsynaptic site (Groh et al., 2012). Until recently, both, the identification of single vesicles and the clear architecture of active zones in PN axonal boutons were below optical resolution even within ultrathin brain sections (Groh et al., 2012) using classical Transmission Electron Microscopy (TEM).

In this study, I started to implement electron tomography (ET) in *C. rufipes* ants, which facilitates high resolution images of synaptic architectures at the ultrastructural level and promotes the understanding of architectural changes in neuronal plasticity processes. I started by testing multiple features for a higher optical resolution of the ET images, like adjusting the slice thickness of tomogram samples and optimizing the tilting angle. With the optimization of both, slice thickness and tilt angle, I am now able to resolve single vesicles

in the cytoplasm of PN axonal boutons in the calyx of *C. rufipes* workers. To do this, I acquired double tilt series of PN axonal boutons with consequent computation of the acquired tilt information. Moreover, adding of 12 nm gold particles onto the grids remarkably improves the automatic alignment of serial micrographs. Hence, the ET provides a powerful tool to understand architectural changes within individual PN axonal boutons in the MB calyx. These architectural changes may be potentially associated with the interior–exterior transition of *C. rufipes* workers

Materials & Methods

In this study, I started to implement ET to increase the synaptic resolution of single PN boutons of the MB calyx to understand architectural changes in neuronal plasticity processes at the ultrastructural level. The animals used in this study were reared as described precisely in **chapter I** (please see part materials & methods).

Tissue Preparation

For ET, ants were immobilized on ice and their heads were cut off. Heads were fixed in dental wax and a window was cut into the head capsule to get access to the brain. Brains were dissected using ice–cold ant ringer solution and immediately incubated into Karnovsky fixation (previously described in Meinertzhagen, 1996; pH 7.2 to 7.4) over night at 4°C. Afterwards, brains were washed in 0.1M cacodylate and 0.04% $CaCl_2$ (3x5 min) and were then fixed in 2% aqueous OsO_4 with 0.2M cacodylate for 2 hours at room temperature. Samples were then washed in distilled water (5x10 min) and contrasted with 0.5% aqueous uranyle acetate over night at 4°C and washed in dest. H_2O (5x10 min) on the next day. Subsequently, the tissues were transferred into 50% EtOH for a couple of seconds before they were further dehydrated on ice in an ascending ethanol series (50%, 70%, 90%, 95% and 2x100%, 10 min per step). Next, the brains were treated with propylene oxide (2x10 min) at room temperature, before they were transferred to a 1:1 mixture of propylene oxide and Epon for four hours under the fume hood. Before final slicing, brains were embedded in fresh Epon and polymerized 48h at 60°C.

For slicing, Epon blocks were first manually trimmed using a razor blade under a dissecting microscope to get access to the region of interest of each brain. Semi– (~500 nm) and ultrathin sections (~100 nm) were then cut in a horizontal plane with a Leica EM UC7 microtome equipped with a histodiamond knife (Diatome, Biel, Switzerland). Semithin sections were stained with toluidine blue and were used to locate and orientate the MB neuropil region under a light microscope. Afterwards, three to four consecutive ultrathin sections were collected on pioloform–slotted 1x2 mm grids (Fig. 1, A; Plano GmbH, Wetzlar, Germany) and further stained and contrasted with 2.5% uranyle acetate solved in ethanol for 10 minutes and Reynolds lead citrate for 10 minutes. Afterwards a carbon coat (~5 nm thickness) was added to the grids with a MED 010 (Balzers Union AG, Balzers,

Liechtenstein). For automatic alignment of tilt series, 12 nm ProtA–Au–beads (Dianova GmbH, Hamburg, Germany) gold particles were added nonspecifically to both sides of the samples by incubation for 5 min in an undiluted solution, followed by a single washing step with dest. H_2O.

Electron Tomography

I used an intermediate–voltage 200 kV JEM 2100 (JEOL, Munich, Germany) transmission electron microscope equipped with a TemCam F416 4k x 4k camera (Tietz Video and Imaging Processing Systems, Gauting, Germany) for tilting series of 20.000x magnification. The tilt series were conducted from –65° to 65° using SerialEM (Mastronarde, 2005) in 1° increment steps (Fig. 1, B). For higher optical resolution, two tilt series were scanned from the same PN bouton by rotating the grid by 90° (Fig. 1, B). Tilt series were aligned and consecutive tomograms were joined using the package eTomo of the IMOD software (Kremer et al., 1996). Every tomogram was reconstructed with a resolution of 1024x1024 pixels (Fig. 1, D).

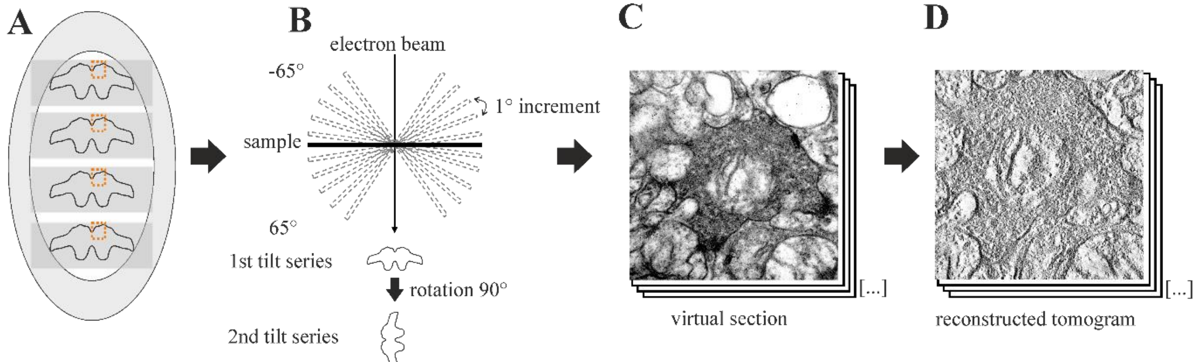


Figure 1: Preparation for ET sections and following image processing. (A) Four consecutive brain slices were placed on a one–hole grid. Orange dotted line indicates the region of interest (projection neuron (PN) axonal bouton). (B) After the same PN bouton was localized at every slice, both tilt series were required from –65° to 65° with 1° increment steps. After acquisition of one tilt series, the second was scanned from the same PN bouton by rotating the grid by 90°. (C) Virtual sections were built of every single image. (D) Reconstructed tomogram of a double tilt series.

Size Measurements of Synaptic Architectures in the MB Calyx

All size measurements were done with the FIJI–win32 software (Schindelin et al., 2012; Wayne Rasband, National Institute of Health, USA) and indicated as mean value ± standard deviation error (SDE). The size of PN boutons was measured as the largest distance between two dots on the lipid membrane and averaged over six different slices. The size of the clear–core and dense–core vesicles as well as of the postsynaptic partners was estimated as the maximal diameter. Additionally, the absolute number of active zones and their potential postsynaptic partners was counted by eye in every single image stack. Graphs and figures were edited using COREL DRAW X8 (Corel Corporation Ltd., Ottawa, Canada).

Results

In this study, I started to reveal the presynaptic architecture of single PN boutons in the MB calyces of *C. rufipes* workers. For the dissection and fixation of the specimen, I used an already published fixation protocol for the honeybee, which was optimized for image recordings with TEM (Groh et al., 2012). In the aforenamed study the optical resolution of presynaptic architectures was below the resolution to identify single vesicles. To come up with this resolution problem, I started to implement ET, which is to date one of the most reliable approaches to obtain a 3D information by electron microscopy (reviewed in Baumeister et al., 1999).

Technical Development of Image Processing

For consecutive slicing through one entire PN bouton, I started with 400 nm semithin slices of the respective specimen, to need as little consecutive slices as possible. The diameter of one PN bouton in the honeybee is described as 1–2 µm (Groh et al., 2012), meaning 4–5 consecutive semithin slices are needed to cut through one entire PN axonal bouton. However, the 400 nm slices were too thick to gain a high resolution of single PN boutons (data not shown). As a next step, I reduced the thickness of the consecutive slices to 250 nm (Fig. 2, A+A´), which resulted in 8–10 consecutive slices through the MB calyx lip region. The resolution I received with the 250 nm semithin slices was higher than in 400 nm semithin slices but still too low to resolve single presynaptic structures (Fig. 2, A´). Finally, I cut 100 nm ultrathin slices (Fig. 2, B+B´), that were thin enough to visualize and identify single synaptic architectures, but 16–18 consecutive brain slices were needed to gain one entire PN axonal bouton (Fig. 2, B´).

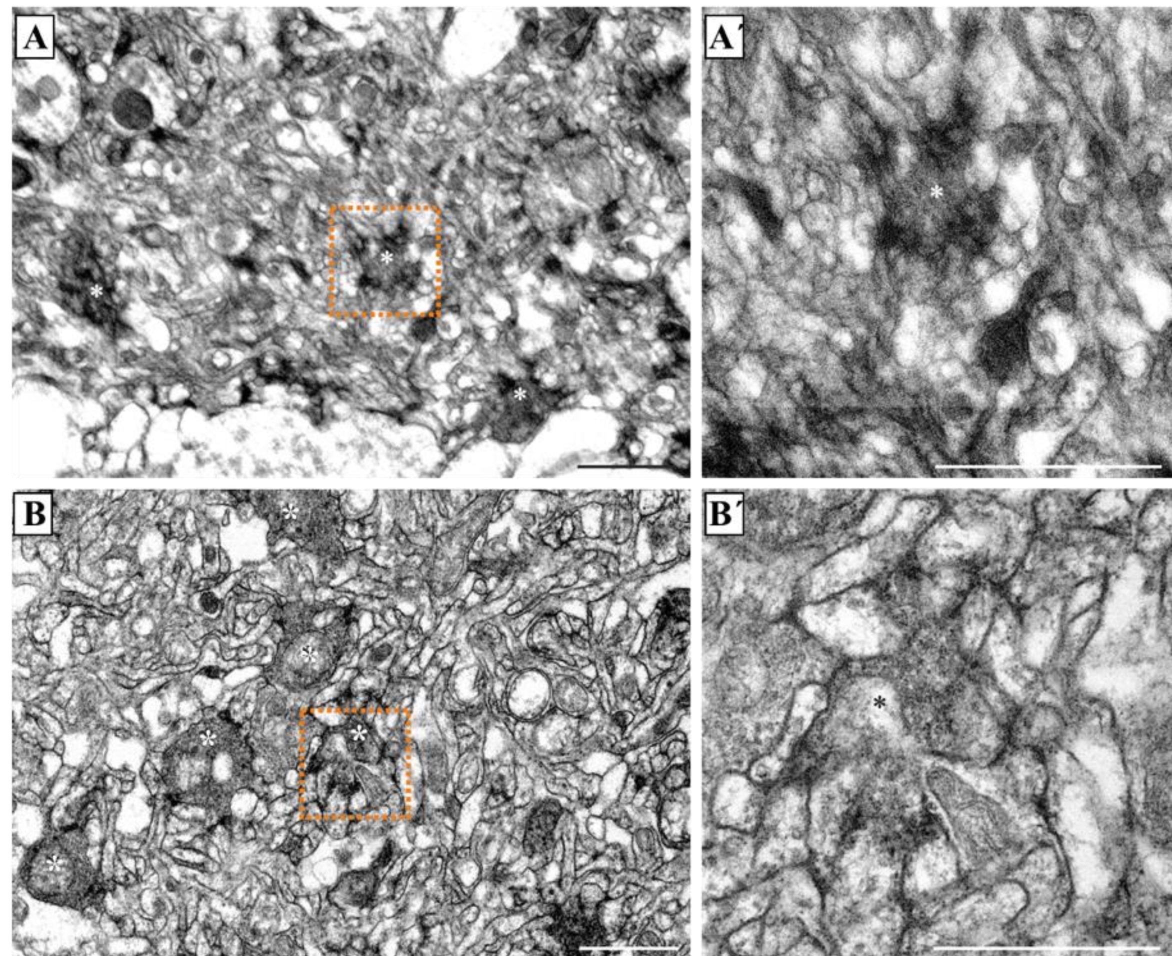


Figure 2: Different thick brain slices used for electron tomography. (A+B) Semithin slice (250 nm; A) and ultrathin slice (100 nm; B) through the olfactory innervated lip region in the mushroom body calyces. Orange dotted line shows magnification of images in A´ and B´. (A´+B´) Magnification of one presynaptic site shown in A and B. Asterisks indicate projection neuron boutons. Scale bars: 1 µm.

As a next step, I adjusted the varying tilting angle of the respective specimen. The maximal tilt angle that can be achieved by the used microscope and tilting stage was ± 70°. I therefore started the tilt series with the maximal angle of 70° and 1° incremental steps. Since the optical resolution of the single images decreased dramatically above an angle of 65°, I decided to set the tilting angle to ± 65°. Furthermore, I started the collection of the single tilt series with a 4k camera and the 2048 x 2048 resolution. This resulted in a huge data size of 4GB per tomogram, which was too large for further alignment of two respective tomograms. I therefore binned the resolution of the tomograms to 1024 x 1024 pixels and used the 2k camera, which reduced the size of one tomogram to 2GB. By comparing the same tomogram scanned with the 4k and 2k cameras I couldn´t detect any differences in the resolution and therefore decided to collect all tomograms with the 2k camera.

Additionally, I started to simplify and automatize the alignment of the collected tilt series by adding 18 nm gold particles randomly on both sides of the grids. As the 18 nm particles were too large for the ultrathin 100 nm slices and contaminated the regions of interest on the grids by large accumulations of single particles (data not shown), I reduced the size of the gold particles to 12 nm (Fig. 3, B). These were large enough to detect during the automatic alignment but small enough not to contaminate the single grids.

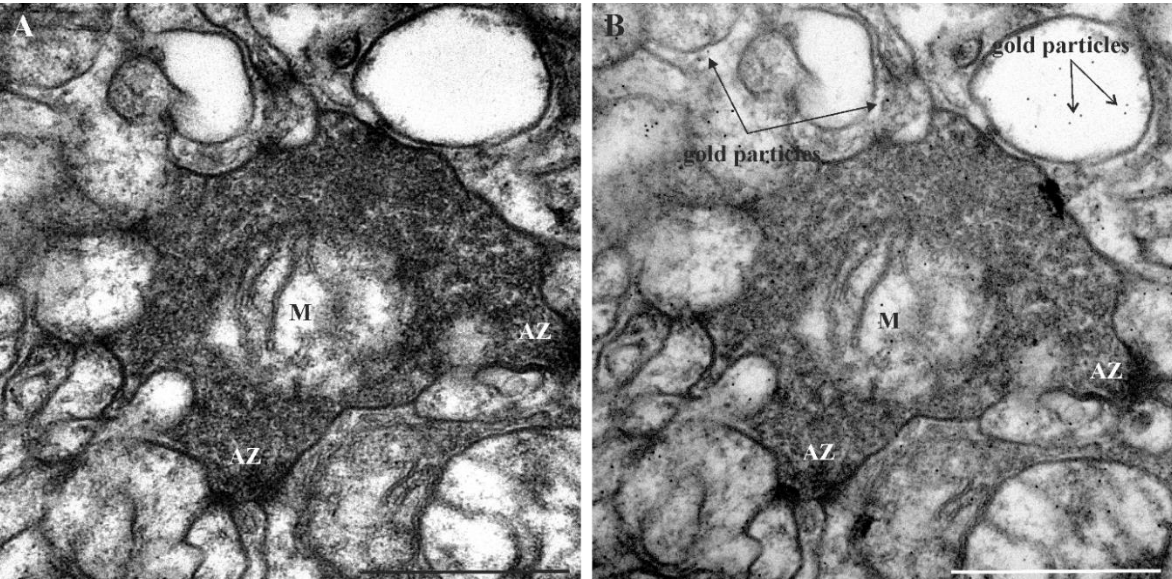


Figure 3: Virtual sections of the same presynaptic terminal without and with gold particles. (A) Virtual section of one projection neuron bouton without gold particles. (B) 12 nm gold particles were added unspecifically to both sides of the grid for better automatic alignment. AZ: active zones; M: mitochondrion. Scale bars: 500 nm.

Data Collection and Alignment

Collection of Double–Tilt Series

Before I started the data collection of the double–tilt series, the eucentricity of the specimen is automatically defined by adjusting the goniometer to the eucentric height. This adjustment places the tilt axis within the specimen plane and therefore minimizes the amount of refocusing during the collection of the tilt series (McEwen and Marko, 1998). Afterwards, the sample stage is tilted to one extreme of the maximal tilt range (in this study to ± 65°) and the region of interest is focused again. The TEM then automatically tilts the stage by the given incremental step (in this study 1°) and refocuses the selected region of each sampled image. The tilt series stops automatically, if 1) the opposite maximal tilt angle is achieved or if 2) the region of interest runs out of focus due to technical disorders of the electron microscope. In the latter case, the operator must manually recenter and refocus the region of interest on the sample and restart the tilt series from the last tilted image that was within the focus. The amount of recentering and –focusing depends on the adjustment of the eucentricity of the sample stage (McEwen and Marko, 1998). After collection of the first tomogram series, I manually removed the rotation holder from the TEM and rotated (by 90°) the grid by hand. Due to the shape of the slot grids (Fig. 1, A) I used the orientation of the slot to estimate the 90° with adequate accuracy. The rotation holder is then channeled into the TEM again and the same position must be identified to record the second tilt series. For simplification, I chose the region of interest e.g. near to the tissue cleft between KC somata and PN boutons.

Ultrastructure of Olfactory PN Axonal Boutons

The most prominent synaptic structures I could identify in the lip region of the MB calyx in *C. rufipes* are the boutons of PNs that are also well described for other eusocial insect species (Ganeshina and Menzel, 2001; Seid and Wehner, 2008, 2009; Groh et al., 2012). The PN boutons were easy to identify via their irregular shape and abundance of synaptic vesicles. In *C. rufipes* workers, they measure 1 to 1.5 µm in diameter (Table 1; mean ± SDE: 1.44 ± 0.08 µm; n=6) and are full of synaptic vesicles (Fig. 4 and 5). I calculated a volume of ~1.56

μm^3 of one PN bouton[1] by using the diameter of the respective PN bouton and the assumption that the PN are almost spherical. The synaptic vesicles were further distinguishable by their size and abundance: the clear–core vesicles are of regular shape and measured 39.36 ± 1.11 nm in diameter (Table 1; mean ± SDE; n=30) and of higher abundance than the dense–core vesicles (Fig. 5). The dense–core vesicles are of larger size (Table 1; diameter: 69.53 ± 3.06 nm; n=13) than the clear–core vesicles and are mostly found in close vicinity to the lipid membrane (Fig. 5).

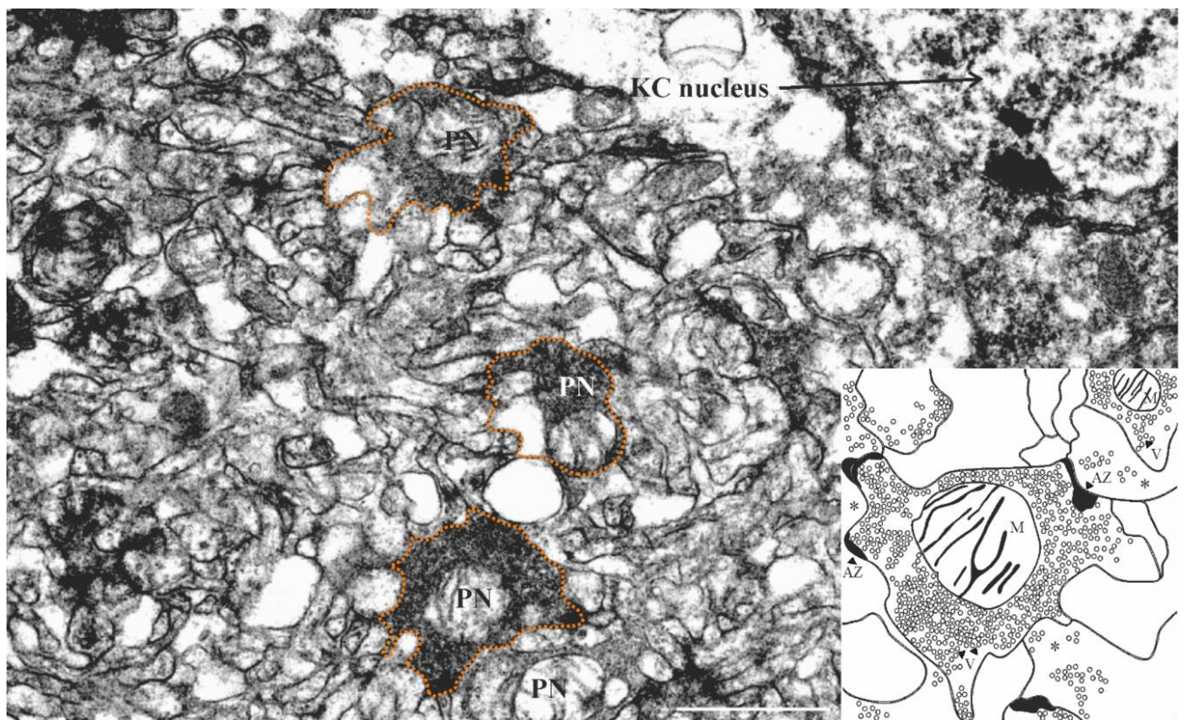


Figure 4: Synaptic architecture of projection neuron boutons in the olfactory lip. High magnification of the olfactory lip region in the mushroom body calyx with Kenyon cell (KC) nuclei and projection neuron (PN) boutons. PNs (boarders highlighted in orange) were readily identifiable by their size and the high packing density of vesicles (V) and bright mitochondria (M). Insert shows schematic drawing of one PN bouton with postsynaptic sides (P) and active zones (AZ); asterisks indicate postsynaptic partners. Scale bar: 1 µm.

Hence, the boutons contain at least one mitochondrion and putative synapses were identified via their electron dense staining along the lipid membrane of the PN boutons (Fig. 4 and 5). In one PN bouton I calculated 31 active zones that have contact to multiple (1 to 3 maximal 4) postsynaptic partners (Fig. 5, white asterisks) of KC dendritic spines (Farris et al., 2001;

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[1] Formula for calculation of the spherical volume: $V = \pi * d^3 * 1/6 = 1.44$ µm
with d: diameter

Ganeshina and Menzel, 2001; Groh et al., 2012). Thus, one PN bouton is surrounded by ~62 postsynaptic profiles. The dendrites of postsynaptic partners measured 0.16 ± 0.02 µm in diameter (Table 1; mean ± SDE; n=6).

Table 1: Size measurements of pre– and postsynaptic architectures.

Diameter PN [mean ± SDE]	PN volume	Diameter clear–core vesicles [mean ± SDE]	Diameter dense–core vesicles [mean ± SDE]	Diameter postsynaptic partners [mean ± SDE]
1.44 ± 0.08 µm (n=6)	1.56 µm^3 (n=1)	39.36 ± 1.11 nm (n=30)	69.53 ± 3.06 nm (n=13)	0.16 ± 0.02 µm (n=6)

Different size measurements are calculated within one projection neuron (PN) axonal bouton of the olfactory lip region. The PN diameter is measured at different depths in one PN bouton and is defined as the maximal distance between two points on the double lipid membrane. SDE: standard deviation error.

Sometimes presynaptic boutons were also in close vicinity to other PN boutons, therefore forming bouton–bouton synapses (Fig. 5, H). As the MG occupied different depths in the MB calyx, a single tomogram section contained a mixture of profile sizes (Fig. 4). To reveal the whole size of one presynaptic bouton, I identified and scanned the same presynaptic bouton on 16 consecutive tomogram samples of the MB olfactory lip region in one *C. rufipes* nurse ant (Fig. 5).

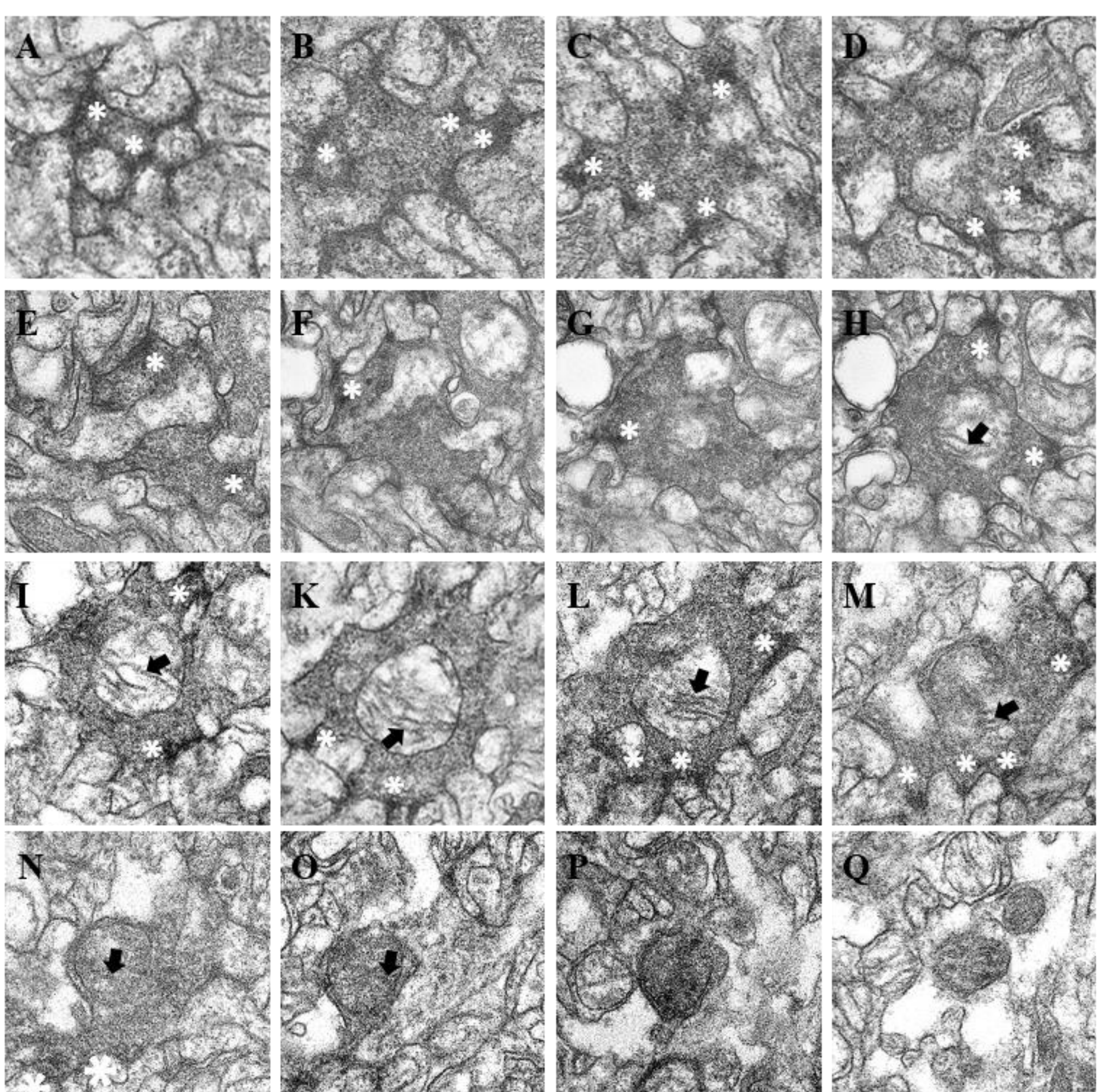

Figure 5: **Consecutive slices of one projection neuron (PN) bouton of the olfactory innervated lip region.** Virtual sections were taken until the selected PN was not visible (data not shown). Black arrows indicate mitochondria, white asterisks the active zones.

Alignment of the Single Tomograms

The most sufficient and time–saving possibility to align the tomograms is to use gold markers as fiducials. During the recording of the single–tilt series, the same gold beats are tracked, if possible in every image (McEwen and Marko, 1998). For alignment, both single–tilt axes are aligned independently, using the same gold particels as fiducial markers in both tilt series (Fig. 6; supplemental video material S1–S4 on attached CD). After alignment of the single tilt series, both series are aligned to each other, by using the 3D coordinates of the fiducial markers in each data set and merging them for every tomogram image independently (supplemental video material S5 on attached CD). A very detailed introduction for the alignment of the tomograms and for joining serial tomograms can be found on the IMOD homepage (*Tomography guide*: http://bio3d.colorado.edu/imod/doc/tomoguide.html and *guide for joining of serial tomograms*: http://bio3d.colorado.edu/imod/doc/tomojoin.html).

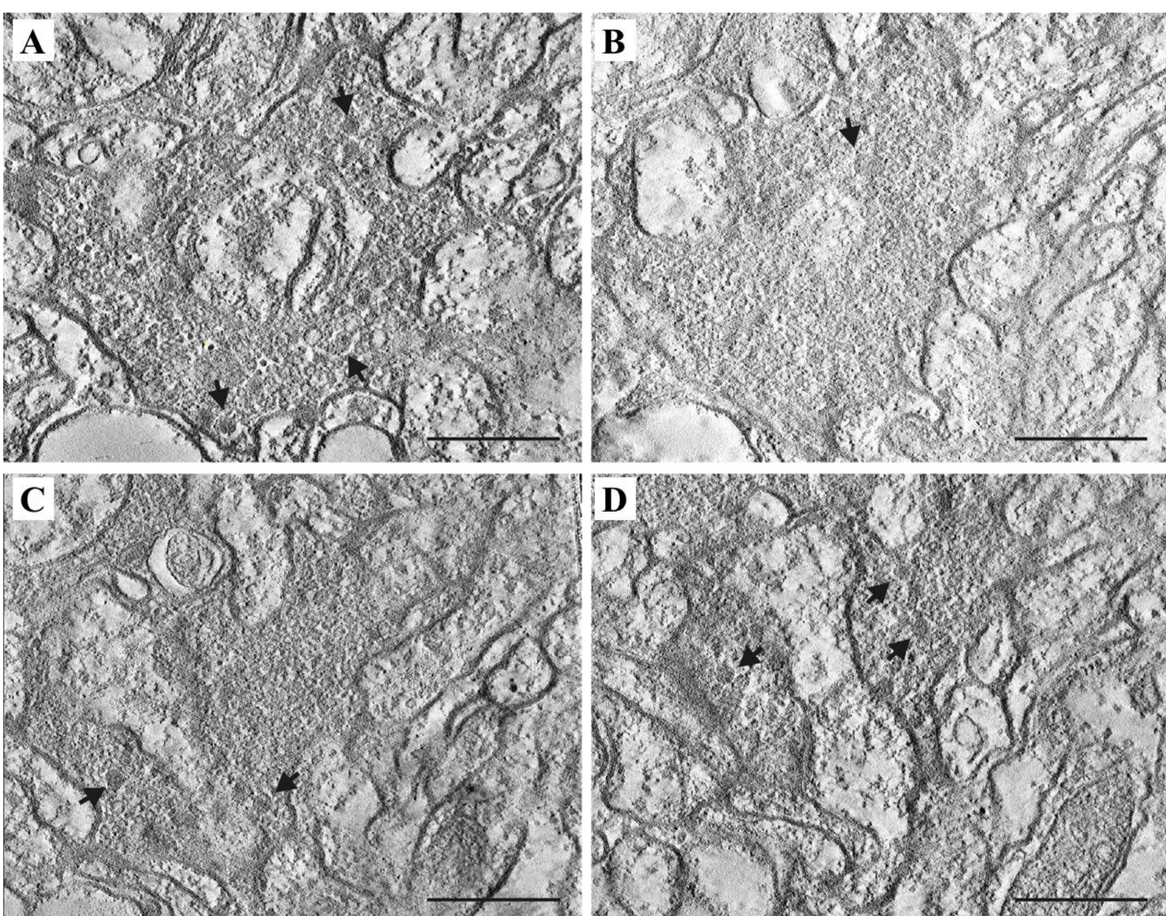

Figure 6: Sections of reconstructed tomograms of four consecutive samples with gold particles. A–D. Tomograms are in order of appearance starting with A. Arrows indicate dense–core vesicles. Scale bars: 500 nm.

Discussion

One main goal of my study is to implement ET to reveal a high optical resolution of presynaptic neuronal profiles in the MB calyces. High resolution of presynaptic components, like mitochondria, active zones and synaptic vesicles facilitates the understanding of architectural changes in neuronal plasticity process that occur with the interior–exterior transition of ant workers.

Advantages and Disadvantages of Electron Tomography

Hitherto, classical TEM was used in earlier studies to reveal the synaptic architecture of neuronal complexes in the insect brain and to associate neuronal maturation with division of labor (*Pheidole dentata*: Seid et al., 2005; *Cataglyphis albicans*: Seid and Wehner, 2008, 2009; *Apis mellifera*: Groh et al., 2012). Using TEM requires high resolution images of cells and tissues that are of interest at the nm–scale in x– and y– dimensions, but is limited in the resolution of the z–dimension. Even though this has the advantage to reveal details of the structure of interest at the ultrastructural level, it has at the same time the disadvantage that structural details from different depths get lost (reviewed in McEwen and Marko, 2001). Since the resolution in the z–dimension is limited in TEM, it would require averaging thousands of copies of a similar motif in one cell to gain a 3D image (reviewed in McEwen and Marko, 2001). As most cellular structures (like active zones and mitochondria) are of complex construction, they cannot be averaged as identical motifs. To deal with this, ET is the only computational 3D reconstruction technique, that is not based on averaging the same image for many times (reviewed in McEwen and Marko, 2001). High resolution image stacks using ET can be required by tilting samples incrementally around an axis vertical to the electron beam (reviewed in Baumeister, 2002). These images received with ET are represented as tomograms, 3D data blocks that are arranged as voxels. The voxels typically have a size of 1–4 nm and are represented with gray values. The grayscale represents the mass density on the corresponding position of the specimen (reviewed in McIntosh et al., 2005). As molecules are negligibly denser than the aqueous contents of the specimen, they interact stronger with the electrons of the electron beam, which is called \`scattering´ (reviewed in McIntosh et al., 2005).

In my study, I varied the tilting angle from –65° to 65° with 1° increment steps. This was shown to obtain the best resolution, as the angular range (± 60° to ± 70°) needs to be as wide as possible with increments (1–2°) as small as possible (reviewed in McEwen and Marko, 2001; Baumeister, 2002). Therefore, electron microscopes must be equipped with an accurate tilt stage and a specimen holder that can adjust the respective high tilting angle (reviewed in McEwen and Marko, 2001). Besides the tilting angle, also the accelerating voltage of the electron microscope is one important consideration to obtain high resolution images. In this study, I used an intermediate–voltage (200 kV) electron microscope, which fit the demands to gain high definition images (reviewed in McEwen and Marko, 2001): if the accelerating voltage is too low, the image quality degrades rapidly at high tilting angles, as at 60° tilting angle the path length of the electron beam is twice the specimen thickness and three times higher at 70°. Therefore, also the slice thickness of the respective specimen should be considered. Due to simple geometrical reasons, a 200 nm thin specimen (at 0° tilt) has a thickness of 540 nm at 70° tilt (Fig. 7, A). As scattering of electrons increases with high tilting angles, the quality of the tomograms and images decreases. Therefore, the quality and resolution of projection samples is decreased in too thick specimens (reviewed in Baumeister, 2002).

In my study, I started with a specimen thickness of 400 nm (data not shown) to reconstruct one individual PN axonal bouton in as few consecutive sections as possible. In the honeybee, PN axonal boutons have been described with a size of 1–2 µm in diameter (Groh et al., 2012). This would mean, that only 4–5 consecutive serial sections are needed in 400 nm thick specimens to cut through one entire PN bouton. Due to the above mentioned limitations of ET, I was not able to resolve high resolution images out of 400 nm thick samples. To calculate the resolution of the tomographic reconstruction, I used a formula[2] given by McEwen and Marko (1998). The resolution at 400 nm sample thickness results in 20.8 nm, with a tilt angle interval of 1° and a maximum tilt angle of 70°. This is about 7 times higher

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[2] $d = 0.035\ aT$ at $\theta_{max} = 60°$

$d = 0.052\ aT$ at $\theta_{max} = 70°$

d: resolution, a: angular interval (in degrees), T: section thickness, θ_{max}: maximal tilting angle

than the resolution that can be achieved with ET (reviewed in McEwen and Marko, 2001). As a next step, I decreased the sample thickness to 250 nm. Using the formula of McEwen and Marko (1998), this resulted in a resolution of 8.7 nm with 1° incremental steps with a maximal tilting angle of 60°. Even though this would have been in the range which is possible to resolve with an intermediate–voltage microscope, the optical resolution was still too low to identify and visualize single vesicles and active zones in the PN axonal bouton of the *C. rufipes* worker (Fig. 2, A+A´). Due to the size of one PN axonal bouton in the honeybee (Groh et al., 2012), this would have meant ~10 serial sections through one entire bouton. Finally, I chose a specimen thickness of 100 nm, as this was thin enough to resolve single vesicles in the MG presynapse but still feasible for consecutive slicing of ~16–18 serial sections. The calculated optical resolution of the ultrathin slices (100 nm) is 3.1 nm with 1° incremental steps and a viewing angle of ± 65° (McEwen and Marko, 1998).

One advantage of the ET is at the same time one disadvantage: due to the tilting of the tomogram samples, the effective electron beam path is increasing rapidly for high tilt angles above 60° (McEwen and Marko, 1998). Therefore, it is nearly impossible to resolve high resolution images from specimens above a tilt angle of 70°. Therefore, most tomogram samples are lacking data between the maximal range of the tilt angle and ± 90° (Fig. 7, B). This problem can be solved by rotating the tomogram sample by 90° and the subsequent collection of a second tilt series (`double tilt series´). This dual axis approach reduces the missing angular range to a minimum and therefore produces a more isotropic optical resolution (Fig. 7, C; Penczek et al., 1995; Mastronarde, 1997). In this study, I could, altogether, scan 16 consecutive tomogram samples with this dual axis approach, resulting in 32 single tomograms of one PN axonal bouton in a *C. rufipes* worker.

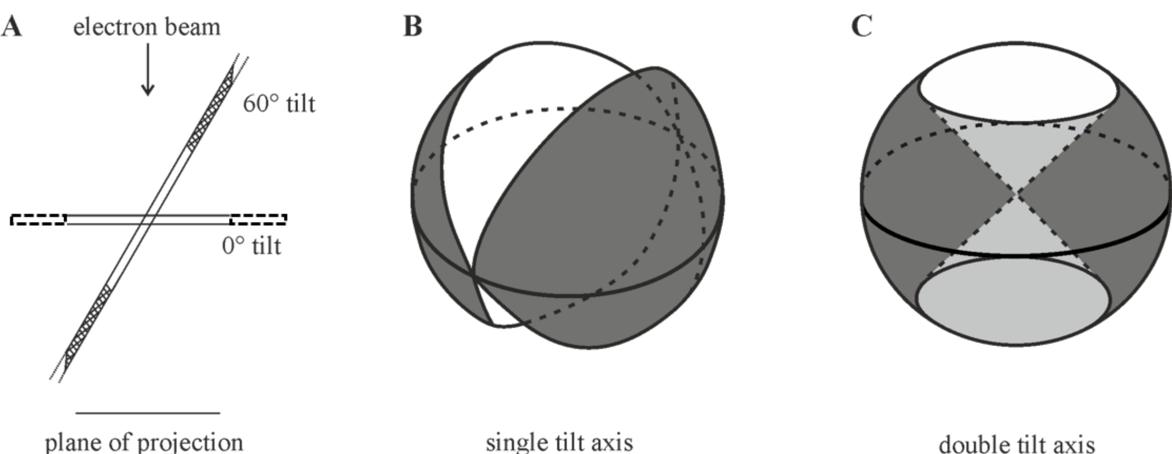

Figure 7: Illustration of the advantages and disadvantages of electron tomography. (A) Solid lines represent the region of interest that is within the viewing area at the respective tilt angle. Hatched area of the specimen depicts material that is within the viewing area at 60° tilt but not at 0°. This area is small since the tilt angle is <60° and the length of the viewing area is five times higher at 60°. (B) Single tilt axis; unshaded (white) area indicates the angular range that is inaccessible due to the geometry of the specimen. (C) Double tilt axis; missing angular information is decreased because the not tilted area is smaller and symmetrically compared with the single tilt axis. Modified after McEwen and Marko, 1998.

After successful tilting of the specimens, the required information needs to be translationally and rotationally aligned to register them for computation of the final 3D–reconstruction. Here, I simplified the 3D alignment of four consecutive tomogram samples by using gold particles as fiducial markers. The colloidal gold beats are placed on both surfaces of the grids before collection of the tilt series, as they can be well localized at random positions on the grids (first described by Fung et al., 1996; Ress et al., 1999). These gold markers can be quite easily found on the tomograms and mostly be tracked throughout the whole image series (Brandt et al., 2001). During the automatic alignment, the 3D position of the gold markers on each tilted image are used to calculate shift vectors and rotations of the respective tilt image that are attributable to the varying tilting angle (Lawrence, 1992; Penczek et al., 1995; Mastronarde, 1997). The size of the used gold beats is dependent of the slice thickness and the used magnification. In general, the lower the magnification and the higher the section thickness, the larger must be the size of the gold markers. For example, at 8.000 – 12.000 x in 250 nm thin sections, the size of 25 nm gold particles is optimal to gain a high quality automatically aligned tomogram (reviewed in McEwen and Marko, 2001). In this study, I therefore started with 18 nm gold beats at the 400 nm sections at 20.000 x and decrease the size of the gold particles to 12 nm in the 100 nm sections at 20.0000 x.

Improvement of the Optical Resolution of Single Tomograms

However, in this study I used a well–established fixation protocol for TEM in the honeybee (Groh et al., 2012), that is based on chemical fixation (Karnovsky fixation in this study), plastic embedding of the respective specimens (Epon in this study) and contrast with heavy metals (Osmium in this study). Nevertheless, the above mentioned methodological approaches implicate some disadvantages for the usage with ET, as they decrease the optical resolution of the tilted images by fixation of artefacts (reviewed in McIntosh et al., 2005): chemical fixation is a slow process resulting in major ultrastructural alterations and staining with heavy metals indeed provides a good contrast but produces a complicated mixture of positive and negative stainings. Additionally, before embedding samples in plastic reagents, they need to be dehydrated and exposed to organic solvents (reviewed in Baumeister, 2002). Even though I could resolve the architecture of single synaptic vesicles, the optical resolution of my tomogram samples still can be improved (for example for 3D reconstruction of the active zones or to visualize the docking of synaptic vesicles at their releasing sites). To deal with this, ET samples are prepared for ultrarapid freezing, by dipping the specimen into liquid ethane. This results in a decrease of their temperature at a rate of 10^4 °Cs^{-1} (Grimm et al., 1998; Ladinsky et al., 1999). With this, living cells are represented accurately as the specimens are frozen within milliseconds and the cells may grow and divide when warmed rapidly up to physiological tolerable temperatures (McIntosh, 2001) during the ET procedure.

One major problem of ultrarapid freezing of biological samples is to avoid damage by ice crystals. The aqueous contents within the specimen will crystallize extremely well, if the temperature is low enough. Consequently, the cytoplasm of cells is destroyed by the expansion that accompanies the freezing of specimens (reviewed in McIntosh et al., 2005). A lot of work was done in the last years to solve this problem. Up to date, the most commonly used method is the high–pressure freezing, where high hydrostatic pressure works as physical cryoprotectant as water expands when it freezes. If the pressure is increased rapidly and the specimen is immediately frozen afterwards, many frozen cells remain in their *status quo* and will not be destroyed through ice crystals. With the high–pressure freezing, samples that are at minimum 200 µm in their diameter can be frozen without ice crystals (Markert et

al., 2017). It was shown that brains of the desert ant *Cataglyphis fortis*, after trimming and cutting them into 90 µm thick sections, fit into the platelets for the high–pressure device (Markert et al., 2017). As this slice thickness is thick enough to contain the whole structure of one PN axonal bouton (1–2 µm in diameter), I would use the `high–pressure freezing´ method to improve the resolution of single PN axonal boutons. With this methodological approach, it is maybe possible, to resolve the ultrastructure of single active zones and potentially also the docking of vesicles at their releasing sites, as this method contains the cells in their *status quo*.

Ultrastructural Profile of Microglomeruli in the Mushroom Body Calyces

In my study, I could visualize the prominent extrinsic neuronal profiles of the sensory PNs due to their size, amount and shape. In the center of the presynaptic sites I could identify at least one mitochondrion, surrounded by a high amount of both, clear– and dense–core vesicles. The calculated PN volume of 1.56 µm^3 is lower than earlier findings in *Cataglyphis fortis* (Bauer, 2015) and *Cataglyphis albicans* (Seid and Wehner, 2008) desert ants, where the mean bouton volume ranged between 2.24 µm^3 and 2.7 µm^3 in the lip region. Also, the PN bouton volumes in the lip region of adult honeybees (Groh et al., 2012) and the fruit fly *Drosophila* average around 4.7 µm^3 (Butcher et al., 2012). This high variation indicates the high variety of the bouton size in different insect species (Seid and Wehner, 2008). The large variety between the calculated volume of PN in *C. rufipes* workers and earlier findings in other insect species is mainly given by the fact, that my study assumes the PN boutons as spherical shapes and estimates their volume, whereas earlier studies reconstructed whole PN boutons and, thus, calculated the volume more accurately. Moreover, I could identify a high number of clear–core vesicles and to a lesser extent dense–core vesicles within one presynaptic profile. The clear–core vesicles are of smaller size (~40 nm) than the dense–core vesicles (~70 nm), which is comparable to size measurements conducted in *Pheidole dentata* ants (clear–core vesicles: 10 to 60 nm; dense–core vesicles: 40 to 65 nm; Seid et al., 2005). The presence of dense–core vesicles in one bouton indicates the contribution of peptidergic and aminergic elements (Watson and Schürmann, 2002), as they may contain biogenic amines (like octopamine, dopamine, and serotonine) or neuropeptides (e.g. FMRFamide) has been described in the honeybee (Schürmann et al., 1989; Kreissl et al., 1994; Blenau et

al., 1999). As most of the dense–core vesicles are located in close vicinity to the lipid membrane in my study, this could support the hypothesis, that dense–core vesicles do not release their neuronal modulators directly at the synaptic contacts and may modulate synaptic transmission (De Camilli and Jahn, 1990; Marder and Thirumalai, 2002). They further are potentially involved in neuromodulatory processes of visual and olfactory stimuli within the MG. To prove this hypothesis, further studies are needed to identify their contents. Of particular interest in neuroscience studies is the question of how synaptic vesicles are recruited, produced and directed towards their releasing sites.

However, the releasing sites of vesicles are termed active zones and are easily identifiable by their high electron density (Fig. 4 + 5). The estimated number of ~31 active zones in one PN axonal bouton in my study is comparable with the amount of ~40 active zones in the fruit fly (Butcher et al., 2012) and ~24 in the desert ant *Cataglyphis fortis* (Bauer, 2015). Compared to the amount of active zones (~70) in the honeybee (Groh et al., 2012), the estimated number seems to be very low. This could be explained by the fact that honeybees are highly olfactory guided, whereas most ant species rely on visual cues. I therefore hypothesize, that the number of active zones in the visually innervated collar region of the MB calyces of *C. rufipes* workers is higher than the amount of active zones in the lip and more comparable to the amount measured in honeybees. The active zones in my study could be identified as non–ribbon synapses, which was also confirmed in earlier studies (*Apis mellifera*: Groh et al., 2012; *Cataglyphis albicans*: Seid and Wehner, 2008; *Cataglyphis fortis*: Bauer, 2015; *Drosophila melanogaster*: Butcher et al., 2012). In honeybee (Groh et al., 2012), *Cataglyphis fortis* (Bauer, 2015), and in the fruit fly (Butcher et al., 2012) in addition ribbon synapses could be detected. The lack of ribbon synapses in this study could be due to the low optical resolution of the active zones in the aligned tomograms. Ribbon synapses are described as being highly accumulated with calcium channels, whereas non–ribbon synapses constitute a more nonfunctional state and become activated by calcium entering the presynaptic site (goldfish: Midorikawa et al., 2007). An alternative explanation may be the existence of two different stages of the synapse that either lost their ribbons or have not established one (Groh et al., 2012). However, just one protein that is involved in the release of neurotransmitters at the active zones has been identified in insects by Fouquet and colleagues in (2009). They identified Bruchpilot (BRP), which belongs to the ERC/Cast

family (Cast= cytomatrix at the active zone associated structural protein, ERC= ELKS–Rab6–interacting protein CAST; Wagh et al., 2006) and involved in the construction of T–bars in *Drosophila* neuromuscular junctions (Kittel, 2006; Wagh et al., 2006). The reduction of BRP levels in *Drosophila* leads to a decreasing rate of neurotransmitters and a wrong localization of Ca^{2+} channels (Kittel, 2006; Wagh et al., 2006; Fouquet et al., 2009). This evidence suggests that BRP is involved in directing synaptic vesicles towards the active zone and also the regulation of transmitter release (Kittel, 2006; Fouquet et al., 2009; Hallermann et al., 2010). Recent studies also identified and localized BRP in the honeybee brain and determined age–related changes of BRP in the MG of the MB calyces (Gehring et al., 2017). They could show, that the amount of BRP increased during the first weeks of an adult life in the MB collar region, but not in the lip. As olfactory neuropils are not fully developed in freshly emerged bees, this might also be accountable for the missing increase of BRP in the MB lip region (Masson and Arnold, 1984; Gascuel and Masson, 1991; Wang et al., 2005).

However, in this study the axonal endings of the sensory PNs could be further identified by the surrounding postsynaptic profiles of the KC dendritic spines (Fig. 5). I could estimate ~2 postsynaptic partners per active zones, resulting in an amount of ~62 postsynaptic partners of one PN bouton. Compared with the amount of postsynaptic partners in the honeybee (~140; Groh et al., 2012) and *Drosophila* (~220; Butcher et al., 2012) the number of postsynaptic partners in *C. rufipes* is comparatively low, but more than two times higher than in *Cataglyphis fortis* (~27; Bauer, 2015). Also the measured mean diameter of the KC dendritic spines (~1.45 µm) is within the range of the size of postsynaptic partners measured in *Pheidole* ants (0.1 to 0.3 µm; Seid et al., 2005). In my study, most presynaptic densities are in contact with two to three, maximal four postsynaptic partners. These structures are termed dyads (two postsynaptic partners) or triads (three postsynaptic partners) and were also found in desert ant *Cataglyphis albicans* and the honeybee *Apis mellifera* (Seid and Wehner, 2008; Groh et al., 2012). This diversity is given due to the fact, that neurogenesis is absent in the adult insect brain (e.g. Fahrbach et al., 1995) and the amount of KCs remains constant during aging (*Camponotus floridanus*: Gronenberg et al., 1996). Therefore, more synaptic contacts between pre– and postsynaptic profiles can exist. Differences in the amount of postsynaptic partners when comparing nurses and foragers are mainly attributed to the outgrowth of KC dendrites (*Apis mellifera*: Farris et al., 2001; Groh et al., 2012). KC

dendritic spines are hypothesized to play a tremendous role in neuronal plasticity processes that occur with division of labor and the prominent switch from nursing to foraging.

Age– and Task–related Plasticity at the Ultrastructural Level

One main goal of my study is the implementation of ET in *C. rufipes* workers. This facilitates high resolution images of synaptic architectures at the ultrastructural level and promotes the understanding of architectural changes in neuronal plasticity. Using this methodological approach, one could investigate possible changes at the ultrastructural level of the PNs of the MB calyx lip and collar region that might be accompanied by division of labor and the behavioral maturation from nursing to foraging in *Camponotus* ants. An age– and experience related plasticity of the nervous system was earlier shown for a broad range of insect species (reviewed in Meinertzhagen, 2001; Fahrbach, 2006), like the honeybee, *Apis mellifera*, where foraging activity was accompanied by vast volumetrically increase of the MB neuropil (Withers et al., 1993; Durst et al., 1994; Fahrbach et al., 1998; Ismail et al., 2006). Furthermore, freshly emerged workers had significantly less synapses, vesicles per synapse and smaller PN boutons than foragers in the MB calyx (Seid et al., 2005), which indicates that this increase in numbers of neuronal architectures from nurse to forager is also accompanied by an increase of the behavioral repertoire (Seid and Traniello, 2006) suggesting the occurrence of axonal pruning. The underlying developmental program of axonal pruning eliminates the axonal outgrowth during maturation processes, which facilitates the proper neuronal wiring and development of brain neuropils (Oland et al., 1999; Raff et al., 2002; Bagri et al., 2003; Awasaki and Ito, 2004; Bishop et al., 2004; Broadie, 2004; Watts et al., 2004). As synaptic pruning occurs in the MB collar region in dark–kept ants that were exposed to light for several days (Stieb et al., 2010, 2012; Yilmaz et al., 2016), pruning processes are maybe the basic mechanism for adapting and preparing the neuronal system for the external environment (Kantor and Kolodkin, 2003; Seid et al., 2005). Recent studies could demonstrate that glial processes mediate this axonal pruning process (Raff et al., 2002; Watts et al., 2004) and are crucial for the maintenance and development of the nervous system (Raff et al., 2002; Awasaki and Ito, 2004; Watts et al., 2004), as well as for strengthening of synaptic connections and removal of toxic components and/ or neurotransmitters (Ventura and Harris, 1999; Slezak and Pfrieger, 2003; Ullian et al., 2004).

In conclusion, this study provides first insight into the complex olfactory neuronal system in the lip neuropil at the ultrastructural level of *Camponotus rufipes* ants. Using ET with consecutive brain slices, I could improve the optical resolution of the architecture of the presynaptic profile of PN terminals remarkably compared to earlier studies in the honeybee (Groh et al., 2012). This method could further be used for a detailed comparison of different age–matched light–exposed or –deprived nurses and foragers of *C. rufipes* to answer the following questions: 1) do worker ants express task–related neuronal plasticity in the MB calyces at the ultrastructural level? 2) Do age–matched individual workers differ in their synaptic architecture (number of vesicles, synapses, postsynaptic partners)? And 3) does the exposure to light for several days lead to a decrease of PN bouton volumes and less vesicles and active zones per PN axonal bouton? Furthermore, by using high–pressure freezing instead of plastic embedded and dehydrated specimens, one could further investigate the actual state of single PN boutons to eventually visualize the docking of synaptic vesicles at their releasing sites.

General Discussion

The experiments conducted in my doctoral research focused on *Camponotus* ants to study behavioral and neurobiological processes within subcolonies and on the individual worker level. By introducing new behavioral approaches, my study provides first insights into the complex behavioral transition from interior to exterior tasks in *C. rufipes* workers. Further neuroanatomical studies revealed that the observed behavioral transition is accompanied by vast volumetrically and synaptic structural changes in higher order brain centers in this species.

I could demonstrate for the first time that *C. rufipes* workers undergo an age– as well as size–related division of labor. Young workers stay inside the dark nest performing nursing tasks first, before they switch to foraging activities at an age of 2–3 weeks. This age–related behavioral switch is further performed by different morphs of the worker caste: while nursing tasks are performed by nearly all workers, mostly media–sized workers switch to foraging duties. As most of the observed foragers are nocturnal with elevated diurnal activity of some individuals, my results further show that the nursing–foraging transition needs precise timing and therefore a proper functional endogenous clock. My study further provides first behavioral results from the natural nesting site (La Coronilla, Uruguay) of these ant species. Additionally, I also measured the foraging activity of the sympatrically living species *C. mus*. My experiments reveal that both *Camponotus* species indeed share comparable environmental conditions, but adapted differently to the local day–night cycle and the ambient temperature. I clearly demonstrate, that workers of *C. mus* are solely diurnal without any foraging activity below 15°C. Interestingly, foragers of *C. rufipes* were predominantly nightactive under a broader temperature range from 9 to 34.5°C. Even though *C. mus* workers don´t display any foraging activity below 15°C in the field, I could demonstrate that both species indeed share the same thermal tolerance values in the laboratory. I therefore hypothesize that temperature and light are the two factors mostly responsible of the daily and seasonal timing of activity rhythms.

The environmental requirements a single worker needs to process, undergo a vast change from complete darkness in the nest side to a visual–enriched environment. The gained visual information (mainly light input) is than further processed in the ants´ brain, mainly by the optic lobes and mushroom bodies. Hence, my experiments focused on cellular changes of both, as well as on subcellular changes of the mushroom body neuropils. My results clearly suggest that the volume of the optic lobes and mushroom bodies, as well as the density of synaptic complexes of the mushroom bodies are affected by both, age and light. I therefore hypothesize that `experience–independent´ and `experience–dependent´ neuronal processes are responsible for the plasticity of the visual system. To date, the underlying neuronal mechanisms that are responsible for the structural remodeling of synaptic architectures are poorly understood. My thesis therefore started to implement the Electron Tomography, which facilitates high resolution images of synaptic architectures at the ultrastructural level and promotes the understanding of synaptic architectures that are involved in neuronal plasticity. With this methodological approach I am able to resolve single vesicles in the PN cytoplasm and to provide a first insight into the complex pre– and postsynaptic architecture of microglomeruli in the mushroom body calyces.

This thesis provides the first comparative study of behavioral and neuronal maturation in *Camponotus* workers, and gives first insights into the complex and flexible behavioral transition and the underlying neuronal plasticity.

Timing of Behavioral Tasks in *Camponotus* ants

Since little was known about the colony structure of *C. rufipes* when I started out my studies, the first part of my PhD thesis was to investigate the timing of the behavioral transition from nursing to foraging duties. In *C. rufipes,* young workers stay inside the nest and are nurtured by older ones during the first 48 hours after emergence, before they start to perform brood care themselves. At an age of two to three weeks, ~53% switch to foraging tasks. This observed age at the onset of foraging is mostly coherent with earlier studies in various ant species (*Messor* and *Myrmica*: Ehrhardt, 1931; *Pheidole dentata*: Muscedere et al., 2009, 2013; *Platythyrea*: Bernadou et al., 2015; *Pogonomyrmex*: Gordon, 1989; Holbrook et al., 2013; *Solenopsis invicta*: Mirenda and Vinson, 1981; *Temnothorax albipennis*: Dornhaus, 2008). Additionally, the *C. rufipes* workers, not undergoing the transition remained nurses throughout their lives. A similar phenomenon, were single individuals show different task frequencies, was described in several ponerine ants: workers either undergo the transition from nursing to foraging or remain nurses and never switch to exterior tasks (*Odontomachus*: Dejean and Lachaud, 1991; *Diacamma*: Nakata, 1995; *Amblypone*: Masuko, 1996). If individual workers switch from nursing to foraging, the transition is correlated with the ants´ body size: foraging activities are mainly performed by media–sized workers, whereas almost all worker morphs are engaged in nursing. This is in line with previous studies in different ant species, where minor and media–sized workers perform mainly foraging tasks (*Solenopsis*: Wilson, 1978; *Orectognathus:* Carlin, 1981; *Camponotus rufipes*: Jaffé and Sanchez, 1984; Soares et al., 2008). One reason of mainly media–sized workers engaged in foraging tasks might be that they are numerous and can run faster and that the loss of minor workers is less affecting the ant colony (Detrain and Pasteels, 1991).

However, colony demands vary over time and can change with colony size. With that the need for specialized tasks like nursing, foraging, nest maintenance and waste management varies (Oster and Wilson, 1978; Mailleux et al., 2003; Thomas and Elgar, 2003). To match the required needs, the number of workers engaged in specific tasks like nursing and foraging fluctuates in adaptation to the given circumstances. Foragers can switch back to nursing tasks if the amount of brood and therefore the demand for brood care increases or if there are too little nurses present (Ehrhardt, 1931; Weir, 1958; McDonald and Topoff, 1985; Symonowicz et al., 2015). Similarly, nurses can start their foraging activity precociously, if the number of foragers present in the colony is insufficient (Lenoir, 1979; Sorensen et al., 1984; McDonald

and Topoff, 1985). In the present study, however, the transition from nursing to foraging might have been prevented by an extended longevity of foraging ants. Under natural conditions, foraging ants are often extremely short–lived since they experience higher soil temperatures and desiccation, and a higher predation pressure compared to intranidal workers (Kwapich and Tschinkel, 2015). Since these factors did not apply to the laboratory experiments, the transition rates from nurse to foragers might have been artificially low as forager numbers were unnaturally high (Porter and Jorgensen, 1981; Gordon and Hölldobler, 1987; Oettler and Johnson, 2009).

Besides different internal colony effects that influence the transition from indoor to outdoor worker, also daily and seasonally changing environmental factors can affect the timing of the forager transition. In subtropical regions, where *C. rufipes* and the sympatrically species *C. mus* populate, the most dominantly changing environmental cues over the day are temperature and light. In addition, while an approximate 12 hour day 12 hour night cycle remains constant throughout the year, temperature minima and maxima underlie seasonal changes. Therefore, I examined how daily light and temperature cycles affect foraging and locomotor activity rhythms under natural conditions in the field during local spring time. I further could replicate these findings under artificial conditions and, moreover, studied how seasonal temperature changes might influence the locomotor activity rhythms of both ant species. Both, field and laboratory studies clearly demonstrate that *C. mus* ants are solely dayactive, irrespective of the seasonal conditions, whereas workers of *C. rufipes* ants showed an almost predominant nightactivity under the thermal summer regime and a pronounced diurnal activity under the thermal winter regime. I therefore speculate, that the two different activity profiles I could observe in the field and the laboratory of *C. rufipes* might be a seasonal adaptation to the more moderate temperature value of the respective season. This was described previously for several other *Camponotus* species (Briese and Macauley, 1980; Cros et al., 1997). Moreover, thermal tolerance values are able to shape foraging activity (Bishop et al., 2017), heat tolerant species (*C. mus* in my study) are mainly diurnal regardless of the season, whereas heat averse species (*C. rufipes* in my study) switch their activity period throughout the year to tolerable temperatures (Cerdá et al., 1998). I therefore hypothesize that *C. rufipes* favors nocturnal foraging to avoid thermal stress. To cope with high temperatures during foraging trips, ants have evolved some physiological adaptations to heat. Desert ants of the species *Cataglyphis bombycina* are known to forage at extremely high temperatures. Therefore their bodies are covered with metallic shimmering hairs that

help decrease the body temperature below the critical temperature level by enhancing optical reflection and dissipation (Shi et al., 2015). The bodies of the invariably day–active *C. mus* workers are covered in metallic shimmering golden hairs that might serve as a thermoregulatory solution. As a result, *C. mus* workers might be able to keep their body temperature below the critical thermal limit when they perform foraging trips during the day at local summer time. Contrary, bodies of *C. rufipes* workers are mostly lacking any hair and are of darker color than *C. mus*. I therefore hypothesize that *C. rufipes* ants prefer foraging at cooler temperatures because their bodies are more likely to warm up at higher temperatures than *C. mus* and, thus, they are more likely to desiccate.

The switch of individual workers from interior to exterior duties requires a drastic transformation of the behavioral repertoire and is further accompanied by immense changes of the sensory environment. Light is completely absent in the dark nest of *Camponotus* ants and is therefore one of the most prominent factors that changes completely when individuals start to forage. These drastic changes of the behavioral repertoire and the environment are most likely reflected in adaptive changes in brain structures associated with the control of behavior and processing of environmental stimuli. Therefore, I investigated how the factors age and light both affect visual brain neuropils at a cellular and subcellular level.

Synaptic Plasticity within the Visual System of *Camponotus rufipes*

Visual Input to the Mushroom Body Calyces

The visual information, that ant workers receive when they start to forage, is mainly processed by visual processing brain centers, mainly the optic lobes (OL) as primary visual brain neuropil and the mushroom bodies (MB) as secondary visual brain neuropil. As nothing is known, however, about the interconnection of both brain neuropils in *C. rufipes* workers, I started out by using anterograde stainings of the visual system how these two neuropils are interconnected. The OL and especially the medulla possess crucial neuronal connections to the MB calyces via the anterior superior optical tract (asot). The asot projects bilaterally through the protocerebrum to the ipsi– and contralateral collar (CO) of the ipsi– and contralateral medial and lateral calyces. The asot then terminates in the contralateral brain hemisphere in the medulla (ME) of the OLs. This was also shown for other ant species and the honeybee (Gronenberg, 1999; Ehmer and Gronenberg, 2002, 2004). Contrary to other studies, I could not show any connection between the lobula (LO) and the MB calyces, or other tracts running from the ME to the MB (Ehmer and Gronenberg, 2002, 2004). Although I cannot completely exclude connections from the LO to the MB with the methods used in my study, these differences of the visual system might be an adaptation to the different lifestyle of various Hymenoptera (Gronenberg, 2001; Ehmer and Gronenberg, 2004). Double staining of the dorsal and ventral part of the ME revealed that neurons originating from the anterior ME terminate predominantly in the posterior part of the CO, whereas neurons from the posterior ME project into the inner part (`dorso–ventral´ layering). This specific layering was also found in other ant species (Gronenberg, 2001) and bumblebees (Paulk and Gronenberg, 2008; Paulk et al., 2009).

Besides the asot, also other neurons originating from the ME and LO project into subordinate visual integration centers in the brain. In *C. rufipes,* my tracer applications into the OLs further reveal the presence of the posterior optic commissure (POC) and the anterior optic tract (AOT). The existence of the POC was further confirmed for honeybees (Hertel and Maronde, 1987; Hertel et al., 1987), where it contains ~200 thin, parallelly arranged neurons that interconnect the MEs of both OLs. Its axons terminate in the dorsoproximal part of the ME with the respective somata located in front of the LO (Hertel et al., 1987). Early studies revealed, that the main function of POC neurons is the analysis of intensity and/ or spectral patterns (Hertel et al., 1987). The AOT, which was also labeled in *Cataglyphis* ants (Schmitt

et al., 2016) and the honeybee (Mota et al., 2011), connects the OL with the anterior optic tubercle (AOTU) and is involved in sky–compass navigation and the detection of polarized light (Pfeiffer et al., 2005; Kinoshita et al., 2007). The staining of the *C. rufipes* visual pathway in the present study revealed a double layering of the AOTU, innervated by ME and LO neurons separately. A similar innervation pattern, where the AOT comprises a mixed innervation pattern of ME and LO neurons was found in the honeybee (Mota et al., 2011), butterflies (Strausfeld and Blest, 1970), flies (Strausfeld and Nässel, 1981) and locusts (Homberg et al., 2003). In the honeybee projections from the MBs into the AOTU could be labeled. Therefore the AOTU might be involved in higher–ordered, cognitive processing (Rybak and Menzel, 1993) of visual information. Even though *C. rufipes* is a mostly nocturnal species with the ability to switch to diurnal activities (Lindenberg et al., *in prep*), the rich diversity of visual projections in both brain hemispheres indicates a prominent role of visual cues.

Synaptic Plasticity within Visual Brain Neuropils Related to Age and Light Exposure

One novel and important aspect of my PhD work was the combination of volumetric measurements and cellular processes underlying neuronal plasticity. With this I was able to reveal volume–dependent and –independent neuronal processes separately during adult maturation of *C. rufipes* ant workers. During the first week of adulthood, the OL and MB calyx volume showed a remarkable volume increase, followed by a decrease of the OL volume. This supports the idea that different brain neuropils are not affected in the same way by age as it was suggested for *C. floridanus* (Gronenberg et al., 1996). Volume increase of the LA, ME and LO could be explained by changes in size and number of neuronal endings of photoreceptor neurons (*Drosophila melanogaster*: Heisenberg et al., 1995; Barth et al., 1997). Besides the initial MB volume increase, also the total number of synaptic complexes in the MB increased significantly in the collar and lip region. Further studies conducted in honeybee foragers suggests, that this enormous intrinsic volumetrical outgrowth of the MB input region is mainly attributed to the branching of the KC dendritic network (Farris et al., 2001). The initial volume increase under DD of primary and secondary visual brain centers likely reflects internal processes that occur without external input (Withers et al., 1995; Fahrbach et al., 1998). This \`experience–independent´ plasticity was further shown in naturally kept honeybee colonies (Farris et al., 2001; Ismail et al., 2006; Muenz et al., 2015),

in bees prevented from foraging (Withers et al., 1995) and in bees as well as ants deprived from visual experience (Fahrbach et al., 1998; *Cataglyphis bicolor*: Kühn–Bühlmann and Wehner, 2006). On a behavioral level, workers of *Camponotus rufipes* ants were shown to switch from indoor nursing tasks to external foraging activities with an age of up to two weeks (own data in **chapter I**). In honeybees, this behavioral period was associated with an increased tendency for first short orientation flight in close vicinity to the hive (Capaldi et al., 2000). During this age, the OL and MB volume as well as the total amount of MG remained constant in *C. rufipes*. This contrasts with earlier studies performed in the honeybee, were the total number of MG starts to decrease while the MB calyx volume continues growing (Muenz et al., 2015). This missing decrease of synaptic complexes, which was mainly attributed to pruning of PN boutons (Seid and Wehner, 2009) and outgrowth of KC dendrites, might be caused by the lack of foraging experience of *C. rufipes* workers in my study (*Apis mellifera*: Farris et al., 2001; Ismail et al., 2006; *Cataglyphis bicolor*: Kühn–Bühlmann and Wehner, 2006). As in *C. rufipes*, the behavioral switch from nursing to foraging occurs approximately at the same age as the observed neuronal plasticity of the central visual nervous system, this most likely reflects the `experience–independent´ neuronal plasticity at the beginning of adulthood.

Moreover, I simulate the transition from nursing to foraging in *C. rufipes* workers. Therefore, 28 day old workers are exposed to light stimuli for 1, 4 or 14 days. With this, I could demonstrate the `experience–dependent´ neuronal process, where exposure to light for four days seems to be the main triggering factor for pruning of synaptic complexes. This process of synaptic pruning in the visually MB collar region in response to light exposure was also shown for the honeybee (Scholl et al., 2014) and desert ants (Stieb et al., 2010, 2012). It is suggested, that synaptic pruning is a characteristic process in adapting and modulating synaptic microcircuits during development and maturation (Truman and Reiss, 1976; Technau and Heisenberg, 1982; Levine and Truman, 1985; Weeks and Truman, 1986; Lee et al., 1999; Raff et al., 2002; Watts et al., 2003). In *C. rufipes* workers, extended light exposure for 14 days leads to an increase of synaptic densities in the collar, independently of the collar volume. In honeybees and ants, a similar volume–independent increase of MG densities was found in the lip after olfactory learning experiments. This suggests that this effect is associated with a transcription–dependent long–term memory formation (Hourcade et al., 2010; Falibene et al., 2015). The synaptic density of the olfactory lip, indeed was

unaffected by light exposure for several days which supports the hypothesis that visual stimuli are mainly processed in visual and not olfactory brain neuropils.

Summarizing this part of my doctoral thesis, the intrinsic neuronal plasticity supports the hypothesis, that the brain of young *C. rufipes* workers undergoes an extended neuronal development of the central visual system, and to a smaller extent in the olfactory subcompartments in the MB calyces. This process could be hypothesized to be `experience–independent´ (Fahrbach et al., 1998) as it occurs before the prominent interior–exterior switch. Hence, this neuronal intrinsic process was followed by an `experience–dependent´ process were synaptic complexes (MG) of the MB were pruned due to light exposure for several days.

Ultrastructural Architecture of the Pre– and Postsynapse in the MB Calyx of *Camponotus rufipes* Ants

The remarkable structural plasticity of the MG (e.g. increase of single PN bouton size and PN bouton to KC spine ratio) suggested an active role played by these synaptic complexes in neuronal plasticity and the promotion of the behavioral transition in *C. rufipes* workers. The processing and integration in the MB calyces of environmental informations and how neuronal mechanisms trigger the task– and age–related plasticity, requires a deeper understanding of the connectivity and synaptic properties of the MG. So far, previous work in the honeybee based on electron microscopic 3D–reconstructions of serial brain sections revealed that the interior–exterior transition is correlated with substantial structural changes in modular synaptic complexes in MB input regions both at the pre– and postsynaptic site (Groh et al., 2012). Using classical Transmission Electron Microscopy (TEM) both, the identification of single vesicles and the clear architecture of active zones in PN axonal boutons were below optical resolution (Groh et al., 2012).

In my PhD thesis, I therefore start to implement the Electron Tomography (ET), which facilitates high resolution images of synaptic architectures at the ultrastructural level and promotes the understanding of architectural changes in neuronal plasticity process. To receive high resolution 3D stacks with ET, the specimens are tilted incrementally around their own axis vertically to the electron beam (reviewed in Baumeister, 2002). The received image stacks are called tomograms (reviewed in McIntosh et al., 2005). To gain a high resolution tomogram, electron microscopes need to be equipped with an accurate tilt stage and a specimen holder that can cope with the maximal tilting angle (reviewed in McEwen and Marko, 2001). However, one of my aims in this part of my doctoral research is, to reconstruct one entire PN axonal bouton in the MB calyx lip in as few and thick slices as possible. As the diameter of one PN bouton in the lip of honeybees was described to range between 1–2 µm (Groh et al., 2012), I started with 400 nm semithin brain slices, resulting in 4–5 consecutive slices. Due to geometrical reasons, the slice thickness at 60° tilt is two times and at 70° tilt three times higher than in the vertical plain at 0° tilt (reviewed in Baumeister, 2002). Therefore, the optical resolution of the tilt series decreased rapidly in the 400 nm brain slices.

A formula[3], developed by McEwen and Marko (1998) calculates the optical resolution of tomographic reconstruction by taking the increment step, maximal tilt angle and slice thickness into account. At 70° maximal tilt angle with 1° incremental steps, the 400 nm brain slices have a resolution of 20.8 nm, which is 7 times higher than the optical resolution that can be achieved with ET (reviewed in McEwen and Marko, 2001). Hence, I decreased the slice thickness finally to 100 nm, which is thin enough to receive a high optical resolution (3.1 nm by using the formula of McEwen and Marco, 1998) and resulted in 16–18 consecutive brain slices. With these consecutive samples, I can scan through one entire PN axonal bouton in the lip region of the MB calyces. Moreover, to gain a better 3D view of the single tomograms, I rotate the grids by 90° and collected a second tilt series of the same PN axonal bouton I used in the first series. As most single tomograms are lacking data between their maximal tilting angle and 90°, collection of a second tilt series results in a reduction of the missing angular range and a better optical resolution (Penczek et al., 1995; Mastronarde, 1997). After the collection of the double tilt series of one PN bouton, my thesis further provides first insights into the automatically alignment of the collected tomograms. I therefore added 12 nm gold particles randomly on both sides of the grids, as they can be easily found on the tomograms and tracked throughout the whole image series (Brandt et al., 2001). During the automatically alignment of the tilt series, the position of the gold beats on each image are used to adjust and rotate the respective tilt image accordingly to following images (Lawrence, 1992; Penczek et al., 1995; Mastronarde, 1997).

With the implementation and further adjusting of single parameters of the ET method, I could scan through one entire PN bouton and to visualize single presynaptic structures, like clear– and dense–core vesicles. The PN boutons in the MB lip are easily identifiable via their size and shape and their high abundance of synaptic vesicles (Ganeshina and Menzel, 2001; Seid and Wehner, 2008, 2009; Groh et al., 2012). The calculated volume of one PN bouton in this study (~1.56 µm^3) is smaller than the volumes of PN boutons in the MB lip of the honeybee (4.7 µm^3; Groh et al., 2012), *Cataglyphis* ants (*C. fortis*: 2.24 µm^3; Bauer, 2015 and *C. albicans*: 2.7 µm^3; Seid and Wehner, 2008) and *Drosophila* (4.7 µm^3; Butcher et al., 2012). This huge difference is probably given due to different calculation methods: In my

[3] d= 0.035 aT at $\theta_{max} = 60°$

d= 0.052 aT at $\theta_{max} = 70°$

d: resolution, a: angular interval (in degrees), T: section thickness, θ_{max}: maximal tilting angle

study, I assume that the PN boutons are of spherical shape and therefore estimated the bouton volume by the formula to calculate spherical volumes. In the other studies, more accurate 3D volume measurements were performed, which additionally show that the shape of the PN boutons is more complex than a sphere (Seid and Wehner, 2008; Butcher et al., 2012; Groh et al., 2012; Bauer, 2015). I further could show, that the PN boutons contain a high number of clear–core vesicles and additionally a smaller amount of dense–core vesicles. The estimated size of the clear–core (~40 nm in diameter) and dense–core (~70 nm in diameter) vesicles fits with the size measurements of both vesicles types in *Pheidole dentata* ants (clear–core vesicles: 10 to 60 nm; dense–core vesicles: 40 to 65 nm; Seid et al., 2005). Dense–core vesicles are described to possibly contain peptidergic and aminergic elements (Watson and Schürmann, 2002) as well as biogenic amines (Schürmann et al., 1989; Kreissl et al., 1994; Blenau et al., 1999). They are further hypothesized to be involved in neuromodulatory processes of sensory stimuli within the MG, but further studies are needed to clearly identify their contents. Whereas the larger dense–core vesicles are described to release their contents away from synapses (De Camilli and Jahn, 1990; Marder and Thirumalai, 2002), the smaller clear–core vesicles are released at so called \`active zones´ that are located at the pre– and postsynaptic membrane and are readily identifiable by their high electron density.

The active zones I could identify in this study in *C. rufipes* are described as non–ribbon synapses. Beside the non–ribbon synapses, also ribbon synapses have been shown in other insect species (*Apis mellifera*: Groh et al., 2012; *Cataglyphis fortis*: Bauer, 2015; *Drosophila melanogaster*: Butcher et al., 2012), that are easily to identify by their t–shaped (\`ribbon´) structure. Non–ribbon synapses are supposed to have either lost their ribbon or do not yet have established one (Groh et al., 2012). In my study, I counted ~31 active zones within one PN bouton which is comparable to the ~40 active zones within one PN bouton of the fruit fly (Butcher et al., 2012) and ~24 active zones measured in *C. fortis* desert ants (Bauer, 2015). But lower than the amount in the honeybee (~70 active zones; Groh et al., 2012). This could be maybe explained by the fact, that honeybees rely more on olfactory cues, whereas *C. rufipes* ants possess a more visual lifestyle. I therefore hypothesize, that the number of active zones in the visual innervated collar region of the MB calyces of *C. rufipes* workers is higher than the number of active zones in the lip and more comparable to the amount measured in honeybees.

Synapses connect the presynaptic side via the active zones with potential postsynaptic partners. In the case of eusocial insects, this postsynaptic partners are built by the KC dendritic spines (Farris et al., 2001; Ganeshina and Menzel, 2001; Groh et al., 2012). The amount (~62) of the postsynaptic KC spines I measured in the lip region of *C. rufipes* workers is lower than the amount in the honeybee (~140; Groh et al., 2012) and *Drosophila* (~220; Butcher et al., 2012), but more than two times higher than in *Cataglyphis fortis* (~27; Bauer, 2015). The mean size of the KC spines in this study (~0.16 µm in diameter) is within the range of earlier studies conducted in *Pheidole dentata* (Seid et al., 2005). Most synapses in my study are in contact with two to three postsynaptic partners, therefore forming dyads and triads. These structures were also found in desert ant *Cataglyphis albicans* and the honeybee *Apis mellifera* (Seid and Wehner, 2008; Groh et al., 2012).

As my study provides the first insight into the complex synaptic architecture of pre– and postsynapse of *Camponotus rufipes* workers, this technique could be further used to investigate possible changes at the ultrastructural level that might be accompanied by behavioral maturation from nursing to foraging. To date, it was shown for freshly emerged worker ants to possess a lower number of synapses, vesicles per synapse and a smaller PN bouton volume compared to foragers in the MB calyx (Seid et al., 2005). Also in the honeybee, KC dendrites in foragers form up to 140 contacts upon a PN axonal bouton compared with only 95 in freshly emerged bees (Groh et al., 2012). Both studies suggest, that the increase in the number of synaptic architectures comparing nurses and foragers is further accompanied by an increase of the behavioral repertoire (Seid and Traniello, 2006). The structural changes of the MG therefore promote behavioral plasticity underlying division of labor (Groh et al., 2012).

I conclude, that workers of the nectar–feeding ant *C. rufipes* aim a highly plastic and flexible visual nervous system, that is affected by both, age and changing visual stimuli. This plasticity comprises the experience–independent and –dependent plasticity as it was further shown for honeybees, ants and wasps (Withers et al., 1995; Fahrbach et al., 1998; O'Donnell et al., 2004; Ismail et al., 2006; Kühn–Bühlmann and Wehner, 2006; Muenz et al., 2015). Even though less is known about the underlying physiological and molecular mechanisms responsible for this neuronal plasticity of the visual system, it likely constitutes one important factor facilitating the interior–exterior switch.

Outlook to Further Studies

This thesis provides first insights into the complex and flexible maturation of the behavior of an ant worker and its underlying neuronal plasticity. The experiments conducted during this work can serve as a first basis for further and more detailed analyses of single aspects. Social insects are known for their age–related division of labor, which is one important factor for their tremendous success (Hölldobler and Wilson, 1990). Workers of *Camponotus rufipes* also switch from interior nursing duties to exterior foraging tasks at an age of 14–20 days (see **chapter I**). As the setup used provides the possibility to observe single ants for 24 hours per day, it is relatively simple to identify the workers´ task allocation and their age. The behavioral analysis provides first insights on how behavioral maturation occurs in this ant species, but the underlying neuronal mechanisms were not studied in this work. Therefore, further quantitative studies with individually marked nurses and foragers are necessary to reveal how this interior–exterior switch influences primary and secondary sensory integration centers of the brain.

My experiments further suggest, that ambient temperature values seem to play a crucial role for daily locomotor and foraging activity in two sympatric *Camponotus* species, *C. mus* and *C. rufipes* (see **chapter II**). In order to reveal first insights into the daily locomotor activity of both species, workers were kept under isolation under the thermal summer and winter regimes. Isolation from the social context is considered as a major stress factor that affects behavior and survival in social insects (Grassé and Chauvin, 1946; Boulay et al., 1999). This becomes clear in studies with *Leptothorax* ants, were whole colonies and groups of ants display daily locomotor activity patterns, whereas isolated workers display arrhythmicity (Cole, 1991). Consequently, isolated workers may not response to light and temperature cycles in the same way as they would within their social context. To cope with this problem, I would design an experimental setup, with a nest chamber under constant conditions that is connected to a foraging arena under 12:12h LD and either thermal winter (15°C–25°C) or summer conditions (25°C–35°C; Fig. 4). In this setup, ants could choose at which daytime and season (winter or summer) they leave the nest in order to perform foraging trips. I would further mark newly emerged workers in a daily manner to easily facilitate their task

allocation (e.g. nurse or forager). Furthermore, the adult behavior of *C. rufipes* workers is affected by temperature values experienced during their pupal development (Weidenmüller et al., 2009), which may promote behavioral flexibility (Jeanson and Weidenmüller, 2014). Further studies are therefore needed to investigate, if e.g. `summer–born´ workers display strict nocturnal activity regardless of the offered thermal regime or if they switch to diurnal foraging activity under thermal winter regimes. This experimental setup also provides the advantage to study the induced neuronal plasticity of sensory neuropils and synaptic complexes that may occur with the interior–exterior switch under different thermal regimes.

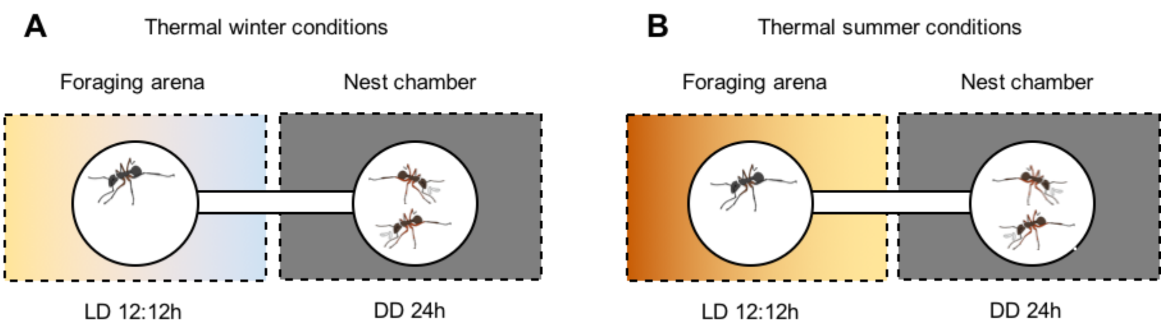

Figure 4: Experimental setup for thermal regimes in the social context. The setup consists of a nest chamber under constant conditions (DD 24h), connected to a foraging arena under a light dark cycle (LD 12:12h) and either thermal winter (A) or summer (B) conditions. Color gradient in the foraging arena indicates thermal regime. Beige: 25°C, blue: 15°C, grey: DD 24h and 25°C; red: 35°C.

The field experiments in La Coronilla, Uruguay in my study where performed during local spring time (see **chapter II**). As the results indicate, nests of *C. rufipes* display mainly nocturnal foraging activity with elevated diurnal activity levels in some nests. I therefore hypothesize that individual workers can shift their foraging activity from nocturnal to diurnal and vice versa. Further field studies during different local seasons need to be done, to proof this hypothesis. Therefore, I would measure the foraging activity of, ideally, the same nests I used during my study to reveal the timing of foraging activity under local summer (January/ February) and winter (July/ August) time.

The remarkable interior–exterior switch of the single workers is associated with an `experience–dependent´ maturation of higher order integration centers in the brain. Even though I could show, that primary and secondary visual brain neuropils are affected by age and light–exposure, until now less is known about the underlying physiological mechanisms that trigger this age–related and light–induced plasticity (see **chapter III**). Recent studies in the honeybee revealed, that titers of juvenile hormone (JH) in the hemolymph increase significantly after light exposure (Scholl et al., 2014). On the other hand, JH does not directly

affect volumetric growth of the MBs (Fahrbach et al., 2003) and the reorganization of synaptic densities (Scholl et al., 2014). Consequently, JH seems to influence behavioral maturation in response to changing environmental conditions. Further studies need to be done to evaluate, if JH levels increase with the switch from interior to exterior work in *C. rufipes* workers and if precocious foragers display elevated JH titers in their hemolymph when compared with normally aged nurses.

Besides the underlying physiological and molecular mechanisms that mediate behavioral plasticity, also little is known about the changes in olfactory and visual PN terminals in the MB neuropil at the ultrastructural level. First results from studies conducted on honeybees revealed first insights into the complex architecture of single PN boutons, but the optical resolution was too low to identify single presynaptic components (Groh et al., 2012). My thesis started to implement ET to gain a higher optical resolution of single neurotransmitter vesicles and active zones (see **chapter IV**). The PN axonal boutons of nurses and foragers were reported to differ in their cytoplasm electron density, referred to as light and dark PN boutons, which most likely display differences in the abundance of clear–core neurotransmitter vesicles (Groh et al., 2012). I therefore hypothesize, that the timing of the interior–exterior transition in *C. rufipes* ants is correlated with an increase in the number of synaptic vesicles in presynaptic terminals in the MB calyces and that the number of activated synapses is increased in foragers. To test this hypothesis, I would compare the number and type of vesicles (dense–core and clear–core vesicles) and active zones in PN boutons of the MB neuropil within different cohorts of *C. rufipes* ant workers: 1) age–matched nurses and foragers that are 2) light–deprived or –exposed and were either 3) strictly nocturnal or with increased diurnal activity.

To examine behavioral and neuronal mechanisms of the timing from interior to exterior work is of high importance to understand the social organization of insect colonies. This thesis provides first important insights into behavioral and neuronal plasticity processes that are accompanied with this polyethism in *Camponotus rufipes* ants. As workers of the nectar–feeding ant *C. rufipes* comprise both forms of division of labor (age– and size–related), they therefore constitute a rewarding model for future experiments to solve these goals and to answer open questions.

Bibliography

Addo–Bediako A, Chown SL, Gaston KJ. 2000. Thermal tolerance, climatic variability and latitude. Proceedings of the Royal Society of London B: Biological Sciences 267:739–745.

Albrecht M, Gotelli NJ. 2001. Spatial and temporal niche partitioning in grassland ants. Oecologia 126:134–141.

Andersen AN. 1983. Species diversity and temporal distribution of ants in the semi-arid mallee region of northwestern Victoria. Austral Ecology 8:127–137.

Andersen AN. 1992. Regulation of "momentary" diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. The American Naturalist 140:401–420.

Anderson C, McShea DW. 2001. Individual versus social complexity, with particular reference to ant colonies. Biological Reviews of the Cambridge Philosophical Society 76:211–37.

Angilletta MJ. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press.

Anjos D V, Caserio B, Rezende FT, Ribeiro SP, Del-Claro K, Fagundes R. 2017. Extrafloral-nectaries and interspecific aggressiveness regulate day/night turnover of ant species foraging for nectar on *Bionia coriacea*. Austral Ecology 42:317–328.

Aschoff J. 1979. Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. Ethology 49:225–249.

Aschoff J. 1986. Anticipation of a daily meal: a process of "learning" due to entrainment. Monitore Zoologico Italiano–Italian Journal of Zoology 20:195–219.

Ashikin N, Hashim R. 2015. Daily activity patterns of *Platythyrea parallela* in Peninsular Malaysia. Asian Myrmecology 7:145–154.

Aso Y, Grübel K, Busch S, Friedrich AB, Siwanowicz I, Tanimoto H. 2009. The mushroom

body of adult *Drosophila* characterized by GAL4 drivers. Journal of Neurogenetics 23:156–172.

Awasaki T, Ito K. 2004. Engulfing action of glial cells is required for programmed axon pruning during *Drosophila* metamorphosis. Current Biology 14:668–677.

Azcárate FM, Kovacs E, Peco B. 2007. Microclimatic conditions regulate surface activity in harvester ants *Messor barbarus*. Journal of Insect Behavior 20:315–329.

Bagri A, Cheng H–J, Yaron A, Pleasure SJ, Tessier–Lavigne M. 2003. Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell 113:285–299.

Barbieri RF, Grangier J, Lester PJ. 2015. Synergistic effects of temperature, diet and colony size on the competitive ability of two ant species. Austral Ecology 40:90–99.

Barnes TC, Kohn HI. 1932. The effect of temperature on the leg posture and speed of creeping in the ant *Lasius*. The Biological Bulletin 62:306–312.

Barth M, Hirsch HVB, Meinertzhagen IA, Heisenberg M. 1997. Experience–dependent developmental plasticity in the optic lobe of *Drosophila melanogaster*. Journal of Neuroscience 17:1493–1504.

Baudier KM, Mudd AE, Erickson SC, O'Donnell S. 2015. Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). Journal of Animal Ecology 84:1322–1330.

Bauer V. 2015. High–resolution localization of synaptic proteins in the insect brain. Masterthesis (University of Würzburg)

Baumeister W. 2002. Electron tomography: towards visualizing the molecular organization of the cytoplasm. Current Opinion in Structural Biology 12:679–684.

Baumeister W, Grimm R, Walz J. 1999. Electron tomography of molecules and cells. Trends in Cell Biology 9:81–85.

Becher MA, Scharpenberg H, Moritz RFA. 2009. Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera L.*). Journal of Comparative Physiology A 195:673–679.

Bell–Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, Zoran MJ. 2005. Circadian rhythms from multiple oscillators: lessons from diverse organisms.

Nature Reviews Genetics 6:544–556.

Bernadou A, Busch J, Heinze J. 2015. Diversity in identity: behavioral flexibility, dominance, and age polyethism in a clonal ant. Behavioral Ecology and Sociobiology: 69:1365–1375.

Bernstein RA. 1976. The adaptive value of polymorphism in an alpine ant, *Formica neorufibarbis* Gelida Wheeler. Psyche: A Journal of Entomology 83:180–184.

Bestelmeyer BT. 1997. Stress tolerance in some Chacoan dolichoderine ants: implications for community organization and distribution. Journal of Arid Environments 35:297–310.

Bestelmeyer BT. 2000. The trade–off between thermal tolerance and behavioral dominance in a subtropical South American ant community. Journal of Animal Ecology 69:998–1009.

Bicker G, Schäfer S, Kingan TG. 1985. Mushroom body feedback interneurones in the honey bee show GABA–like immunoreactivity. Brain Research 360:394–397.

Bishop DL, Misgeld T, Walsh MK, Gan W–B, Lichtman JW. 2004. Axon branch removal at developing synapses by axosome shedding. Neuron 44:651–661.

Bishop TR, Robertson MP, Rensburg BJ, Parr CL. 2017. Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants. Ecological Entomology 42:105–114.

Blenau W, Schmidt M, Faensen D, Schürmann F–W. 1999. Neurons with dopamine–like immunoreactivity target mushroom body Kenyon cell somata in the brain of some Hymenopteran insects. International Journal of Insect Morphology and Embryology 28:203–210.

Bloch G. 2009. Plasticity in the circadian clock and the temporal organization of insect societies. In: Organization of Insect Societies: From Genome to Sociocomplexity. p 402–432.

Bloch G, Robinson GE. 2001. Chronobiology. Reversal of honeybee behavioural rhythms. Nature 410:1048.

Blüthgen N, Fiedler K. 2004. Competition for composition: Lessons from nectar–feeding ant communities. Ecology 85:1479–1485.

Bolton B. 1995. A new general catalogue of the ants of the world. Harvard University Press Cambridge, MA.

Bonavita–Cougourdan A, Clement J–L, Lange C. 1993. Functional subcaste discrimination (foragers and brood–tenders) in the ant *Camponotus vagus* scop.: polymorphism of cuticular hydrocarbon patterns. Journal of Chemical Ecology 19:1461–1477.

Boomsma JJ, Leusink A. 1981. Weather conditions during nuptial flights of four European ant species. Oecologia 50:236–241.

Boulay R, Quagebeur M, Godzinska EJ, Lenoir A. 1999. Social isolation in ants: evidence of its impact on survivorship and behavior in *Camponotus fellah* (Hymenoptera: Formicidae). Sociobiology 33:111–124.

Brady J. 1974. The physiology of insect circadian rhythms. Advances in Insect Physiology 10:1–115.

Brandon JG, Coss RG. 1982. Rapid dendritic spine stem shortening during one–trial learning: the honeybee's first orientation flight. Brain Research 252:51–61.

Brandt S, Heikkonen J, Engelhardt P. 2001. Automatic alignment of transmission electron microscope tilt series without fiducial markers. Journal of Structural Biology 136:201–213.

Brent C, Peeters C, Dietmann V, Crewe R, Vargo E. 2006. Hormonal correlates of reproductive status in the queenless ponerine ants, *Streblognathus peetersi*. Journal of Comparative Physiology A 192:315–320.

Briese DT, Macauley BJ. 1980. Temporal structure of an ant community in semi–arid Australia. Australian Journal of Ecology 5:121–134.

Broadie K. 2004. Axon pruning: an active role for glial cells. Current Biology 14:302–304.

Busher CE, Calabi P, Traniello JFA. 1985. Polymorphism and division of labor in the neotropical ant *Camponotus sericeiventris* Guerin (Hymenoptera: Formicidae). Annals of the Entomological Society of America 78:221–228.

Butcher NJ, Friedrich AB, Lu Z, Tanimoto H, Meinertzhagen IA. 2012. Different classes of input and output neurons reveal new features in microglomeruli of the adult *Drosophila* mushroom body calyx. Journal of Comparative Neurology 520:2185–2201.

De Camilli P, Jahn R. 1990. Pathways to regulated exocytosis in neurons. Annual Review

of Physiology 52:625–645.

Capaldi EA, Smith AD, Osborne JL, Fahrbach SE. 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature 403:537.

Carlin NF. 1981. Polymorphism and division of labor in the dacetine ant *Orectognathus versicolor* (Hymenoptera: Formicidae). Psyche 88:231–244.

Cerdá X, Retana J. 2000. Alternative strategies by thermophilic ants to cope with extreme heat: individual versus colony level traits. Oikos 89:155–163.

Cerdá X, Retana J, Cros S. 1997. Thermal disruption of transitive hierarchies in Mediterranean ant communities. Journal of Animal Ecology 66:363–374.

Cerdá X, Retana J, Cros S. 1998. Critical thermal limits in Mediterranean ant species: Trade–off between mortality risk and foraging performance. Functional Ecology 12:45–55.

Charbonneau D, Dornhaus A. 2015. Workers “specialized” on inactivity: Behavioral consistency of inactive workers and their role in task allocation. Behavioral Ecology and Sociobiology 69:1459–1472.

Christian KA, Morton SR. 1992. Extreme thermophilia in a central Australian ant, *Melophorus bagoti*. Physiological Zoology 65:885–905.

Clémencet J, Cournault L, Odent A, Doums C. 2010. Worker thermal tolerance in the thermophilic ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Insectes Sociaux 57:11–15.

Cole BJ. 1986. The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. Behavioral Ecology and Sociobiology 18:165–173.

Cole BJ. 1991. Is Animal Behaviour Chaotic? Evidence from the Activity of Ants. Proceedings of the Royal Society of London Series B: Biological Sciences 244:253–259.

Collett TS, Dillmann E, Giger A, Wehner R. 1992. Visual landmarks and route following in desert ants. Journal of Comparative Physiology A 170:435–442.

Coss RG, Brandon JG, Globus A. 1980. Changes in morphology of dendritic spines on honeybee and foraging experiences. Brain Research 192:49–59.

Cros S, Cerdá X, Retana J. 1997. Spatial and temporal variations in the activity patterns of Mediterranean ant communities. Ecoscience 4:269–278.

Danks H V. 2003. Studying insect photoperiodism and rhythmicity: Components, approaches and lessons. European Journal of Entomology 100:209–221.

Dejean A, Lachaud J–P. 1991. *Odontomachus troglodytes*: Interaction of age and interindividual variability. Sociobiology 18:177–196.

Del–Claro K, Oliveira PS. 1999. Ant–homoptera interactions in a neotropical savanna: The honeydew–producing treehopper, *Guayaquila xiphias* (Membracidae), and its associated ant fauna on *Didymopanax vinosum* (Araliaceae). Biotropica 31:135–144.

Del–Claro K, Oliveira PS. 2000. Conditional outcomes in a neotropical treehopper–ant association: temporal and species–specific variation in ant protection and homopteran fecundity. Oecologia 124:156–165.

Detrain C, Pasteels JM. 1991. Caste differences in behavioral thresholds as a basis for polyethism during food recruitment in the ant, *Pheidole pallidula* (Nyl.) (Hymenoptera: Myrmicinae). Journal of Insect Behavior 4:157–176.

Devaud J–M, Papouin T, Carcaud J, Sandoz J, Grünewald B. 2015. Neural substrate for higher–order learning in an insect : Mushroom bodies are necessary for configural discriminations. Proceedings of the National Academy of Sciences 122:5854–5862.

Devlin PF. 2002. Signs of the time: environmental input to the circadian clock. Journal of Experimental Botany 53:1535–1550.

Dew HE, Michener CD. 1981. Division of labor among workers of *Polistes metricus* (Hymenoptera: Vespidae): Laboratory foraging activities. Insectes Sociaux 28:87–101.

Dobrin SE, Fahrbach SE. 2012. Rho GTPase activity in the honey bee mushroom bodies is correlated with age and foraging experience. Journal of Insect Physiology 58:228–234.

Dobrin SE, Herlihy JD, Robinson GE, Fahrbach SE. 2011. Muscarinic regulation of Kenyon cell dendritic arborizations in adult worker honey bees. Arthropod Structure & Development 40:409–419.

Dolezal AG, Brent CS, Hölldobler B, Amdam GV. 2012. Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. Journal of Experimental Biology 215:454–460.

Dornhaus A. 2008. Specialization does not predict individual efficiency in an ant. PLoS Biology 6:2368–2375.

Dornhaus A, Holley J–A, Franks NR. 2009. Larger colonies do not have more specialized workers in the ant *Temnothorax albipennis*. Behavioral Ecology 20:922–929.

Drees BM, Summerlin B, Vinson SB. 2007. Foraging activity and temperature relationship for the red imported fire ant. Southwestern Entomologist 32:149–155.

Dreisig H. 1985. A time budget model of thermoregulation in shuttling ectotherms. Journal of Arid Environments 8:191–205.

Dujardin F. 1850. Mémoire sur le système nerveux des insectes. Ann Sci Nat Zool 14:195–206.

Dunlap JC, Loros JJ, Decoursey PJ. 2003. Sinauer Associates; Sunderland. In: Chronobiology: Biological Timekeeping.

Durst C, Eichmüller S, Menzel R. 1994. Development and experience lead to increased volume of subcompartments of the honey bee mushroom body. Behavioral and Neural Biology 62:259–263.

Edmunds LN. 1988. Models and mechanisms for circadian timekeeping. In: Cellular and Molecular Bases of Biological Clocks. Springer–Verlag New York. p 497.

Ehmer B, Gronenberg W. 2002. Segregation of visual input to the mushroom bodies in the honeybee (*Apis mellifera*). Journal of Comparative Neurology 451:362–373.

Ehmer B, Gronenberg W. 2004. Mushroom body volumes and visual interneurons in ants: Comparison between sexes and castes. The Journal of Comparative Neurology 469:198–213.

Ehrhardt S. 1931. Über Arbeitsteilung bei *Myrmica*–und *Messor*–arten. Zoomorphology 20:755–812.

Esch C, Jimenez JP, Peretz C, Uno H, O'Donnell S. 2017. Thermal tolerances differ between diurnal and nocturnal foragers in the ant Ectatomma ruidum. Insectes Sociaux 64:1–6.

Fagundes M, Neves FS, Fernandes GW. 2005. Direct and indirect interactions involving ants, insect herbivores, parasitoids, and the host plant *Baccharis dracunculifolia* (Asteraceae). Ecological Entomology 30:28–35.

Fahrbach SE. 2006. Structure of the mushroom bodies of the insect brain. Annual Review of Entomology 51:209–232.

Fahrbach SE, Dobrin SE. 2008. The how and why of structural plasticity in the adult honeybee brain. In: Dukas R, Ratcliff JM, editors. Cognitive Ecology II. p 27–46.

Fahrbach SE, Farris SM, Sullivan JP, Robinson GE. 2003. Limits on volume changes in the mushroom bodies of the honey bee brain. Journal of Neurobiology 57:141–151.

Fahrbach SE, Moore D, Capaldi EA, Farris SM, Robinson GE. 1998. Experience–expectant plasticity in the mushroom bodies of the honeybee. Learning & Memory 5:115–123.

Fahrbach SE, Strande JL, Robinson GE. 1995. Neurogenesis is absent in the brains of adult honey bees and does not explain behavioral neuroplasticity. Neuroscience Letters 197:145–148.

Falibene A, Josens R. 2014. Environmental temperature affects the dynamics of ingestion in the nectivorous ant *Camponotus mus*. Journal of Insect Physiology 71:14–20.

Falibene A, Roces F, Rössler W. 2015. Long–term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf–cutting ants. Frontiers in Behavioral Neuroscience 9:1–13.

Falibene A, Roces F, Rössler W, Groh C. 2016. Daily thermal fluctuations experienced by pupae via rhythmic nursing behavior increase numbers of mushroom body microglomeruli in the adult ant brain. Frontiers in Behavioral Neuroscience 10:1–12.

Farris SM. 2008. Structural, functional and developmental convergence of the insect mushroom bodies with higher brain centers of vertebrates. Brain, Behavior and Evolution 72:1–15.

Farris SM, Robinson GE, Davis RL, Fahrbach SE. 1999. Larval and pupal development of the mushroom bodies in the honey bee, *Apis mellifera*. Journal of Comparative Neurology 414:97–113.

Farris SM, Robinson GE, Fahrbach SE. 2001. Experience– and age–related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. The Journal of Neuroscience 21:6395–6404.

Fellers JH. 1987. Interference and exploitation in a guild of woodland ants. Ecology 68:1466–1478.

Fellers JH. 1989. Daily and seasonal activity in woodland ants. Oecologia 78:69–76.

Fleischmann PN, Christian M, Müller VL, Rössler W, Wehner R. 2016. Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. Journal of Experimental Biology 219:3137–3145.

Fleischmann PN, Grob R, Wehner R, Rössler W. 2017. Species–specific differences in the fine structure of learning walk elements in *Cataglyphis ants*. Journal of Experimental Biology 220:2426–2435.

Fouquet W, Owald D, Wichmann C, Mertel S, Depner H, Dyba M, Hallermann S, Kittel RJ, Eimer S, Sigrist SJ. 2009. Maturation of active zone assembly by *Drosophila* Bruchpilot. The Journal of Cell Biology 186:129–145.

Frambach I, Rössler W, Winkler M, Schürmann FW. 2004. F–actin at identified synapses in the mushroom body neuropil of the insect brain. Journal of Comparative Neurology 475:303–314.

Fung JC, Liu W, De Ruijter WJ, Chen H, Abbey CK, Sedat JW, Agard DA. 1996. Toward fully automated high–resolution electron tomography. Journal of Structural Biology 116:181–189.

Gadagkar R, Joshi N V. 1984. Social organisation in the Indian wasp *Ropalidia cyathiformis* (Fab.)(Hymenoptera: Vespidae). Ethology 64:15–32.

Galle L. 1986. Habitat and niche analysis of grassland ants (Hymenoptera: Formicidae). Entomologia Generalis 11:197–211.

Ganeshina O, Menzel R. 2001. GABA-immunoreactive neurons in the mushroom bodies of the honey bee: An electron microscopic study. Journal of Comparative Neurology 437:335–349.

Ganeshina O, Schäfer S, Malun D. 2000. Proliferation and programmed cell death of neuronal precursors in the mushroom bodies of the honeybee. Journal of Comparative Neurology 417:349–365.

Gascuel J, Masson C. 1991. Developmental study of afferented and deafferented bee antennal lobes. Developmental Neurobiology 22:795–810.

Gehring KB, Heufelder K, Depner H, Kersting I, Sigrist SJ, Eisenhardt D. 2017. Age–associated increase of the active zone protein Bruchpilot within the honeybee

mushroom body. PloS one 12:e0175894.

Gehring WJ, Wehner R. 1995. Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. Proceedings of the National Academy of Sciences 92:2994–2998.

Giebultowicz JM. 2001. Peripheral clocks and their role in circadian timing: insights from insects. Philosophical Transactions of the Royal Society 356:1791–1799.

Giurfa M. 2007. Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. Journal of Comparative Physiology A 193:801–824.

Goni B, De Zolessi LC, Imai HT. 1983. Karyotypes of thirteen ant species from Uruguay (Hymenoptera, Formicidae). Caryologia 36:363–371.

Gordon DM. 1983a. The relation of recruitment rate to activity rhythms in the harvester ant, *Pogonomyrmex barbatus* (F. Smith) (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 56:277–285.

Gordon DM. 1983b. Daily rhythms in social activities of the harvester ant, *Pogonomyrmex badius*. Psyche 90:413–423.

Gordon DM. 1986. The dynamics of the daily round of the harvester ant colony (*Pogonomyrmex barbatus*). Animal Behaviour 34:1402–1419.

Gordon DM. 1989. Dynamics of task switching in harvester ants. Animal Behaviour 38:194–204.

Gordon DM, Chu J, Lillie A, Tissot M, Pinter N. 2005. Variation in the transition from inside to outside work in the red harvester ant *Pogonomyrmex barbatus*. Insectes Sociaux 52:212–217.

Gordon DM, Hölldobler B. 1987. Worker longevity in harvester ants (*Pogonomyrmex*). Psyche 94:341–346.

Grassé PP, Chauvin R. 1946. L’effet de groupe et la survie des neutres dans les sociétés d’insectes. Revue Scientifique 7:461–464.

Greenough WT, Larson JR, Withers GS. 1985. Effects of unilateral and bilateral training in a reaching task on dendritic branching of neurons in the rat motor–sensory forelimb cortex. Behavioral and Neural Biology 44:301–314.

Greenough WT, Volkmar FR. 1973. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. Experimental Neurology 40:491–504.

Greiner B, Narendra A, Reid SF, Dacke M, Ribi WA, Zeil J. 2007. Eye structure correlates with distinct foraging–bout timing in primitive ants. Current Biology 17:879–880.

Grimm R, Singh H, Rachel R, Typke D, Zillig W, Baumeister W. 1998. Electron tomography of ice–embedded prokaryotic cells. Biophysical Journal 74:1031–1042.

Groh C, Ahrens D, Rössler W. 2006. Environment– and age–dependent plasticity of synaptic complexes in the mushroom bodies of honeybee queens. Brain, Behavior and Evolution 68:1–14.

Groh C, Kelber C, Grübel K, Rössler W. 2014. Density of mushroom body synaptic complexes limits intraspecies brain miniaturization in highly polymorphic leaf–cutting ant workers. Proceedings of the Royal Society B 281:20140432.

Groh C, Lu Z, Meinertzhagen IA, Rössler W. 2012. Age–related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. Journal of Comparative Neurology 520:3509–3527.

Groh C, Rössler W. 2011. Comparison of microglomerular structures in the mushroom body calyx of neopteran insects. Arthropod Structure & Development 40:358–367.

Groh C, Tautz J, Rössler W. 2004. Synaptic organization in the adult honey bee brain is influenced by brood–temperature control during pupal development. Proceedings of the National Academy of Sciences of the United States of America 101:4268–4273.

Gronenberg W. 1999. Modality–specific segregation of input to ant mushroom bodies. Brain, Behavior and Evolution 54:85–95.

Gronenberg W. 2001. Subdivisions of Hymenopteran mushroom body calyces by their afferent supply. Journal of Comparative Neurology 435:474–489.

Gronenberg W. 2008. Structure and function of ant (Hymenoptera : Formicidae) brains: Strength in numbers. Myrmecological News 11:25–36.

Gronenberg W, Heeren S, Hölldobler B. 1996. Age–dependent and task–related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. The Journal of Experimental Biology 199:2011–2019.

Gronenberg W, Hölldobler B. 1999. Morphologic representation of visual and antennal

information in the ant brain. Journal of Comparative Neurology 412:229–240.

Grünewald B. 1999. Morphology of feedback neurons in the mushroom body of the honey bee, *Apis mellifera*. Journal of Comparative Neurology 404:114–126.

Hallermann S, Kittel RJ, Wichmann C, Weyhersmüller A, Fouquet W, Mertel S, Owald D, Eimer S, Depner H, Schwärzel M. 2010. Naked dense bodies provoke depression. Journal of Neuroscience 30:14340–14345.

Hammer M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. Nature 366:59–63.

Hansen LD, Akre RD. 1990. Biology of carpenter ants. Applied Myrmecology: A World Perspective 274–280.

Hardin PE. 2011. Molecular genetic analysis of circadian timekeeping in *Drosophila*. Advances in Genetics 74:141–173.

Hastings JW, Rusak B, Boulos Z. 1991. Circadian rhythms: The physiology of circadian rhythms. In: Prosser L, editor. Neural and Integrative Animal Physiology. p 535–546.

Hastings JW, Sweeney BM. 1957. On the mechanism of temperature independence in a biological clock. Proceedings of the National Academy of Sciences 43:804–811.

Heinrich B. 1996. The thermal warriors: strategies of insect survival. Library Journal 121:90.

Heisenberg M. 2003. Mushroom body memoir: from maps to models. Nature Reviews Neuroscience 4:266–275.

Heisenberg M, Heusipp M, Wanke C. 1995. Structural plasticity in the *Drosophila* brain. Journal of Neuroscience 15:1951–1960.

Helfrich–Förster C, Yoshii T, Wülbeck C, Grieshaber E, Rieger D, Bachleitner W, Cusumano P, Rouyer F. 2007. The lateral and dorsal neurons of *Drosophila melanogaster*: New insights about their morphology and function. Cold Spring Harbor Press 72:517–525.

Herbers JM. 1983. Social organization in *Leptothorax* ants: within– and between–species patterns. Psyche 90:361–386.

Hertel H, Maronde U. 1987. The physiology and morphology of centrally projecting visual interneurons in the honeybee brain. Journal of Experimental Biology 133:301–315.

Hertel H, Schäfer S, Maronde U. 1987. The physiology and morphology of visual commissures in the honeybee brain. Journal Experimental Biology 133:283–300.

Hinze B, Leuthold RH. 1999. Age related polyethism and activity rhythms in the nest of the termite *Macrotermes bellicosus* (Isoptera, Termitidae). Insectes Sociaux 46:392–397.

Hölldobler B. 1961. Zur Frage der Oligogynie bei *Camponotus ligniperda* Latr. und *Camponotus herculeanus* L. (Hym. Formicidae). Zeitschrift für Angewandte Entomologie 49:337–352.

Hölldobler B. 1980. Canopy orientation: a new kind of orientation in ants. Science 210:86–88.

Hölldobler B, Wilson EO. 1990. The ants. Cambridge: Harvard University Press.

Hölldobler B, Wilson EO. 2009. The superorganism: the beauty, elegance and strangeness in insect colonies. WW Norton & Company.

Holbrook CT, Eriksson TH, Overson RP, Gadau J, Fewell JH. 2013. Colony-size effects on task organization in the harvester ant *Pogonomyrmex californicus*. Insectes Sociaux 60:191–201.

Holt SJ. 1955. On the foraging activity of the wood ant. The Journal of Animal Ecology 24:1–34.

Homberg U, Hofer S, Pfeiffer K, Gebhardt S. 2003. Organization and neural connections of the anterior optic tubercle in the brain of the locust, *Schistocerca gregaria*. Journal of Comparative Neurology 462:415–430.

Houadria M, Salas–Lopez A, Orivel J, Blüthgen N, Menzel F. 2015. Dietary and temporal niche differentiation in tropical ants–can they explain local ant coexistence? Biotropica 47:208–217.

Hourcade B, Muenz TS, Sandoz J–C, Rössler W, Devaud J–M. 2010. Long–term memory leads to synaptic reorganization in the mushroom bodies: a memory trace in the insect brain? The Journal of Neuroscience 30:6461–6465.

Hu XP, Appel AG. 2004. Seasonal variation of critical thermal limits and temperature tolerance in Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). Environmental Entomology 33:197–205.

Ismail N, Robinson GE, Fahrbach SE. 2006. Stimulation of muscarinic receptors mimics

experience–dependent plasticity in the honey bee brain. Proceedings of the National Academy of Sciences of the United States of America 103:207–211.

Ito K, Shinomiya K, Ito M, Armstrong JD, Boyan G, Hartenstein V, Harzsch S et al. 2014. A systematic nomenclature for the insect brain. Neuron 81:755–765.

Jáffe K, Sanchez C. 1984. Comportamiento alimentario y sistema de reclutamiento en la hormiga *Camponotus rufipes* (Hymenoptera: Formicidae). Acta Cientifica Venezolana 35:270–277.

Jandt JM, Huang E, Dornhaus A. 2009. Weak specialization of workers inside a bumble bee (*Bombus impatiens*) nest. Behavioral Ecology and Sociobiology 63:1829–1836.

Jayatilaka P, Narendra A, Reid SF, Cooper P, Zeil J. 2011. Different effects of temperature on foraging activity schedules in sympatric *Myrmecia* ants. Journal of Experimental Biology 214:2730–2738.

Jeanne RL. 1986a. The evolution of the organization of work in social insects. Monitore Zoologico Italiano 20:119–133.

Jeanne RL. 1986b. The organization of work in *Polybia occidentalis*: costs and benefits of specialization in a social wasp. Behavioral Ecology and Sociobiology 19:333–341.

Jeanson R, Weidenmüller A. 2014. Interindividual variability in social insects – proximate causes and ultimate consequences. Biological Reviews 89:671–687.

Jensen TF, Nielsen MG. 1975. The influence of body size and temperature on worker ant respiration. Natura Jutlandica 18:21–25.

Johnson CH, Elliott JA, Foster R. 2003. Entrainment of circadian programs. Chronobiology International 20:741–774.

Jones JC, Helliwell P, Beekman M, Maleszka R, Oldroyd BP. 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. Journal of Comparative Physiology A 191:1121–1129.

Josens RB. 2002. Nectar feeding and body size in the ant *Camponotus mus*. Insectes Sociaux 49:326–330.

Kantor DB, Kolodkin AL. 2003. Curbing the excesses of youth: molecular insights into axonal pruning. Neuron 38:849–852.

Kaspari M, Byrne MM. 1995. Caste allocation in litter *Pheidole*: lessons from plant defense theory. Behavioral Ecology and Sociobiology 37:255–263.

Kay A, Rissing SW. 2005. Division of foraging labor in ants can mediate demands for food and safety. Behavioral Ecology and Sociobiology 58:165–174.

Kay CAR, Whitford WG. 1978. Critical thermal limits of desert honey ants: possible ecological implications. Physiological Zoology 51:206–213.

Kenyon FC. 1896. The brain of the bee: A preliminary contribution to the morphology of the nervous system of the Arthropoda. Journal of Comparative Neurology 6:133–210.

Kinoshita M, Pfeiffer K, Homberg U. 2007. Spectral properties of identified polarized–light sensitive interneurons in the brain of the desert locust *Schistocerca gregaria*. Journal of Experimental Biology 210:1350–1361.

Kipyatkov VE. 1993. Annual cycles of development in ants: diversity, evolution, regulation. Proceedings of the Colloquia on Social Insects 2:25–48.

Kipyatkov VE. 1995. Role of endogenous rhythms in the regulation of annual cycles of development in ants (Hymenoptera, Formicidae). Entomological Review 74:1–15.

Kipyatkov VE. 2001. Seasonal life cycles and the forms of dormancy in ants (Hymenoptera: Formicidea). Acta Societatis Zoologicae Bohemicae 65:211–238.

Kirschner S, Kleineidam CJ, Rybak R, Grünewald B, Zube C, Rössler W. 2006. Dual olfactory pathway in the honey bee, *Apis mellifera*. The Journal of Comparative Neurology 499:933–952.

Kittel RJ. 2006. Bruchpilot promotes active zone assembly, Ca2+ channel clustering, and vesicle release. Science 312:1051–1054.

Klein BA, Gibbs AG, Larsen KMF. 2003. Signatures of sleep in the paper wasp *Polistes flavus*. The 2003 ESA Annual Meeting and Exhibition.

Klein BA, Klein A, Wray MK, Mueller UG, Seeley TD. 2010. Sleep deprivation impairs precision of waggle dance signaling in honey bees. Proceedings of the National Academy of Sciences 107:22705–22709.

Kolb B, Whishaw IQ. 1998. Brain plasticity and behavior. Annual Review of Psychology 49:43–64.

Konopka RJ, Pittendrigh C, Orr D. 1989. Reciprocal behaviour associated with altered homeostasis and photosensitivity of *Drosophila* clock mutants. Journal of Neurogenetics 6:1–10.

Koto A, Mersch D, Hollis B, Keller L. 2015. Social isolation causes mortality by disrupting energy homeostasis in ants. Behavioral Ecology and Sociobiology 69:583–591.

Kreissl S, Eichmüller S, Bicker G, Rapus J, Eckert M. 1994. Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. Journal of Comparative Neurology 348:583–595.

Kremer JR, Mastronarde DN, McIntosh JR. 1996. Computer visualization of three–dimensional image data using IMOD. Journal of structural biology 116:71–76.

Kühn–Bühlmann S, Wehner R. 2006. Age–dependent and task–related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. InterScience 66:511–521.

Kumar Sharma V, Rahman Lone S, Mathew D, Goel A, Chandrashekaran MK. 2004. Possible evidence for shift work schedules in the media workers of the ant species *Camponotus compressus*. Chronobiology International 21:297–308.

Kusnezov N. 1952. El género *Camponotus* en la Argentina (Hymenoptera, Formicidae). Acta Zoologica Lilloana 8:427–456.

Kusnezov N. 1963. Zoogeografía de las hormigas en Sudamerica. Acta Zoologica Lilloana 19:25–186.

Kwapich CL, Tschinkel WR. 2015. Limited flexibility and unusual longevity shape forager allocation in the Florida harvester ant (*Pogonomyrmex badius*). Behavioral Ecology and Sociobiology 70:1045.

Ladinsky MS, Mastronarde DN, McIntosh JR, Howell KE, Staehelin LA. 1999. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. The Journal of Cell Biology 144:1135–1149.

Lakin–Thomas PL. 2000. Circadian rhythms: new functions for old clock genes. Trends in Genetics 16:135–142.

Land MF. 1997. Visual acuity in insects. Annual review of entomology 42:147–177.

Lavine BK, Vander Meer RK, Morel L, Gunderson RW, Han JH, Stine A. 1990. False color

data imaging: A new pattern recognition technique for analyzing chromatographic profile data. Microchemical Journal 41:288–295.

Lawrence MC. 1992. Least–squares method of alignment using markers. In: Electron tomography. Springer. p 197–204.

Lee T, Lee A, Luo L. 1999. Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 126:4065–4076.

Leiss F, Groh C, Butcher NJ, Meinertzhagen IA, Tavosanis G. 2009. Synaptic organization in the adult *Drosophila* mushroom body calyx. Journal of Comparative Neurology 517:808–824.

Leitch B, Laurent G. 1996. GABAergic synapses in the antennal lobe and mushroom body of the locust olfactory system. The Journal of Comparative Neurology 372:487–514.

Lenoir A. 1979. Feeding behaviour in young societies of the ant *Tapinoma Erraticum* L.: Trophallaxis and polyethism. Insectes Sociaux 26:19–37.

Lessard J–P, Dunn RR, Sanders NJ. 2009. Temperature–mediated coexistence in temperate forest ant communities. Insectes Sociaux 56:149–156.

Levine RB, Truman JW. 1985. Dendritic reorganization of abdominal motoneurons during metamorphosis of the moth, *Manduca sexta*. The Journal of Neuroscience 5:2424–2431.

Lighton JRB, Feener Jr DH. 1989. Water–loss rate and cuticular permeability in foragers of the desert ant *Pogonomyrmex rugosus*. Physiological Zoology 62:1232–1256.

Lindauer M. 1952. Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. Journal of Comparative Physiology A 34:299–345.

Lindauer M, Watkin B. 1953. Division of labour in the honeybee colony. Bee World 34:63–73.

MacKay WP, Sassaman CA. 1984. Paradoxical acclimation and seasonal comparisons of oxygen consumption rates in three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). Journal of Comparative Physiology B 154:399–407.

Mailleux AC, Deneubourg JL, Detrain C. 2003. How does colony growth influence communication in ants? Insectes Sociaux 50:24–31.

Marder E, Thirumalai V. 2002. Cellular, synaptic and network effects of neuromodulation. Neural Networks 15:479–493.

Markert SM, Bauer V, Muenz TS, Jones NG, Helmprobst F, Britz S, Sauer M, Rössler W, Engstler M, Stigloher C. 2017. 3D subcellular localization with superresolution array tomography on ultrathin sections of various species. Methods in Cell Biology 140:21–47.

Marsh AC. 1985. Microclimatic factors influencing foraging patterns and success of the thermophilic desert ant, *Ocymyrmex barbiger*. Insectes Sociaux 32:286–296.

Marsh AC. 1988. Activity patterns of some Namib desert ants. Journal of Arid Environments 14:61–73.

Masson C, Arnold G. 1984. Ontogeny, maturation and plasticity of the olfactory system in the workerbee. Journal of Insect Physiology 30:7–14.

Mastronarde DN. 1997. Dual–axis tomography: an approach with alignment methods that preserve resolution. Journal of Structural Biology 120:343–352.

Mastronarde DN. 2005. Automated electron microscope tomography using robust prediction of specimen movements. Journal of Structural Biology 152:36–51.

Masuko K. 1996. Temporal division of labor among workers in the ponerine ant, *Amblyopone silvestrii* (Hymenoptera: Formicidae). Sociobiology 28:131–150.

McCluskey ES. 1967. Circadian rhythms in female ants, and loss after mating flight. Comparative Biochemistry and Physiology 23:665–677.

McCluskey ES, Carter CE. 1969. Loss of rhythmic activity in female ants caused by mating. Comparative Biochemistry and Physiology 31:217–226.

McDonald P, Topoff H. 1985. Social regulation of behavioral development in the ant, *Novomessor albisetosus* (Mayr). Journal of Comparative Psychology 99:3.

McEwen BF, Marko M. 1998. Three–dimensional transmission electron microscopy and its application to mitosis research. Methods in Cell Biology 61:81–111.

McEwen BF, Marko M. 2001. The emergence of electron tomography as an important tool for investigating cellular ultrastructure. Journal of Histochemistry & Cytochemistry 49:553–563.

McIntosh JR. 2001. Electron microscopy of cells. The Journal of Cell Biology 153:25–32.

McIntosh JR, Nicastro D, Mastronarde D. 2005. New views of cells in 3D: an introduction to electron tomography. Trends in Cell Biology 15:43–51.

Meinertzhagen IA. 2001. Plasticity in the insect nervous system. Advances in Insect Physiology 28:84–167.

Meinertzhagen IA. 1996. Ultrastructure and quantification of synapses in the insect nervous system. Journal of Neuroscience Methods 69:59–73.

Midorikawa M, Tsukamoto Y, Berglund K, Ishii M, Tachibana M. 2007. Different roles of ribbon–associated and ribbon–free active zones in retinal bipolar cells. Nature Neuroscience 10:1268-1276.

Mildner S, Roces F. 2017. Plasticity of daily behavioral rhythms in foragers and nurses of the ant *Camponotus rufipes*: influence of social context and feeding times. PLoS ONE 12:e0169244.

Mirenda JT, Vinson SB. 1981. Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta buren*. Animal Behaviour 29:410–420.

Mistlberger RE. 2009. Food–anticipatory circadian rhythms: Concepts and methods. European Journal of Neuroscience 30:1718–1729.

Mobbs PG. 1982. The brain of the honey bee *Apis mellifera*. I. The connections and spatial organization of the mushroom bodies. Philosophical Transactions of the Royal Society 1091:309–354.

Mobbs PG. 1984. Neural networks in the mushroom of the honeybee. Journal of Insect Physiology 30:43–58.

Moore D, Rankin MA. 1983. Diurnal changes in the accuracy of the honeybee foraging rhythm. The Biological Bulletin 164:471–482.

Moore D, Rankin MA. 1993. Light and temperature entrainment of a locomotor rhythm in honeybees. Physiological Entomology 18:271–278.

Mota T, Yamagata N, Giurfa M, Gronenberg W, Sandoz J–C. 2011. Neural organization and visual processing in the anterior optic tubercle of the honeybee brain. The Journal of Neuroscience 31:11443–11456.

Muenz TS, Groh C, Maisonnasse A, Le Conte Y, Plettner E, Rössler W. 2015. Neuronal plasticity in the mushroom body calyx during adult maturation in the honeybee and possible pheromonal influences. Developmental Neurobiology 75:1368–1384.

Müller S. 2012. Circadiane Rhythmen in Lokomotion und Bruttrageverhalten bei *Camponotus* Ameisen: Zeitgeber und Synchronisation. Masterthesis (University of Würzburg)

Muscedere ML, Djermoun A, Traniello JFA. 2013. Brood–care experience, nursing performance, and neural development in the ant *Pheidole dentata*. Behavioral Ecology and Sociobiology 67:775–784.

Muscedere ML, Willey TA, Traniello JFA. 2009. Age and task efficiency in the ant *Pheidole dentata*: young minor workers are not specialist nurses. Animal Behaviour 77:911–918.

Nakata K. 1995. Age polyethism, idiosyncrasy and behavioural flexibility in the queenless ponerine ant, *Diacamma sp.* Journal of Ethology 13:113–123.

Neder R. 1957. Allometrisches Wachstum von Hirnteilen bei drei verschieden großen Schabenarten. Doctoral Dissertation.

Nene WA, Rwegasira GM, Nielsen MG, Mwatawala M, Offenberg J. 2016. Nuptial flights behavior of the African weaver ant, *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) and weather factors triggering flights. Insectes Sociaux 63:243–248.

Nielsen MG. 1981. Diurnal foraging activity of two ant species, *Myrmica schencki* Emery and *Formica rufibarbis* F., in a sandy heath area. Natura Jutlandica 19:49–52.

Nobua Behrmann B, Lopez de Casenave J, Milesi F, Farji–Brener A. 2017. Coexisting in harsh environments: temperature–based foraging patterns of two desert leafcutter ants (Hymenoptera: Formicidae: *Attini*). Myrmecological News 25:41–49.

North RD. 1993. Entrainment of the circadian rhythm of locomotor activity in wood ants by temperature. Animal Behaviour 45:393–397.

O'Donnell S, Bulova SJ. 2007. Worker connectivity: a review of the design of worker communication systems and their effects on task performance in insect societies. Insectes Sociaux 54:203–210.

O'Donnell S, Donlan NA, Jones TA. 2004. Mushroom body structural change is associated with division of labor in eusocial wasp workers (*Polybia aequatorialis*, Hymenoptera:

Vespidae). Neuroscience Letters 356:159–162.

Oberg EW, Del Toro I, Pelini SL. 2012. Characterization of the thermal tolerances of forest ants of New England. Insectes sociaux 59:167–174.

Oettler J, Johnson RA. 2009. The old ladies of the seed harvester ant *Pogonomyrmex rugosus*: foraging performed by two groups of workers. Journal of Insect Behavior 22:217–226.

Oland LA, Marrero HG, Burger I. 1999. Glial cells in the developing and adult olfactory lobe of the moth Manduca sexta. Cell and Tissue Research 297:527–545.

Oliveira PS, Klitzke C, Vieira E. 1995. The ant fauna associated with the extrafloral nectaries of *Ouratea hexasperma* (Ochnaceae) in an area of cerrado vegetation in Central Brazil. Entomologist's Monthly Magazine 131:77–82.

Oster G, Wilson EO. 1978. Caste and ecology in the social insects. Princeton University Press, Princeton, NJ.

Paranjpe DA, Sharma VK. 2005. Evolution of temporal order in living organisms. Journal of Circadian Rhythms 3:7.

Pasch E, Muenz TS, Rössler W. 2011. CaMKII is differentially localized in synaptic regions of kenyon cells within the mushroom bodies of the honeybee brain. Journal of Comparative Neurology 519:3700–3712.

Paulk AC, Dacks AM, Phillips–Portillo J, Fellous J–M, Gronenberg W. 2009. Visual processing in the central bee brain. The Journal of Neuroscience 29:9987–9999.

Paulk AC, Gronenberg W. 2008. Higher order visual input to the mushroom bodies in the bee, *Bombus impatiens*. Arthropod Structure & Development 37:443–458.

Paulk AC, Phillips–Portillo J, Dacks AM, Fellous J–M, Gronenberg W. 2008. The processing of color, motion, and stimulus timing are anatomically segregated in the bumblebee brain. The Journal of Neuroscience 28:6319–6332.

Peakin GJ, Josens G. 1978. Respiration and energy flow. International Biological Programme.

Penczek P, Marko M, Buttle K, Frank J. 1995. Double–tilt electron tomography. Ultramicroscopy 60:393–410.

Peng R, Nielsen MG, Offenberg J, Birkmose D. 2013. Utilisation of multiple queens and pupae transplantation to boost early colony growth of weaver ants *Oecophylla smaragdina*. Asian Myrmecology 5:177–184.

Pfeiffer K, Kinoshita M, Homberg U. 2005. Polarization–sensitive and light–sensitive neurons in two parallel pathways passing through the anterior optic tubercle in the locust brain. Journal of Neurophysiology 94:3903–3915.

Pittendrigh CS. 1954. On temperature independence in the clock system controlling emergence time in *Drosophila*. Proceedings of the National Academy of Sciences 40:1018–1029.

Pittendrigh CS. 1960. Circadian rhythms and the circadian organization of living systems. In: Cold Spring Harbor symposia on quantitative biology. Vol. 25. Cold Spring Harbor Laboratory Press. p 159–184.

Porter SD, Jorgensen CD. 1981. Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? Behavioral Ecology and Sociobiology 9:247–256.

Porter SD, Tschinkel WR. 1985. Fire ant polymorphism: the ergonomics of brood production. Behavioral Ecology and Sociobiology 16:323–336.

Porter SD, Tschinkel WR. 1987. Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): Effects of weather and season. Environmental Entomology 16:802–808.

Raff MC, Whitmore A V, Finn JT. 2002. Axonal self–destruction and neurodegeneration. Science 296:868–871.

Ress D, Harlow ML, Schwarz M, Marshall RM, McMahan UJ. 1999. Automatic acquisition of fiducial markers and alignment of images in tilt series for electron tomography. Microscopy 48:277–287.

Retana J, Cerdá X. 1990. Social organization of *Cataglyphis cursor* ant colonies (Hymenoptera, Formicidae): Inter- and intraspecific comparisons. Ethology 84:105–122.

Retana J, Cerdá X. 2000. Patterns of diversity and composition of Mediterranean ground ant communities tracking spatial and temporal variability in the thermal environment. Oecologia 123:436–444.

Ribeiro PL, Camacho A, Navas CA. 2012. Considerations for assessing maximum critical

temperatures in small ectothermic animals: insights from leaf–cutting ants. PLoS One 7:e32083.

Robinson EJH, Feinerman O, Franks NR. 2009. Flexible task allocation and the organization of work in ants. Proceedings Biological Sciences/ The Royal Society 276:4373–4380.

Robinson GE. 1992. Regulation of division of labour in insect societies. Annual Review of Entomology 37:637–665.

Roces F. 1995. Variable thermal sensitivity as output of a circadian clock controlling the bimodal rhythm of temperature choice in the ant *Camponotus mus*. Journal of Comparative Physiology A 177:637–643.

Roces F, Núñez JA. 1989. Brood translocation and circadian variation of temperature preference in the ant *Camponotus mus*. Oecologia 81:33–37.

Roces F, Núñez JA. 1995. Thermal sensitivity during brood care in workers of two *Camponotus* ant species – circadian variation and its ecological correlates. Journal Of Insect Physiology 41:659–669.

Roces F, Núñez JA. 1996. A circadian rhythm of thermal preference in the ant *Camponotus mus*: masking and entrainment by temperature cycles. Physiological Entomology 21:138–142.

Roenneberg T, Daan S, Merrow M. 2003. The art of entrainment. Journal of Biological Rhythms 18:183–194.

Roenneberg T, Foster RG. 1997. Twilight times: Light and the circadian system. Photochemistry and Photobiology 66:549–561.

Rosengaus RB, Traniello JFA. 1991. Biparental care in incipient colonies of the dampwood termite *Zootermopsis angusticollis* Hagen (Isoptera: Termopsidae). Journal of Insect Behavior 4:633–647.

Rosengren R. 1971. Route fidelity, visual memory and recruitment behaviour in foraging wood ants of the genus *Formica* (Hymenoptera, Formicidae). Acta Zoologica Fennica 133:1–101.

Rwegasira RG, Mwatawala M, Rwegasira GM, Mogens GN, Offenberg J. 2015. Comparing different methods for trapping mated queens of weaver ants (*Oecophylla longinoda*; Hymenoptera: Formicidae). Biocontrol Science and Technology 25:503–512.

Rybak J, Menzel R. 1993. Anatomy of the mushroom bodies in the honey bee brain: the neuronal connections of the alpha–lobe. The Journal of Comparative Neurology 334:444–465.

Santini G, Tucci L, Ottonetti L, Frizzi F. 2007. Competition trade-offs in the organisation of a Mediterranean ant assemblage. Ecological Entomology 32:319–326.

Saunders DS. 2002. Insect clocks. Elsevier.

Savolainen R, Vepsäläinen K. 1988. A competition hierarchy among boreal ants: impact on resource partitioning and community structure. Oikos 51:135–155.

Savolainen R, Vepsäläinen K. 1989. Niche differentiation of ant species within territories of the wood ant *Formica polyctena*. Oikos 56:3–16.

Sawyer LA, Hennessy JM, Peixoto AA, Rosato E, Parkinson H, Costa R, Kyriacou CP. 1997. Natural variation in a *Drosophila* clock gene and temperature compensation. Science 278:2117–2120.

Schindelin J, Arganda–Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B. 2012. Fiji: an open–source platform for biological–image analysis. Nature Methods 9:676–682.

Schlichting M, Helfrich–Förster C. 2015. Photic entrainment in *Drosophila* assessed by locomotor activity recordings. Methods in Enzymology 552:105–123.

Schmid–Hempel P. 1990. Reproductive competition and the evolution of work load in social insects. The American Naturalist 135:501–526.

Schmid B, Helfrich–Förster C, Yoshii T. 2011. A new ImageJ plug–in "ActogramJ" for chronobiological analyses. Journal of biological rhythms 26:464–467.

Schmitt F, Stieb SM, Wehner R, Rössler W. 2016. 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*. Developmental Neurobiology 76:309–404.

Schmitt F, Vanselow JT, Schlosser A, Kahnt J, Rössler W, Wegener C. 2015. Neuropeptidomics of the carpenter ant *Camponotus floridanus*. Journal of Proteome Research 14:1504–1514.

Scholl C, Kübert N, Muenz TS, Rössler W. 2015. CaMKII knockdown affects both early and late phases of olfactory long–term memory in the honeybee. Journal of

Experimental Biology 218:3788–3796.

Scholl C, Wang Y, Krischke M, Mueller MJ, Amdam GV, Rössler W. 2014. Light exposure leads to reorganization of microglomeruli in the mushroom bodies and influences juvenile hormone levels in the honeybee. Developmental Neurobiology 74:1141–1153.

Schultz TR. 2000. In search of ant ancestors. Proceedings of the National Academy of Sciences 97:14028–14029.

Schürmann FW. 1973. Über die Struktur der Pilzkörper des Insektenhirns. Zeitschrift für Zellforschung und mikroskopische Anatomie 285:247–285.

Schürmann FW, Elekes K, Geffard M. 1989. Dopamine–like immunoreactivity in the bee brain. Cell and Tissue Research 256:399–410.

Schwander T, Rosset H, Chapuisat M. 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. Behavioral Ecology and Sociobiology 59:215–221.

Seid MA, Harris KM, Traniello JFA. 2005. Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. Journal of Comparative Neurology 488:269–277.

Seid MA, Traniello JFA. 2006. Age–related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): A new perspective on temporal polyethism and behavioral plasticity in ants. Behavioral Ecology and Sociobiology 60:631–644.

Seid MA, Wehner R. 2008. Ultrastructure and synaptic differences of the boutons of the projection neurons between the lip and collar regions of the mushroom bodies in the ant, *Cataglyphis albicans*. Journal of Comparative Neurology 507:1102–1108.

Seid MA, Wehner R. 2009. Delayed axonal pruning in the ant brain: A study of developmental trajectories. Developmental Neurobiology 69:350–364.

Sendova–Franks AB, Hayward RK, Wulf B, Klimek T, James R, Planqué R, Britton NF, Franks NR. 2010. Emergency networking: famine relief in ant colonies. Animal Behaviour 79:473–485.

Shapley H. 1920. Thermokinetics of *Liometopum apiculatum Mayr*. Proceedings of the National Academy of Sciences 6:204–211.

Sharma VK. 2003. Period responses to zeitgeber signals stabilize circadian clocks during entrainment. Chronobiology International 20:389–404.

Sharma VK, Lone SR, Goel A, Chandrashekaran MK. 2004. Circadian consequences of social organization in the ant species *Camponotus compressus*. Naturwissenschaften 91:386–390.

Sheata MN, Kaschef AH. 1971. Foraging activities of *Messor aegyptiacus emery* (Hym., Formicidae). Insectes sociaux 18:215–226.

Shi NN, Tsai C, Camino F, Gary D. 2015. Keeping cool: Enhanced optical reflection and heat dissipation in silver ants. Science 349:298–301.

Slezak M, Pfrieger FW. 2003. New roles for astrocytes: regulation of CNS synaptogenesis. Trends in Neurosciences 26:531–535.

Soares PAO, Delabie JHC, Zanuncio J, Serrao J. 2008. Neural plasticity in the brain of workers of the carpenter ant *Camponotus rufipes* (Hymenoptera: Formicidae). Sociobiology 51:705–718.

Sorensen A, Busch TM, Vinson SB. 1984. Behavioral flexibility of temporal subcastes in the fire ant, *Solenopsis invicta*, in response to food. Psyche 91:319–332.

Steiger U. 1967. Über den Feinbau des Neuropils im Corpus pedunculatum der Waldameise. Cell and Tissue Research 81:511–536.

Stephan FK. 2002. The "other" circadian system: food as a Zeitgeber. Journal of Biological Rhythms 17:284–292.

Stieb SM, Hellwig A, Wehner R, Rössler W. 2012. Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. Developmental Neurobiology 72:729–742.

Stieb SM, Muenz TS, Wehner R, Rössler W. 2010. Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Developmental Neurobiology 70:408–423.

Strausfeld NJ. 1976. Some quantitative aspects of the fly's brain. In: Atlas of an Insect Brain. Springer. p 49–55.

Strausfeld NJ. 1989. Beneath the compound eye: neuroanatomical analysis and physiological correlates in the study of insect vision. In: Facets of vision. Springer. p

317–359.

Strausfeld NJ. 2002. Organization of the honey bee mushroom body: representation of the calyx within the vertical and gamma lobes. Journal of Comparative Neurology 450:4–33.

Strausfeld NJ, Blest AD. 1970. Golgi studies on insects. 1. Optic lobes of Lepidoptera. Philosophical Transactions of the Royal Society of London Series B 258:81-134.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. Learning & Memory 5:11–37.

Strausfeld NJ, Nässel DR. 1981. Neuroarchitectures serving compound eyes of Crustacea and insects. In: Autrum H, editor. Handbook of Sensory Physiology. Springer Berlin Heidelberg. p 34–41.

Strausfeld NJ, Obermayer M. 1976. Resolution of intraneuronal and transynaptic migration of cobalt in the insect visual and central nervous systems. Journal of Comparative Physiology A 110:1–12.

Symonowicz B, Kieruzel M, Szczuka A, Korczyńska J, Wnuk A, Mazurkiewicz PJ, Chiliński M, Godzińska EJ. 2015. Behavioral reversion and dark–light choice behavior in workers of the red wood ant *Formica polyctena*. Journal of Insect Behavior 28:245-256.

Tautz J, Maier S, Groh C, Rössler W, Brockmann A. 2003. Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proceedings of the National Academy of Sciences 100:7343–7347.

Technau G, Heisenberg M. 1982. Neural reorganization during metamorphosis of the corpora pedunculata in *Drosophila melanogaster*. Nature 295:405–407.

Thomas ML, Elgar MA. 2003. Colony size affects division of labour in the ponerine ant *Rhytidoponera metallica*. Die Naturwissenschaften 90:88–92.

Thomas ML, Holway DA. 2005. Condition-specific competition between invasive Argentine ants and Australian *Iridomyrmex*. Journal of Animal Ecology 74:532–542.

Trujillo–Cenóz O, Melamed J. 1962. Electron microscope observations on the calyces of the insect brain. Journal of Ultrastructure Research 7:389–398.

Truman JW, Reiss SE. 1976. Dendritic reorganization of an identified motoneuron during

metamorphosis of the tobacco hornworm moth. Science 192:477–479.

Ullian EM, Christopherson KS, Barres BA. 2004. Role for glia in synaptogenesis. Glia 47:209–216.

Uylings HBM, Kuypers K, Veltman WAM. 1978. Environmental influences on the neocortex in later life. Progress in Brain Research 48:261–274.

Ventura R, Harris KM. 1999. Three–dimensional relationships between hippocampal synapses and astrocytes. Journal of Neuroscience 19:6897–6906.

Volkmar FR, Greenough WT. 1972. Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science 176:1445–1447.

Vowles DM. 1955. The structure and connexions of the *Corpora pedunculata* in bees and ants. Quarterly Journal of Microscopical Science 96:239–255.

Waddington SJ, Hughes WOH. 2010. Waste management in the leaf–cutting ant *Acromyrmex echinatior*: the role of worker size, age and plasticity. Behavioral Ecology and Sociobiology 64:1219–1228.

Wagh DA, Rasse TM, Asan E, Hofbauer A, Schwenkert I, Dürrbeck H, Buchner S, Dabauvalle M–C, Schmidt M, Qin G. 2006. Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in *Drosophila*. Neuron 49:833–844.

Wagner D, Brown MJF, Broun P, Cuevas W, Moses LE, Chao DL, Gordon DM. 1998. Task–related differences in the cuticular hydrocarbon composition of harvester ants, *Pogonomyrmex barbatus*. Journal of Chemical Ecology 24:2021–2037.

Wagner D, Tissot M, Cuevas W, Gordon DM. 2000. Harvester ants utilize cuticular hydrocarbons in nestmate recognition. Journal of Chemical Ecology 26:2245–2257.

Wagner D, Tissot M, Gordon D. 2001. Task–related environment alters the cuticular hydrocarbon composition of harvester ants. Journal of Chemical Ecology 27:1805–1819.

Wang S, Zhang S, Sato K, Srinivasan MV. 2005. Maturation of odor representation in the honeybee antennal lobe. Journal of Insect Physiology 51:1244–1254.

Warrant EJ, McIntyre PD. 1990. Limitations to resolution in superposition eyes. Journal of Comparative Physiology A 167:785–803.

Waser NM. 1998. Task–matching and short–term size shifts in foragers of the harvester ant, *Messor pergandei* (Hymenoptera: Formicidae). Journal of Insect Behavior 11:451–462.

Watson AHD, Schürmann F. 2002. Synaptic structure, distribution, and circuitry in the central nervous system of the locust and related insects. Microscopy Research and Technique 56:210–226.

Watts RJ, Hoopfer ED, Luo L. 2003. Axon pruning during *Drosophila* metamorphosis: Evidence for local degeneration and requirement of the ubiquitin–proteasome system. Neuron 38:871–885.

Watts RJ, Schuldiner O, Perrino J, Larsen C, Luo L. 2004. Glia engulf degenerating axons during developmental axon pruning. Current Biology 14:678–684.

Weber NA. 1946. Dimorphism in the african *Oecophylla* worker and an anomaly (Hym.: Formicidae). Annals of the Entomological Society of America 39:7–10.

Weeks JC, Truman JW. 1986. Steroid control of neuron and muscle development during the metamorphosis of an insect. Journal of Neurobiology 17:249–267.

Wehner R. 2003. Desert ant navigation: How miniature brains solve complex tasks. Journal of Comparative Physiology A 189:579–588.

Wehner R, Herrling PL, Brunnert A, Klein R. 1972. Periphere Adaptation und Zentralnervöse Umstimmung im Optischen System von *Cataglyphis bicolor* (Formicidae, Hymenoptera). Revue Suisse de Zoologie.

Wehner R, Meier C, Zollikofer C. 2004. The ontogeny of foraging haviour in desert ants, *Cataglyphis bicolor*. Ecological Entomology 29:240–250.

Wehner R, Rössler W. 2013. Invertebrate learning and memory: bounded plasticity in the desert ant's navigational tool kit. Elsevier Inc, Chapter 39.

Weidenmüller A, Mayr C, Kleineidam CJ, Roces F. 2009. Preimaginal and adult experience modulates the thermal response behavior of ants. Current Biology 19:1897–1902.

Weir JS. 1958. Polyethism in workers of the ant *Myrmica*. Insectes Sociaux 5:97–128.

Wheeler DE. 1986. Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. The American Naturalist 128:13–34.

Wheeler DE. 1991. The developmental basis of worker caste polymorphism in ants. The

American Naturalist 138: 1218–1238.

Wheeler WM. 1933. Colony–founding among ants. Harvard University Press.

Whitford WG, Ettershank G. 1975. Factors affecting foraging activity in Chihuahuan desert harvester ants. Environmental Entomology 4:689–696.

Wilson EO. 1968. The ergonomics of caste in the social insects. American Naturalist 102:41–66.

Wilson EO. 1971. The insect societies. (Harvard University/Belknap Press, Cambridge M, editor.).

Wilson EO. 1978. Division of labor in fire ants based on physical castes (Hymenoptera: Formicidae: *Solenopsis*). Journal of the Kansas Entomological Society 51:615–636.

Wilson EO. 1980. Caste and division of labor in leaf–cutter ants (Hymenoptera: Formicidae: *Atta*). Behavioral Ecology and Sociobiology 7:143–156.

Winston ML. 1991. The biology of the honey bee. Harvard University Press.

Withers GS, Fahrbach SE, Robinson GE. 1993. Selective neuroanatomical plasticity and division of labour in the honey bee. Nature 364:238–240.

Withers GS, Fahrbach SE, Robinson GE. 1995. Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. Journal of Neurobiology 26:130–144.

Witthöft W. 1967. Absolute Anzahl und Verteilung der Zellen im Hirn der Honigbiene. Zeitschrift für Morphologie der Tiere 61:160–184.

Yasuyama K, Meinertzhagen IA, Schürmann F. 2002. Synaptic organiozation of the mushroom body calyx in *Drosophila melanogaster*. Journal of Comparative Neurology 445:211–226.

Yilmaz A, Aksoy V, Camlitepe Y, Giurfa M. 2014. Eye structure, activity rhythms, and visually–driven behavior are tuned to visual niche in ants. Frontiers in Behavioral Neuroscience 8:205.

Yilmaz A, Lindenberg A, Albert S, Grübel K, Spaethe J, Rössler W, Groh C. 2016. Age–related and light–induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar–feeding ant *Camponotus rufipes*. Developmental

Neurobiology 76:1041–1057.

Solutions for Immunohistochemistry

Solutions for Immunofluorescence Stainings

Ant Ringer

Sol A, dissolved in 900 ml dest.:		Sol B, dissolved in 100 ml dest.:	
	Molar mass [mM]		Molar mass [mM]
NaCl	130.00	Na_2HPO_4	8.00
KCl	5.00	KH_2PO_4	1.40
$CaCl_2$	5.00		

After mixing Sol A and Sol B, Tes and Trehalose was added and the pH was adjusted to 7.2.

Formaldehyde Fixative (FA)

10 ml of 16% formaldehyde (EM Grade, No. 15710, Electron Microscopy Sciences, Washington) diluted in 30 ml phosphate buffered saline (PBS; pH 7.2).

Phosphate Buffered Saline (PBS)

	Molar mass [mM]
Sodium chloride (NaCl)	130.00
Potassium chloride (KCl)	2.70
Disodium phosphate (Na_2HPO_4)	8.00
Monopotassium phosphate (KH_2PO_4)	1.40

Distilled water was adjusted to pH 7.2 with sodium hydroxide (NaOH) and hydrogen chloride (HCl)

Phosphate Buffered Saline with Triton X–100 (PBST)

0.2% Triton X–100 (AppliChem GmbH, Darmstadt, Germany) diluted in PBS (pH 7.2).

Solutions for Electron Tomography

Karnovsky Fixation

2% paraformaldehyde, 2.5% glutardialdehyde, 0.1 M cacodylate (pH 7.2); 2% DMSO

To produce 4% formaldehyde stock solution, 1g paraformaldehyde is mixed with 25 ml 0.2M cacodylatebuffer (pH 7.2) and heated with a Bunsen burner until the paraformaldehyde is solved.

Producing 20 ml Fixanz	Amount [ml]
4% Formaldehyde in 0.2 M Cacodylate	10.00
25% Glutardialdehyde	2.00
H_2O dest.	7.60
2% DMSO	0.40

2% Osmium

Use 500 µl osmium (4% stock solution in H_2O bidest.) and mix with 330 µl cacodylate (0.3 M) and 170 µl H_2O bidest.

Epon

Epoxy mixture	Component	Amount [g]
Glycidether	A	97.10
Dodecenylsuccinic anhydride (DDSA)	A	130.80
Glycidether	B	90.00
Methylnadicanhydride (MNA)	B	81.37

To produce component A, mix glycidether and DDSA; for component B mix glycidether with MNA. For hardening mix 3 parts of A with 7 parts of B and add 1.5 – 2.0% dimethylaminomethylphenol (DMP–30).

List of Abbreviations

AL	antennal lobe
AOT	anterior optic tract
AOTU	anterior optic tubercle
asot	anterior superior optical tract
BRP	bruchpilot
CaMKII	calcium/calmodulin–dependent protein kinase II
CB	central body
CO	collar
CT_{max}	maximal critical temperature limits
CT_{min}	minimal critical temperature limits
D	dense region
DD	constant darkness
E	entrainment
ET	electron tomography
F	free–run
f–actin	filamentous actin
GLMM	general linearized mixed model
IOC	inferior optic commissure
IQR	interquartile range
JH	juvenile hormone
KC	Kenyon cell

LA	lamina
lALT	lateral antennal lobe tract
LCA	lateral calyx
LD	light–dark
LI	lip
LO	lobula
LTM	long term memory
MB	mushroom body
MCA	medial calyx
ME	medulla
MG	microglomeruli
MS	methyl salicylate
ND	non–dense region
OL	optic lobe
PBS	phosphate buffered saline
PED	peduncle
PN	projection neuron
POC	posterior optic commissure
rH	relative humidity
S	phase–shift
SDE	standard deviation error
TEM	transmission electron microscopy
ZT	zeitgeberzeit

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Full Paper

Yilmaz A*, **Lindenberg A***, Albert S, Grübel K, Späthe J, Rössler W and Groh C (2016) Age–related and light–induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar–feeding ant *Camponotus rufipes.* Developmental Neurobiology 76: 1041–1057 (* equally contributing first authors)

Conference Abstracts

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Affidavit

I hereby confirm that my thesis entitled “Timing of Sensory Preferences in *Camponotus* Ants” is the result of my own work. I did not receive any help or support from commercial consultants. All resources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Würzburg, 29.08.2017

Place, Date   Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation „Zeitliche Anpassung sensorischer Präferenzen in *Camponotus* Ameisen“ eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Würzburg, 29.08.2017

Ort, Datum   Unterschrift