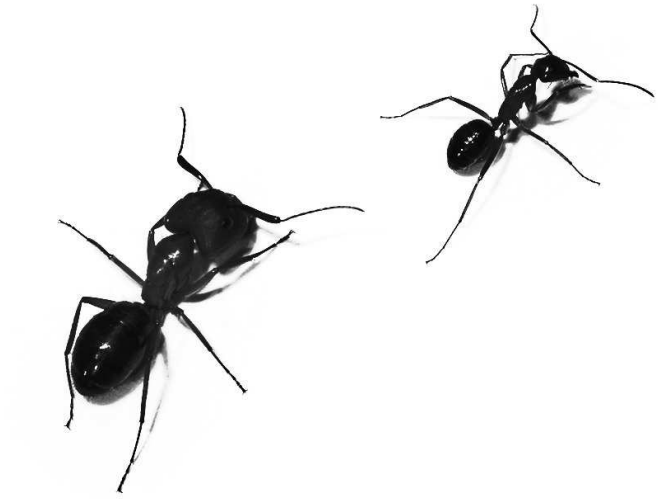
NEURONAL REPRESENTATION AND PROCESSING OF CHEMOSENSORY COMMUNICATION SIGNALS IN THE ANT BRAIN



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Vorgelegt von

Christina Zube

aus Wuppertal

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"Whatever course you decide upon, there is always someone to tell you that you are wrong. There are always difficulties arising which tempt you to believe that your critics are right. To map out a course of action and follow it to an end requires courage."

- Ralph Waldo Emerson -

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ABSTRACT

Ants heavily rely on olfaction for communication and orientation and ant societies are characterized by caste- and sex-specific division of labor. Olfaction plays a key role in mediating caste-specific behaviours. I investigated whether caste- and sex-specific differences in odor driven behavior are reflected in specific differences and/or adaptations in the ant olfactory system. In particular, I asked the question whether in the carpenter ant, *Camponotus floridanus*, the olfactory pathway exhibits structural and/or functional adaptations to processing of pheromonal and general odors.

To analyze neuroanatomical specializations, the central olfactory pathway in the brain of large (major) workers, small (minor) workers, virgin queens, and males of the carpenter ant *C. floridanus* was investigated using fluorescent tracing, immunocytochemistry, confocal microscopy and 3D-analyzes. For physiological analyzes of processing of pheromonal and non-pheromonal odors in the first odor processing neuropil , the antennal lobe (AL), calcium imaging of olfactory projection neurons (PNs) was applied.

Although different in total glomerular volumes, the numbers of olfactory glomeruli in the ALs were similar across the female worker caste and in virgin queens. Here the AL contains up to ~460 olfactory glomeruli organized in 7 distinct clusters innervated via 7 antennal sensory tracts. The AL is divided into two hemispheres regarding innervations of glomeruli by PNs with axons leaving via a dual output pathway. This pathway consists of the medial (m) and lateral (l) antenno-cerebral tract (ACT) and connects the AL with the higher integration areas in the mushroom bodies (MB) and the lateral horn (LH). M- and l-ACT PNs differ in their target areas in the MB calyx and the LH. Three additional ACTs (mediolateral - ml) project to the lateral protocerebrum only. Males had ~45% fewer glomeruli compared to females and one of the seven sensory tracts was absent. Despite a substantially smaller number of glomeruli, males possess a dual PN output pathway to the MBs. In contrast to females, however, only a small number of glomeruli were innervated by projection neurons of the m-ACT. Whereas all glomeruli in males were densely innervated by serotonergic processes, glomeruli innervated by sensory tract six lacked serotonergic innervations in the female castes. It appears that differences in general glomerular organization are subtle among the female castes, but sex-specific differences in the number, connectivity and neuromodulatory innervations of glomeruli are substantial and likely to promote differences in olfactory behavior.

Calcium imaging experiments to monitor pheromonal and non-pheromonal processing in the ant AL revealed that odor responses were reproducible and comparable across individuals.

Calcium responses to both odor groups were very sensitive (10^{-11} dilution), and patterns from both groups were partly overlapping indicating that processing of both odor classes is not spatially segregated within the AL. Intensity response patterns to the pheromone components tested (trail pheromone: nerolic acid; alarm pheromone: n-undecane), in most cases, remained invariant over a wide range of intensities (7-8 log units), whereas patterns in response to general odors (heptanal, octanol) varied across intensities. Durations of calcium responses to stimulation with the trail pheromone component nerolic acid increased with increasing odor concentration indicating that odor quality is maintained by a stable pattern (concentration invariance) and intensity is mainly encoded in the response durations of calcium activities. For n-undecane and both general odors increasing response dynamics were only monitored in very few cases.

In summary, this is the first detailed structure-function analyses within the ant's central olfactory system. The results contribute to a better understanding of important aspects of odor processing and olfactory adaptations in an insect's central olfactory system. Furthermore, this study serves as an excellent basis for future anatomical and/or physiological experiments.

INTRODUCTION

The olfactory sense represents the most important sensory modality for a large variety of species. Odors provide information about food quality and environmental cues as well as mating partners or kinship. Detection and identification of odors, therefore, is in most instances of essential impact for survival.

Olfaction and its underlying neuronal mechanisms are investigated in a large variety of species including vertebrates and invertebrates. The resulting knowledge revealed some basic similarities between the various systems. Insects, in general, are advantageous model systems for an integrative approach to investigate the neuronal basis of behavior. Their relatively small and easily accessible brains together with elaborated behaviors can be used for novel neuroethological approaches. Within the insects it is the ants that provide a very powerful group of highly olfactory oriented animals showing a large variety of complex olfactory-guided behaviors. However, the olfactory system in the ant brain has rarely been investigated so far, although ants provide highly interesting model systems to study neuronal olfactory processing in a behavioral context.

Behavioral complexity in ants is conditioned by olfactory stimuli to a great extent (Hölldobler, 1995). Odors play an essential role in ant communication and orientation. Ants utilize an enormous variety of pheromones in addition to environmental odors and cuticular substances controlling social behavior in ant colonies. Their proper recognition and processing, therefore, is essential for survival and reproductive success of an ant colony and requires a very precise sensory machinery and neuronal network in the brain of each individual. Another prominent feature within the ants is the appearance of morphologically different castes responsible for different tasks. Queens, males and workers as well as different worker castes differ in size and display different behaviors, which offers the unique opportunity to correlate morphological differences with differences in brain anatomy and/or brain physiology.

The high impact of olfaction together with these intraspecific polymorphisms associated with polyethism is what makes ants excellent model systems for a better understanding of olfaction and its underlying neuronal mechanisms.

1. THE OLFACTORY SENSE

In general, three categories of chemical communication signals can be determined for all species dealing with odor information:

1. **General odors** are mainly food odors or environmental cues. They provide information about food sources or the quality of food itself, they may help finding adequate nesting sites or are indicators for climate changes and threatening situations elicited by natural disasters or threads by enemies. These chemicals signals are not actively released by an individual in terms of communication but provide essential information about an individual's environment.

2. Chemical signals that are produced and released by individuals for explicit intra-species communication are termed **pheromones**. Lüscher and Karlson (1959) defined a pheromone as "a substance secreted to the outside of an individual and received by a second individual of the same species in which they release a specific reaction, for example a definite behavior or developmental process". A few years later in 1963 the definition was more specified by Wilson and Bossert who further classified pheromones as primer and releaser pheromones with releaser pheromones eliciting a very specific behavior and primer pheromones inducing longer lasting physiological changes to endocrine state or development of the receiving individual.

   These communication signals appear as relatively small molecules of high volatility to act as long distance signals. Such odors of high volatility allow for an efficient recruitment of many individuals at the same time and over a large spatial range which is the case in alarm- or recruitment pheromones in honeybees or ants. Furthermore, these odors enable single individuals to get in touch with mating partners over long distances in areas of low population density. In moths for example the female sex pheromone may travel a distance of several kilometers to reach a male individual.

3. Chemical communication signals acting over short distances appear as long chained and thus low volatile chemicals and serve primarily as **recognition signals**. They are highly species- or individual specific and provide detailed information about the releasing individual, acting as primer and/or releaser pheromone. In a large variety of species they are used as kin recognition signals. Ants were shown to use long chained hydrocarbons spread over the whole cuticle as for example information cue about kinship concerning

other colony members. Even slight variations in the cuticular hydrocarbon profile prove non kinship and elicit immediate aggressive behavior towards the non-nestmate. The queen within an ant colony takes advantage of such odors and transfers her own cuticular profile on her eggs to signalize her presence to the workers which thus are prevented from own reproduction (Endler et al., 2004). In the vertebrate system such low volatile chemicals serve as information cues about reproductive state or even indicate the genetic equipment concerning the immune system, and thus serve as mating cues when searching for adequate mating partners (Brennan and Zufall, 2006). In this respect the major histocompatibility complex (MHC), a group of genes coding for proteins involved in histocompatibility and immune reaction, may play an important role in mating behavior in vertebrates and will be discussed in the following.

2. PHEROMONE COMMUNICATION IN VERTEBRATES

The vertebrate olfactory system is made up of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), a blind-ended tube in the nasal epithelium. The MOE contains ciliated olfactory sensory neurons which project to the main olfactory bulb (MOB), the vomeronasal sensory neurons in the VNO have microvillar morphology and project to the accessory olfactory bulb (AOB) (see Fig. 1). Mice were shown to have almost 1,300 different olfactory receptors expressed on receptor neurons in the MOE (Zhang and Firestein, 2002).

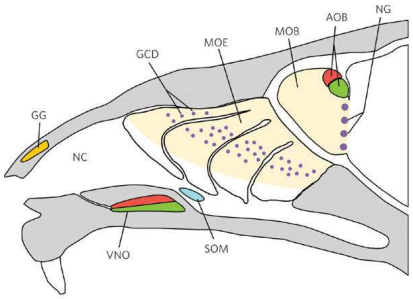

Fig. 1: The mouse olfactory system (Brennan and Zufall, 2006) is made up of the vomeronasal organ (VNO) with its target region, the accessory olfactory bulb (AOB), and the main olfactory epithelium (MOE) with its target region, the main olfactory bulb (MOB).

For a long time it was assumed that pheromone- and general odor processing is strictly separated in vertebrates. The MOB was thought to process general odors, whereas the VNO was said to exclusively process pheromones. This separation was questioned over the years when experiments with lesioned individuals showed that the MOB as well as the VNO system responded to pheromones as well as other low volatile chemicals or general odors. The nipple-search pheromone in rabbit pups, for example, is still detected after VNO lesion (Hudson and Distel, 1986).

In the vertebrate olfactory system chemicals of small molecular size and high volatility mostly serve as attractant or alarm pheromones. One of the most prominent substances in this context is Methylthiomethanethiol (MTMT), which is part of the male mouse urine and attracts female investigation (Lin et al., 2005). Low volatiles, such as proteins and peptides, serve as recognition pheromones in terms of mate choice and parent-offspring recognition. Here the major histocompatibility complex (MHC) plays a crucial role. The MHC represents a group of genes coding for proteins involved in histocompatibility and immune reaction. These proteins or their degradation products were found in urine or sweat, and their presence was shown to influence mating and kin recognition behavior. Laboratory mice for example preferentially mate with mice with dissimilar MHC as their own (Jordan and Bruford, 1998), and female mice preferentially feed pups of their own MHC genotype (Yamazaki et al., 2000). Even learning effects, such as olfactory imprinting, were shown to be MHC coupled in some cases. Here the "Bruce Effect" in mice represents the most common MHC dependent behavior. Female mice learn the male urinary chemical composition during mating. After being exposed to a different male or its urine, pregnancy is selectively blocked and the female mouse quickly becomes fertile again to mate with the new male (Leinders-Zufall et al., 2004).

3. PHEROMONE COMMUNICATION IN INSECTS

In non-social insects pheromone communication is mostly restricted to communication with putative mating partners via sex pheromones. By now one of the best investigated model system for sex pheromone processing is the moth olfactory system. Male moths have developed specific neuroanatomical and -physiological adaptations for the detection and processing of female sex-pheromones (Hansson et al., 1991; Mustaparta, 1996; Hildebrand and Shepherd, 1997). This system will be described in detail further below. Besides the moth pheromone system, very few other non-social species such as bugs, locusts or aphids were shown to use pheromone communication (Pickett et al., 1992; Torto et al., 1994; Cruz-Lopez et al., 2001). Sex-pheromones during mating is present in these species, too, but living in small groups, here without any significant social interaction, these species also use aggregation or alarm pheromones to organize their "living together" and to guarantee for species survival. One might assume that these systems may represent a pre-stage to chemically mediated organization of social behavior as it is found in social insects.

Social insects such as social bees, ants, social wasps and termites have extended and refined intraspecific communication via chemical cues. They are evolutionary very successful due to their social organization, and the underlying complex chemical communication system may play a crucial role in this context (Hölldobler, 1990; Hölldobler and Wilson, 2005). Division of labor is an important feature allowing an effective organization of different colony tasks. In social insect colonies it is mainly reflected in the appearance of morphologically different castes responsive for different tasks. Queens, males and workers as well as different worker castes express different sizes as well as different behaviors such as reproduction, rearing young, foraging for food, defending the nest or exploring new foraging grounds. The underlying chemical communication system is mainly mediated by pheromonal communication and helps to maintain the colony structure and productivity. Both are essential for the survival of the colony.

By now, honeybees and several species of ants represent the best investigated model systems for pheromone communication in social insects (Hölldobler, 1990; Le Conte and Hefetz, 2008.). In honeybees for example a prominent pheromone is the queen mandibular pheromone (QMP). Released by the queen it acts both as releaser and as primer pheromone, and in the latter suppresses amongst others the ovary development of female workers, thus preventing them from reproduction (Hoover et al., 2003; Slessor et al., 2005). A similar mutual control of reproduction (worker policing) has recently been shown in the carpenter ant *Camponotus floridanus* where cuticular hydrocarbons serve as queen recognition signal and eliminate worker reproduction (Endler et al., 2004).
In terms of defending the colony against predators or other threats each individual may use alarm pheromones to set the colony to an alerted state and induce aggressive behavior. Other pheromones such as aggregation pheromones induce aggregation behavior during swarming and are used as nest entrance or food source marker, just to mention the most important domains of pheromones in social insects (Schmidt et al., 1993; Deneubourg et al., 2002).

4. THE ANT OLFACTORY COMMUNICATION SYSTEM

Ant societies are characterized by a highly sophisticated caste system (queen, male and worker caste) with distinct differences in behavior which heavily depends on chemical communication and species-specific pheromones (Hölldobler and Wilson 1990). As mentioned before, trail-following behavior, alertness, recruitment or the signaling of the reproductive state are coordinated by the action of pheromones (Hölldobler, 1995). In addition, substances on the body-surface (cuticular hydrocarbons) serve as intra- and interspecific recognition cues affecting nestmate recognition as well as intra- and interspecies aggressive interactions (e.g. Singer, 1998; Lahav et al. 1999; Lenoir et al., 1999).

Ant communication systems are largely based on a variety of pheromones released from different glands that are distributed over the whole body (cp. Fig. 2). The different pheromones are usually composed of multiple components in different ratios that create a species-specific pheromone (Hölldobler, 1995). In general, they contain at least one or few major components which are already sufficient to initiate the behavior when exposed to an individual (Hölldobler, 1995).

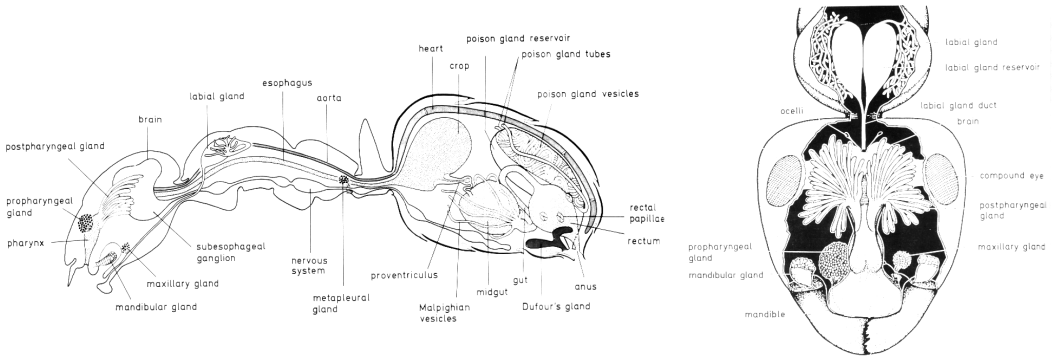

Fig. 2: Schematic drawing of a sagittal section through a *Formica* worker and a *Formica* worker head showing the major organs and exocrine glands (based on Otto, 1962 and Gösswald, 1985 and modified from Emmert, 1968).

In ants several types of pheromone interactions can be distinguished including worker-worker interaction, queen-worker communication, brood-coupled pheromones or pheromones released from the queen or males to manipulate mating partners or other queens in the colony (Van der Meer et al., 1998). In terms of worker-worker interaction alarm-pheromones and trail- or recruitment pheromones play an essential role in colony organization (Hölldobler, 1990). Alarm pheromones released from the mandibular-, the Dufour's- and the poison glands, as it is present in Formicine ants, are released in

response to stress or perturbations of the colony in order to alert colony members (Blum, 1985). Alarm behaviors may vary from species to species and reach from simple attractant to the pheromone source up to aggressive behavior such as biting, stinging or even spraying of poison gland products (Wilson, 1962; Hölldobler et al., 1990). In *Atta texicana* or *Pogonomyrmex badius* even an alarm pheromone-concentration dependent behavior was described (Wilson, 1958a; Moser et al., 1968). At low concentrations the ants are highly attracted to the pheromone source, whereas their behavior switches to aggressive alarm behavior with increasing concentrations. Based on these observations the term "active space" of alarm pheromones in ant communication was established. In many cases alarm-pheromone release is elicited as a consequence of the recognition of a non-colony member that intruded the colony. Such recognition processes are triggered via species- or colony-specific cuticular odor profiles termed as "cuticular hydrocarbon profiles" providing detailed information about the individual's origin (Fletcher and Michener, 1987).

As recruitment or trail-following signals worker ants use (in addition to invitational movements towards other colony members) chemical blends from their rectal- or Dufour's gland. After release of such pheromones nestmates are intended to either help with handling food or prey from incoming foragers or even follow foragers on the trail to profitable food sources to forage on their own (Hölldobler, 1990). Regarding social organization and the characteristic polythethism within the worker caste these odors appear as the most important chemical regulators.

Male and queen odor driven behavior is regulated in terms of mating behavior and worker reproduction. During mating periods males as well as females use pheromones to find an adequate mating partner. During the so called female-calling syndrome the females release pheromones to attract males. This was observed in species where females are often wingless or just fertile workers and do not move far away from the nest (Hölldobler, 1990). In winged species such as *Pheidole* queens gather in aerial swarms and attract males with pheromones (Hölldobler, 1990). In other so called male-aggregation syndromes it is the males that release pheromones from their mandibular glands when gathering at specific mating sites to attract virgin queens (seen in *Lasius* and *Solenopsis* – Hölldobler, 1990) and perform their nuptial flights. This emphasizes the highly variable mating systems across ant species and clearly shows that in insects it has not strictly to be the female caste which attracts the mating partner. In addition, queens were shown to manipulate other queens as well as workers via specific queen recognition pheromones. With such pheromones they indicate their presence

in the colony and thus prevent other queens and workers from self-reproduction (Hölldobler, 1990; LeConte and Hefetz, 2008). Here, the queen pheromone acts specifically as a primer pheromone and induces for example the suppression of ovary development in female workers. In contrast, the queen pheromone can also fulfill releaser pheromone function. Released from, for example, the poison gland, queen recognition pheromones lead to a strong attraction of workers towards the queen, formation of a dense cluster around her and initiate feeding and guarding behavior (Glancey et al., 1982; Edwards and Chambers 1984; Glancey, 1986). Besides this, the queen transfers a mixture of low volatile long chained cuticular hydrocarbons similar to her own cuticular profile on her eggs to manifest her presence within the whole colony (Endler et al., 2004). The awareness of queen presence within the colony may not only prevent workers but also other queens, as it is the case in polygynous colonies with at least two queens, from major reproduction (Keller and Nonacs, 1993). Under these circumstances the queen strengthens her dominant control position within the colony and thus claims the hierarchical structure.

5. THE OLFACTORY PATHWAY

5.1. Comparison of the organization and functional properties of primary olfactory centers in vertebrates and insects

The vertebrate nose and the insect antenna house several thousands of olfactory receptor neurons (ORNs) where odorant molecules are received and the chemical stimulus is transformed into an electrical signal. The first level of transformation of an olfactory stimulus starts with the reception of odorant molecules by odorant receptor proteins. These membrane-associated proteins are expressed in the ORNs. Each of these receptor neurons was shown to express one or two odorant receptor proteins (Clyne et al., 1999; de Bryune et al., 1999; Touhara et al. 1999; Rawson et al., 2000). Several approaches indicate that most odorant receptors have a broad molecular receptive range and can be activated by more than one odorant (Duchamp-Viret, 1999; Araneda et al., 2000; Stoertkuhl et al., 2001), others show a very narrow tuning and allow binding of only one specific molecule (Sakurai et al., 2004; Wilson and Meinen, 2006; de Bruyne and Baker, 2008). The latter very often belong to pheromone detecting systems. The way how odors or odorant molecules are recognized, therefore, depends on the kind of available receptor types and their activation profile. As a result an odor is characterized by a variable map of activated odorant receptors within the

olfactory epithelium. In the next step of olfactory transduction successful binding of an odorant molecule to a receptor protein results in an activation of the ORN.

Olfactory information is further transferred via the antennal nerve/olfactory nerve to the first odor processing neuropil, the antennal lobe (AL) in insects and the olfactory bulb (OB) in vertebrates (cp. Fig. 3). Both neuropils have a strikingly similar substructure, small spheroidal compartments of densely packed neuropil called glomeruli. Their number varies from species to species and ranges from approximately 50 - 450 in the fruitfly and the ant to up to 1000 in mice. It was shown in several species that ORNs expressing one receptor type converge on one or few common glomeruli, thus creating an odor specific glomerular map within the AL or OB, which is refined and shaped by interneurons within the AL neuronal network (Vassar et al., 1994; Mombaerts et al., 1996; Clyne et al. 1999; Rössler et al., 1999a, b; Vosshall et al 1999, 2000; Gao et al. 2000; Xu et al., 2000; Carlsson et al., 2002; Wang et al., 2003). Within glomeruli ORN axons converge on two types of second order neurons such as local neurons (LN) and projection neurons (PN) in insects and periglomerular cells and mitral cells in the vertebrate system (Hildebrand and Shepherd, 1997). Local neurons/periglomerular cells appear in uni- or multiglomerular shape and provide connections between single glomeruli within the AL/OB neuropil (Flanagan and Mercer, 1989a; Strausfeld and Hildebrand, 1999). Most of them were proven to be GABAergic and thus act in an inhibitory way in the neuronal network of the AL (Watson, 1990; Hildebrand et al., 1992; Bicker, 1999; Lledo et al., 2004). Recently also excitatory (cholinergic) local neurons were shown, extending the complexity of the glomerular odor processing network (Olsen et al., 2007; Shang et al., 2007).

Glomeruli, therefore, represent central structural and functional units within the AL and OB, the first odor processing stations (e.g. Hildebrand and Shepherd, 1997; Hansson and Anton, 2000; Galizia and Menzel, 2001; Sandoz, 2006). Functional imaging studies in insects and vertebrates demonstrated that the spatio-temporal activity pattern of AL glomeruli is essential for coding odor information in the central nervous system. For further processing and integration with other sensory modalities and for learning and memory processes the coded odor information is transferred to higher integration areas such as the mushroom bodies (MB) and the lateral protocerebral lobe (LPL) in insects and the olfactory cortex in vertebrates via olfactory PNs in insects and mitral and tufted cells in vertebrates. PNs and mitral/tufted cells usually have dendritic ramifications in a single glomerulus where they receive synaptic information from ORNs as well as LNs/periglomerular cells. The presence of these common organizational and physiological properties of the primary

olfactory centers in vertebrates and insects demonstrates that glomeruli have evolved as a key element in olfactory processing in both taxa. It is highly suggestive that glomeruli in insects and vertebrates are the result of convergent evolution (Hildebrand and Shepherd 1997).

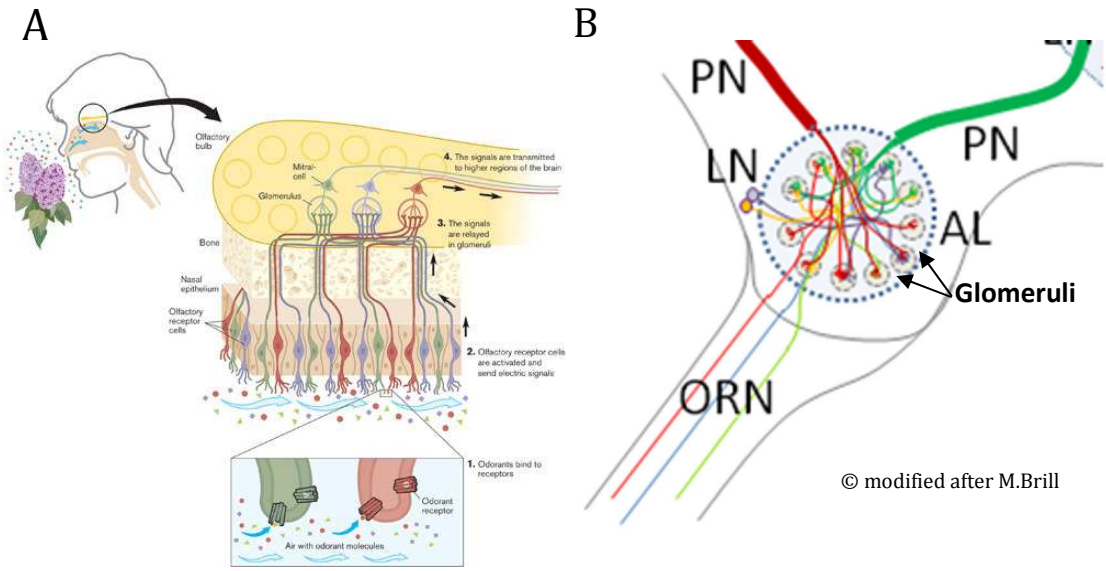

Fig. 3: The primary olfactory centers of vertebrates and insects. **A:** The primary vertebrate olfactory center, the OB (from Buck and Axel, 2004). **B:** The primary insect olfactory center, the AL. AL: antennal lobe; LN: local neurons; ORN: olfactory receptor neurons; PN: projection neuron.

5.2. The insect olfactory pathway: From the antenna to higher integration areas in the insect brain

Insect odor reception begins at the pairwise antennae, in many species consisting of a basal element – the scapus- , a pedicellus and a distal flagellum made of several antennal segments. On each antenna specialized sensilla house olfactory sensory neurons with specific odorant receptors. In the honeybee around 60000 receptor neurons (Esslen and Kaissling, 1967) are distributed in ~2600 olfactory *Sensilla placodea*. In *Drosophila* ~62 odorant receptors on 1300 ORNs mediate the odor reception (Shanbag et al. 1999; Vosshall, 2000; Hallem et al., 2004). Within the antenna ORN axons bundle to form the antennal nerve (AN) which proceeds to the AL. Upon entrance of the AL, ORN axons are sorted into glomerular specific fascicles (Rössler et al., 1990b). In the honeybee the AN splits into four antennal sensory tracts innervating specific groups of glomeruli, thus dividing the AL into distinct clusters (Suzuki, 1975; Abel et al. 2001; Kirschner et al, 2006). The information transfer from ORNs to second order neurons within the AL follows a strong convergence as it was shown, for

example, in the honeybee where ~60000 ORNS (Esslen and Kaissling, 1976) synapse on ~4000 LNs (Witthöft, 1967) and ~800 PNs (Bicker et al., 1993) emphasizing the integrative function of the AL neuronal network.

The processed odor information is subsequently transferred to higher integration areas via olfactory PNs that synapse onto ~ 170000 Kenyon Cells (KC) (in the honeybee) - MB intrinsic neurons, which transfer the information to the MB lobes (Strausfeld, 2002). Here integration with other sensory modalities as well as learning and memory foundation occurs (e.g. Strausfeld et al., 1998; Menzel, 2001; Strausfeld, 2002; Heisenberg, 2003). Two types of PNs were described in the insect olfactory system, the uni-glomerular and multi-glomerular PNs. Uni-glomerular PNs show dense ramifications in one single glomerulus and project to higher integration areas such as the MB calyces and the lateral horn (LH), a prominent structure in the lateral protocerebrum (Abel et al., 2001; Kirschner et al., 2006).

The MBs in the insect brain appear as paired structures consisting of a calyx, peduncle and lobes (Mobbs, 1982). The calyx represents a prominent sensory input zone and is a secondary odor processing neuropil within the insect brain. KC dendrites have intense ramifications within the calycal neuropil and send the received information to the MB peduncle and vertical and medial lobes (cp. Fig. 4) (Mobbs, 1982; Strausfeld, 2002).

The calyces within the honeybee brain appear as cupshaped structures that enclose ~ 170000 KC somata, with only few somata located at the outside of the calyces (Kenyon, 1896; Witthöft, 1967). The calyces here are further subdivided in lip, collar and basal ring region with the lip region as target region of olfactory PNs, the collar receiving exclusively visual information, and the basal ring as synaptic input region of both sensory modalities (see Fig. 4) (Mobbs, 1982; Gronenberg and Hölldobler, 1999; Gronenberg, 1999; Gronenberg and Lopez-Riquelme, 2004). Visual information is transferred to the collar and basal ring region via visual PNs from the ipsilateral lobula and the ipsi- and contralateral medulla (Gronenberg, 2001). These neurons were shown to proceed via a tract called the anterior superior optical tract (Gronenberg, 2001). It appears unique in social hymenoptera that the calyx receives visual input.

5.3. The dual olfactory pathway: A special feature in Hymenoptera?

In most insects investigated so far olfactory PNs project odor information from the AL to the MB and the LH mainly via one prominent tract, the medial or inner antenno-cerebral-tract (m-ACT or i-ACT). In the honeybee a "dual olfactory pathway" was characterized showing that odor information is transferred via two PN output tracts to the MBs and LH (cp. Fig. 4) (Abel et al., 2001; Kirschner et al., 2006). Besides the m-ACT, which runs through the medial part of the protocerebrum to the MB and then to the LH, a prominent lateral antenno-cerebral tract (l-ACT) proceeds through the lateral part of the protocerebrum to the LH first and then to the MB. Within the AL and the higher integration centers, m- and l-ACT PNs show a characteristic innervation pattern which reflects the duality of this pathway (Kirschner et al., 2006). Within the AL, glomeruli innervated by l-ACT PNs are exclusively located in the anterior part of the AL, whereas m-ACT innervated glomeruli form the posterior part of the AL, resulting in a hemispherical division of the AL glomeruli. This division is maintained in the MB where m-ACT PNs exclusively innervate the outer rim of the calyx lip region with only few synaptic boutons in the inner part of the lip. L-ACT PNs synapse more densely in the inner part of the lip but lack innervations in the outer rim (Kirschner et al., 2006). Segregation of PN input is also present in the LH with a club shaped region exclusively innervated by the m-ACT and an adjacent neuropil supplied only by l-ACT PNs indicating that olfactory information from glomeruli in the two AL hemispheres is transferred and processed via two uni-glomerular PN output channels (Kirschner et al., 2006). Comparison with other insect olfactory systems indicates that this "dual pathway" may be a special feature in Hymenoptera.

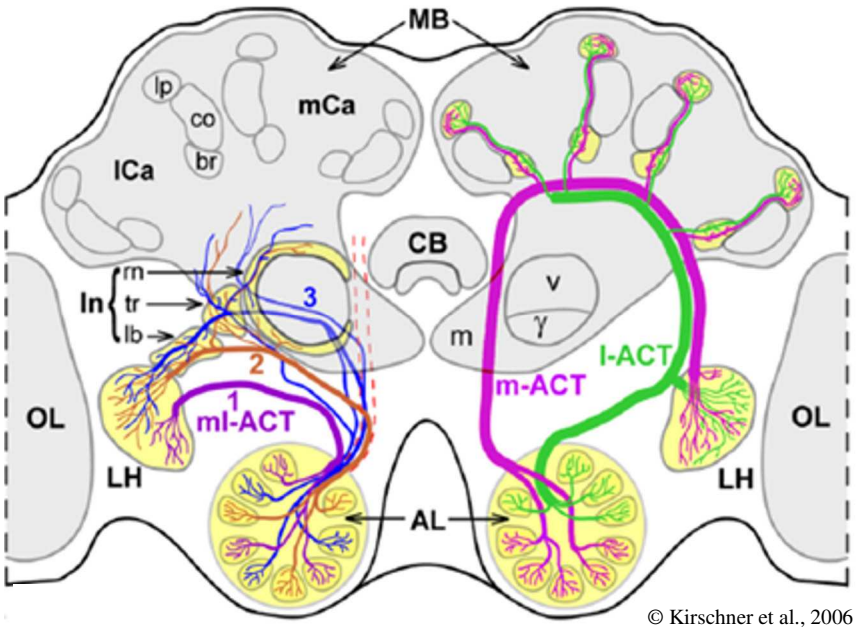

Fig. 4: Schematic drawing of the central olfactory pathway in the honeybee brain. On the left side the three multiglomerular ml-ACTs with their terminal projections in the LH and the LPL are shown. The right side shows the projections of uniglomerular PNs from the AL to the MB and the LH via the two m- and l-ACT tracts (for details see Kirschner et al., 2006).

6. PROCESSING OF OLFACTORY INFORMATION IN THE INSECT ANTENNAL LOBE/ THE VERTEBRATE OLFACTORY BULB

6.1. Optical imaging studies

A common feature of vertebrate and invertebrate odor processing within the OB and the AL is that the quality of an odor is reflected in an odor specific activation of AL glomeruli (glomerular chemotopic map). Each odor thereby activates a specific pattern of AL glomeruli in a combinatorial way (Rodrigues, 1988; Lieke, 1993; Ressler et al., 1994; Vassar et al., 1994; Joerges et al., 1996; Mombaerts et al., 1996; Friedrich and Korsching, 1997; Rubin and Katz, 1999; Uchida, 2000; Meister and Bonhoeffer, 2001; Carlsson et al., 2002). Such odor induced activation patterns were shown to be conserved between individuals and appear bilaterally symmetrical (Joerges et al., 1997; Galizia et al., 1998; Carlsson et al., 2001). Interestingly calcium imaging studies of AL glomerular activity comparing odorant ORN input and PN output pattern in the honeybee revealed a strong sharpening of the PN output pattern (Sachse and Galizia, 2002). The reason for the refined PN pattern is hypothesized to be found in the LNs and their contrast enhancing impact (Sachse and Galizia, 2002).

Since the odorant world consists of different types and classes of chemical stimuli the sensory network has evolved special coding properties to map functional groups and related odors. Chemically related odors are represented within the AL in adjacent patterns depending of chain length or chemical class (Joerges et al., 1997; Johnson and Leon, 2000; Wachowiak et al., 2000, 2002). Similarly, functional groups seem to activate specific but not necessarily adjacent areas within the odor processing neuropil (Sachse et al., 1999; Johnson and Leon, 2000; Meister and Bonhoeffer, 2001; Couto et al., 2007;).

Under natural conditions such odor stimuli may vary in signal intensity, so the quantity of an odor might also be reflected in the glomerular activation pattern. Several studies on vertebrates and invertebrates revealed that with increasing concentration the glomerular activation pattern was modified, resulting in a recruitment of additional glomeruli due to activation of unspecific receptors at the sensory neuron level (Rubin and Katz, 1999; Johnson and Leon, 2000; Fuss and Korsching, 2001; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Friedrich et al, 2002; Carlsson and Hansson, 2003; Wang et al., 2003). Although this is by now the most frequently observed mechanism, other studies report a concentration invariant activation pattern, at least over some concentrations (Joerges et al., 1997; Johnson and Leon, 2000; Wachowiak et al., 2000, 2002). Besides concentration dependent changes of the glomerular pattern, single glomeruli may vary in their response intensity when stimulated with different odor concentrations. In general, single glomeruli show an increase in signal intensity with increasing odor concentration (Friedrich and Korsching, 1997; Rubin and Katz, 1999; Meister and Bonhoeffer, 2001; Sachse and Galizia, 2003; Wang et al., 2003;) but in some species additionally a shift of the strongest activated glomeruli to neighboring glomeruli was described (Galizia et al., 2000; Fried et al., 2002; Carlsson and Hansson, 2003).

In addition, odor concentration can also be reflected in the duration of the neuronal signal. With increasing odor concentration the signal duration was described to be prolonged for up to several seconds until the basic level is reached again (Friedrich and Korsching, 1997; Rubin and Katz, 1999; Meister and Bonhoeffer, 2001; Sachse and Galizia, 2003; Wang et al., 2003). This clearly shows that odor concentration is not solely represented via one neuronal coding modality but appears to be represented by a combinatorial coding system.

6.2. Electrophysiological studies

Odor processing in the AL does not only occur in the described combinatorial way of glomerulus-activity, induced by simple neuronal on-off reactions with either excitatory and/or inhibitory effect. Assuming that a natural odorant is composed of many different single components the glomerular pattern enlarges with increasing blend composition and sooner or later reaches a spatial maximum of glomerulus activation. To overcome such limitations of odor processing, the central nervous system has to extend its coding properties to a second level, the temporal processing of odor information. There are evidences by different electrophysiological studies on the central nervous system that temporal patterns of neuronal activity additionally contribute to a far more complex odor coding mechanism (reviewed in Laurent, 1996). In this context it is not only the basic features of neuronal activity such as phasic, tonic or irregular action potential generation (spiking) and the associated spike frequency that physiologically characterize an odor stimulus. The functional and behavioral impact of temporal odor coding, however, are still a matter of debate (e.g. Christensen et al., 2000).

At single neuron level each odor elicits an odor specific activation pattern encoding specific odorant properties. Interestingly neurons of similar properties may in some cases be grouped together and may form united cell structures. This was indicated in the honeybee brain where the few PNs measured, running through either m- or l-ACT, elicited tract specific odor processing properties. Here neurons in the m-ACT coded odors via latency differences or specific inhibitory phases combined with excitatory phases, whereas neurons of the l-ACT coded odors by spike rate differences (Müller et al., 2002). However, further studies on this subject are required to strengthen this observation.

Reaching further at network level, important properties of odor processing appear to be oscillation and synchronization of odor induced activated cells. LNs and PNs are hypothesized to play an important role in terms of sharpening and particularizing the AL information for the MB and other higher brain centers. Different odor features appear to be represented by changing firing rates, changing temporal patterns or via synchronous firing of specific sets of PNs (Harrison and Scott, 1986; Heinbockel et al., 1998; Stopfer and Laurent, 2004; Kay and Stopfer, 2006). Depending on odorant identity and intensity odor specific subgroups of LNs and PNs show periods of increased and decreased activity and PNs frequently develop oscillatory synchronization (Laurent and Davidowitz, 1994; Wehr and Laurent, 1999; Laurent et al., 2001; Vickers et al., 2001). This might be induced by either ORNs (Wehr and Laurent, 1999) or influenced and controlled via for example GABAergic LNs (Bazhenov et al., 2001).

Here one argues that the network oscillations mediate and modulate the synchronized firing of PNs (Friedrich and Stopfer, 2001; Friedrich and Laurent, 2001) whereas other studies demonstrated that synchronous firing is odor and oscillation independent (Christensen et al., 2000; Lei et al., 2000; Vickers et al., 2001). The behavioral consequences of a disruption of such synchronization mechanisms were shown in the honeybee. Here the ability of odor discrimination after a desynchronization of PN answers led to strong deficits in discrimination of closely related odors (Stopfer et al., 1997; Hosler et al., 2000). Hence, an accurate temporal coding of chemical stimuli may be essential for fine odor discrimination and, in a next step, for proper odor learning and memory.

6.3. Processing of odor mixtures in the antennal lobe/olfactory bulb

Natural odorant stimuli rarely appear as one single component but, in general, are a complex mixtures of several components mostly represented at different relative concentrations (reviewed for example in Hölldobler and Wilson, 1995). How such complex odor compositions are represented in the nervous system has been investigated and discussed in vertebrates (Laing and Francis, 1989; Giraudet et al., 2002; Duchamp-Viret et al., 2003; Kay et al., 2003, 2005; Wiltrout et al., 2003; Tabor et al., 2004; Cometto-Muniz et al., 2005;) and invertebrates (Christensen and Hildebrand, 1987; Akers and Getz, 1993; Derby et al., 1996; Smith, 1998; Derby, 2000; Deisig et al., 2003; Heinbockel et al., 2004; Deisig et al., 2006; Carlsson et al., 2007; Silbering and Galizia, 2007). Two main theories may be formulated. One may assume on the one hand that odor mixtures are reflected in an activation pattern made up of a simple addition of the activation patterns of the single components. On the other hand a mixture may be represented in a neuronal activation pattern independent from the single odor patterns. To date optical imaging studies on mixture processing in the AL indicate that most mixture responses consist of a combination/addition of their single odor responses. In most cases the mixture patterns contain the response pattern of at least one component and, thus, are most likely predictable (Joerges et al., 1997; Giraudet et al., 2002; Deisig et al., 2006). It also occured that one component always dominated in the mixture (Giraudet et al., 2002). However, since the temporal and physiological properties of glomeruli and single neurons vary from single component to mixture it is difficult to predict a mixture response from its single component responses at the temporal level (Belluscio and Katz, 2001; Duchamp-Viret et al., 2003; Tabor et al., 2004; Deisig et al., 2006; Silbering and Galizia, 2007). In this context suppressive, synergistic or hypoadditive interactions at ORN up to PN level were observed and discussed to contribute to such complex mixture representations.

Data from optical imaging studies and electrophysiological experiments mostly revealed suppressive effects in the representation of the mixture compared to the single odors (Derby et al., 1985; De Jong and Visser, 1988; Joerges et al., 1997; Carlsson and Hansson, 2002; Silbering and Galizia, 2007). This means that the signal response to the mixture is significantly weaker than the strongest response to one component presented alone. In the honeybee this effect increased, i.e. the suppression was stronger, with increasing mixture components, but here primarily a hypoadditive effect dominated in binary mixtures (Deisig et al., 2006) which means that the mixture response is equivalent to the strongest single component. The responsibility for suppressive or synergistic effects at AL/OB level is thought to lie in the inhibitory effects of LNs which shape odor processing through lateral inhibition. At ORN level competitive binding mechanisms or inhibitory influences between transduction cascades may as well induce such phenomena at the very periphery (Ache et al.; 1988; Getz and Acers, 1994; Kang and Caprio, 1997). Due to studies that have been carried out so far, mixture processing at AL level is far from being understood.

7. MORPHOLOGICAL AND PHYSIOLOGICAL ASPECTS OF PHEROMONE PROCESSING IN THE INSECT CENTRAL NERVOUS SYSTEM

Within the insect AL some species have evolved significantly enlarged glomeruli, so called macroglomeruli, where pheromone processing occurs. Macroglomeruli are hypothesized to have evolved from ordinary glomeruli by ingrowth of increasing numbers of odor specific ORNs and thus obtain a higher sensitivity for specific odors. Macroglomeruli were first described in the moth olfactory system where sex-pheromone specific glomeruli form a macroglomerular complex (MGC) at the entrance of the male AL. The male AL contains an MGC of four enlarged glomeruli (cp. Fig. 5C) which exclusively process components of the female sex pheromone blend (e.g. Christensen and Hildebrand, 1987; Hansson et al., 1991; Hildebrand and Shepherd, 1997; Vickers et al., 1998; Hansson and Anton, 2000; Rospars and Hildebrand, 2000). Within each of those four glomeruli one specific pheromone component is represented indicating a very high specificity of this MGC system (Hansson et al., 1991; Hansson et al., 1992; Ochieng et al., 1995). Macroglomeruli were also described in the cockroach and honeybee drones (Fig. 5A) and here were also shown to be involved in sex-pheromone processing (Arnold et al., 1985; Malun et al., 1993; Anton and Homberg, 1999; Brockmann and Brückner, 2001; Sandoz, 2006). Similar to the moth MGC,

the honeybee drone MGs were shown to selectively process single components of the queen pheromone (Sandoz, 2006).

Macroglomeruli in connection with non-sexual pheromone processing were by now only described in leafcutter ants (Kleineidam et al., 2005). In female workers of the leafcutter ant species *Atta sexdens* and *Atta vollenweideri* a macroglomerulus is located at the entrance of the AL (Fig. 5B) and is supposed to play a crucial role in trail following behavior (Kleineidam et al., 2007). Interestingly, the macroglomerulus was absent in small workers indicating caste-specific and thus behavioral-specific differences in AL organization in leafcutter ants (Kleineidam et al., 2005).

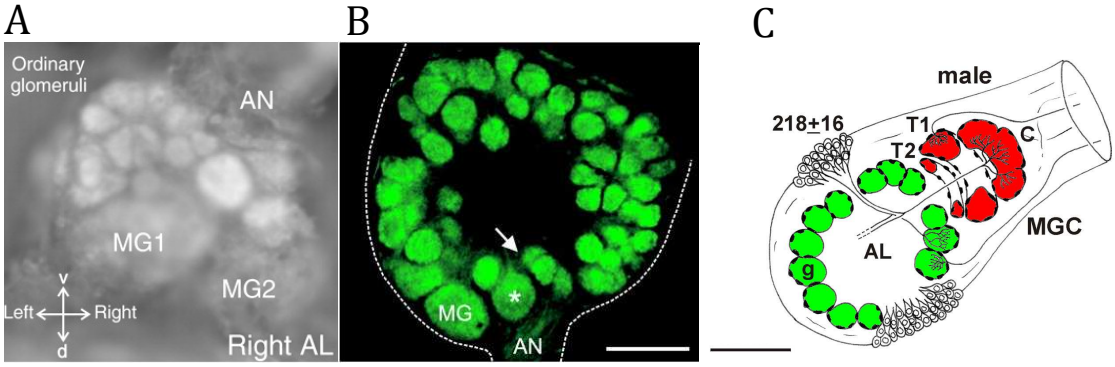

Fig. 5: Macroglomeruli (MG in **A** and **B**; MGC in **C**) in the insect AL: Macroglomeruli in the honeybee drone (**A**) (Sandoz, 2006), in the female worker ant (**B**) (Kleineidam et al., 2005) and in the male moth AL (**C**) (Rössler et al., 1999). Scale in B = 50µm and in C= 200µm.

As one might assume, not all insect species using pheromone communication may have evolved AL macroglomeruli for pheromone processing. This seems to be true for some other ant species, such as the carpenter ant *Camponotus obscuripes*, which is by now the only field of research on this topic (Yamagata et al., 2006). Intracellular recordings and stainings of alarm-pheromone responsive PNs in this species revealed selective innervations of one specific cluster of normally sized glomeruli within the posterior part of the AL. This indicates at least some degree of anatomical segregation of pheromone processing in the ant AL (Yamagata et al., 2006).

At the physiological level specific adaptations have evolved to facilitate highly specific odor selectivity and odor reliability. Pheromone sensitive ORNs with highly sensitive and narrow tuning to specific pheromones or pheromone components have been described in different species (Kaissling and Priesner, 1970; Berg et al., 1998; Nakagawa et al., 2005; Ozaki, 2005; Schlief and Wilson, 2007). In the moth MGC and in the fly, for example, they synapse on PNs

which were shown to exclusively respond to pheromone stimulation either, indicating that the sensitivity of a PN is correlated with/close to the sensitivity of its ORNs (Christensen and Hildebrand, 1987; Kanzaki et al., 1991, 1998; Mustaparta, 1996; Heinbockel and Hildebrand, 1998; Schlief and Wilson, 2007). Such fast and direct odor transmission was termed as a "labeled line" processing. Over the years this labeled line model was weakened by studies which showed that the physiological properties of the AL sex pheromone processing neurons can be largely variable and heterogeneous due to network-dependent interactions. PNs thus may receive input from several different ORNs and ORNs themselves may share odorant information from other AL glomeruli via the AL network.

Most information on this field of research is provided, again, from the moth olfactory system. At the ORN level, for example, firing rates were synchronized when the pheromone was presented in a binary mixture with a host plant component (Ochieng et al., 2002). At PN level it was shown that even pheromone processing neurons respond to non-pheromonal odors if the concentration is high enough (Hansson et al., 1989; Carlsson and Hansson, 2002), and although pheromone processing PNs are still highly selective to a single pheromone component, firing patterns of such PNs can be modulated by neurons innervating neighboring glomeruli, even by non-pheromone processing neurons/glomeruli (Christensen and Hildebrand, 1987; Christensen et al., 1991; Heinbockel et al., 1998; Vickers et al., 1998; Namiki et al., 2008). One study in the honeybee supported these findings by showing that PNs were activated by pheromones as well as non-pheromonal stimuli (Müller et al., 2002). These findings support at least some level of combinatorial coding properties rather than a strict labeled line system.

8. OBJECTIVES

My PhD-project focuses on the question of how olfactory stimuli are represented within the ant brain and what coding properties may account for odor processing. For this purpose the neuroanatomical and neurophysiological properties of the olfactory system in the carpenter ant, *Camponotus floridanus* were investigated. The colony structure of this highly eusocial ant species exhibits a pronounced polymorphism and polyethism among the queen, males and major and minor worker castes (see Fig. 6 - male not shown).

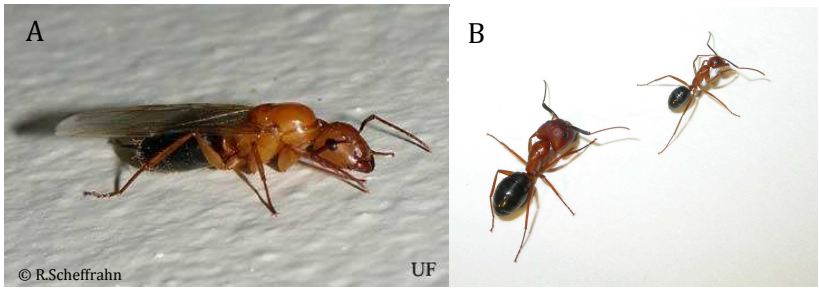

Fig. 6: Winged virgin queen (**A**) and major and minor workers (**B**) in the carpenter ant *Camponotus floridanus.*

The polymorphism and polyethism provides the advantage of correlating morphological differences in brain structure and physiological differences with caste specific differences in behavior. Thus, individuals of a *C. floridanus* colony (i.e. worker castes, males and young winged queens) were compared neuroanatomically to monitor common features and possible sex or task related differences in odor processing. Neurophysiological experiments were carried out on major workers to investigate general principles of pheromonal and non-pheromonal odor processing in the ant AL.

In my analyses of the olfactory pathway and neuronal odor representation within this ant species, the main emphasis was placed on the first olfactory neuropil, the AL. For a detailed characterization of this brain structure and its neuronal pathways to higher centers such as the MBs and LH, neuroanatomical and neurophysiological techniques were used.

Two main subjects of interest were investigated:

1. **Do caste- and sex-specific adaptations exist in the central olfactory pathway that may promote differences in olfactory processing and behavior?**

   A detailed neuroanatomical characterization of the AL of the different castes was performed to reveal anatomical differences between worker castes, winged females (young queens) and males within this first order relay station of olfactory information processing. Using confocal microscopy and 3D-reconstruction software a quantification of spatial organization and volumes of olfactory glomeruli within the AL provided detailed information about caste dependent plasticity within this brain region.
   Furthermore a detailed characterization of the input-output connections of the AL was performed using neuronal tracing techniques to reveal caste- and sex-specific differences in the central olfactory pathway. This was combined with immunostainings of one prominent neuromodulator, serotonin. Here, detailed analysis of serotonergic innervated glomeruli and the innervation pattern of the MB calyces were analyzed.

2. **How are pheromonal and non-pheromonal odors processed within the AL?**

   a. Does the AL have segregated regions for processing of pheromonal and non-pheromonal odors?
   b. Is the ant olfactory system specialized to detect minimal quantities of pheromonal signals, and is the system able to respond appropriately to large ranges of intensities as it is required for pheromone detection?
   c. Do pheromones receive a dominant representation in binary mixtures of pheromonal and non-pheromonal odors?

   Physiological investigation of neuronal activity within the AL of the major worker caste during odor stimulation were carried out to give an insight in how odors are represented and processed within the glomerular system of the AL. Here, optical registration of neuronal activity was performed using calcium imaging tools. Representation profiles of various odorants, such as general odors and pheromones are aimed to find out how the ant neuronal network processes olfactory information, how odor discrimination or categorization may take place at this processing level and what mechanisms may underlie the internal representation of different odor qualities.

MATERIALS AND METHODS

1. ANIMALS

Virgin queens, males and major and minor workers of the carpenter ant, *Camponotus floridanus*, were used for the experiments. For neuroanatomical experiments large workers (head width > 3mm; n=21), small workers (head width of < 1.5mm; n=25), virgin queens (head width > 2.7 mm; n=7) and males (head width < 1mm; n=70) were used. Workers and virgin queens were taken from a laboratory colony originally collected in Sugarloaf Shores, Florida, USA containing a single-mated founding queen. In two queenless subcolonies of the Sugarloaf Shore colony egg-laying workers provided the males for all experiments. For neurophysiological experiments ants were taken from a colony with a single-mated founding queen collected in Orchid Island, Florida, USA. Only large workers (head width > 3 mm, body length ~10 mm; n= 1083) were used. The colonies were reared under constant conditions in an environmental chamber at 25°C and 85% humidity in a 12 h / 12 h photoperiod. Animals were fed twice a week with honey water, bathkar and dead cockroaches. Fresh water was given once a week. Ants were anaesthetized with CO_2 and prepared for anatomical and physiological experiments as described below.

2. NEUROANATOMICAL PROCEDURES

2.1. Whole mount preparations

For visualization of brain structures, especially AL glomeruli, tissue autofluorescence caused by glutaraldehyde fixation was utilized. The ants were decapitated, and the head capsule fixed in dental-wax coated dishes. The head capsule was opened by cutting a window between the compound eyes, and the brain was rinsed immediately using fresh ant-saline solution (127 mM NaCl, 7 mM KCl, 1.5 mM $CaCl_2$, 0.8 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 4.8 mM TES, 3.2 mM Trehalose, pH 7.0). Glands and tracheae were removed, and the brains were dissected out and fixed immediately in cold 1% glutaraldehyde in phosphate-buffered saline (PBS; pH 7.2) for 4 days at 4°C. The brains were then washed in PBS (5 x 10 min) and dehydrated in an ascending series of ethanol (30%, 50%, 70%, 90%, 95%, 3 x 100%, 10 min each step). Finally, the brains were cleared in methylsalicylate (M-2047, Sigma Aldrich, Steinheim, Germany) and mounted in methylsalicylate in special aluminium slides with a central hole covered by thin cover slips from both sides. Whole mounts were stored at -20°C.

2.2. Tracer application and tissue preparation

For antennal backfill preparations, all animals were fixed in customized acrylic blocks, and the heads and antennae were stabilized with dental wax. Both antennae were cut at the lower part of the pedicellus. The cut surface was immediately covered with a drop of tetramethylrhodamindextrane with biotin (3000 MW, lysine-fixable; Microruby, D 7162, Molecular Probes, Eugene, USA) dissolved in distilled water, and the preparation was kept in a moistened chamber for five hours up to over night to let the dye diffuse. The brains were then dissected and fixed immediately in 4% formaldehyde in 0.1M PBS over night at 4°C. After washing with 0.1M PBS (3 x 10 minutes), the brains were dehydrated in an ascending alcohol series (30%, 50%, 70%, 90%, 95%, 3 x 100%, 10 min each step) and cleared and mounted in methylsalicylate (Fischer Scientific GmbH, Schwerte, Germany) on customized aluminium slides.

For labeling of the antenno-cerebral tracts (ACTs) to higher centers within the protocerebrum the ants were fixed in dental-wax dishes. The heads were fixed in an anterior preparation to access the front of the brain. A cut between the compound eyes was made to open the head capsule and glands and tracheae were removed. The tracer was applied into specific tissue areas to stain the tracts of interest (Kirschner et al., 2006). The dextran conjugate tetramethylrhodamine dextran with biotin was used for all experiments. The tracer was applied as follows: glass micropipettes were pulled with a horizontal laser-electrode puller (P2000, Sutter Instruments Co., Novato, USA) using borosilicate capillaries (1B100F-3, Precision Instruments, Sarasota, USA). The broken tip of each glass electrode was coated with small dye crystals (~200 µm in diameter). Prior to inserting the dye coated pipettes into brain tissue, the region of interest was carefully perforated with an unbroken glass pipette. Subsequently the dye electrode was plunged into the perforated area and remained for up to 10 seconds in the target area. The pipette was removed and the brain immediately rinsed with fresh ant-ringer solution to wash out excessive dye. To investigate the AL connections within the protocerebrum, dye was inserted directly into the AL neuropil (Fig. 7 position 2). The m-ACT was labeled by insertion of the dextran tracer in the medial-caudal protocerebrum at the position where the m-ACT exits the AL (compare position 3 in Fig. 7; Kirschner et al., 2006). After dye insertion, all preparations were kept in a moistened chamber for 4 hrs to let the dye diffuse and were then prepared for confocal microscopy as described above.

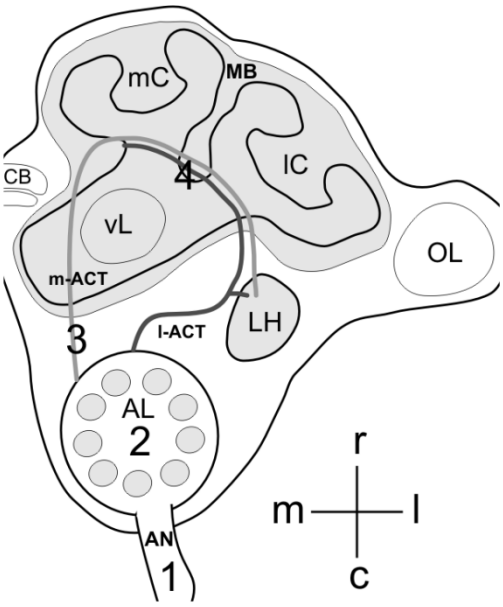

Fig. 7: Schematic drawing of the tracer application sites within the ant brain. The numbers indicate the locations of tracer application within the brain regions of interest. For further details see text. AN, antennal nerve; AL, antennal lobe; OL, optic lobes; LH, lateral horn; vL, vertical lobe; CB, central body; MB, mushroom body; lC, lateral calyx; mC, medial calyx; m- and l-ACT, medial and lateral antenna-cerebral tract; r, rostral; c, caudal; m, medial; l, lateral.

2.3. Immunocytochemistry - Serotonin labeling

Ants were anaesthetized and waxed in dishes as described above. The heads were covered with fresh ant ringer solution and the head capsule opened. After dissection, brains were immediately immersed in cold 4% formaldehyde in PBS overnight at 4°C. For sectioning the brains were then rinsed in PBS (3x 10 min), embedded in 5% low melting point agarose (Agarose II, No. 210-815, Amresco, Solon, OH) and carefully adjusted in a frontal plane. Brains were treated as whole mounts or sectioned at 100 µm thickness using a vibrating microtome (Leica VT 1000S, Nussloch, Germany), pre-incubated in PBS containing 0.2% Triton-X (PBST) and 5% normal goat serum (NGS; ICN Biomedicals, No. 191356, Orsay, France) for 1h at room temperature (Rössler et al., 2002). Sections and whole mounts were then incubated with a rabbit antiserum against serotonin (1:40000, DiaSorin, Stillwater, MN, Cat. No. 20080, Lot No. 051007) in PBST for two days at 4°C. After primary antibody incubation, agarose sections (or whole mounts) were rinsed in PBS (4 x 10 min) and incubated in Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (1:250; Molecular Probes, A-11008) in 1% NGS in PBS for 2h at room temperature. Sections were finally rinsed in six changes of PBS, transferred to 60% glycerol in PBS for 30 minutes, and mounted on slides in 80% glycerol in PBS. Whole mounts were dehydrated in an ascending ethanol series and cleared and mounted in methylsalicylate (see above).

2.4. Confocal laser-scanning microscopy and 3D-reconstructions of glomeruli and tracts

All brains were viewed using two confocal laser scanning microscopes (Leica TCS LP and Leica TCS SP2 AOBS; Leica Microsystems AG, Wetzlar, Germany) equipped with an argon/krypton and helium-neon laser. Excitation wavelengths were 568nm for tetramthylrhodamine dextran and 488nm for Alexa Fluor 488. Two different HC PL APO objective lenses were used for image acquisition (10 x 0.4 NA imm and 20 x 0.7 NA imm), and optical sections were taken at distances between 1-10 µm. In certain cases a digital zoom of 2-3x was applied. All confocal image stacks were viewed and processed with the 3D-reconstruction software AMIRA 3.1 (Mercury Computer Systems, Berlin, Germany). To obtain a better signal to noise ratio, some preparations were deconvoluted using the AMIRA deconvolution algorithm. Images were scaled, and snapshots were taken from single optical sections or complete stacks. Screenshots were further processed in Adobe Photoshop 6.0 and 7.0 software (Adobe Systems Inc. San José, USA) and adjusted for brightness and contrast. Anatomical directions refer to Strausfeld (2002) and Kirschner et al. (2006). Single glomeruli were clearly visible as densely packed neuropil structures and were outlined in all focal planes (yz,xy,xz). 3D-reconstructions of individual glomeruli were done using the Amira 3.1 feature “wrap”. Antennal sensory input tracts were rendered using the “interpolate” and “automatic threshold” feature. Individual glomeruli or cluster of glomeruli associated with the same antennal sensory input tracts (T1-T7) were color coded.

3. NEUROPHYSIOLOGICAL PROCEDURES

3.1. Preparation and dye loading for calcium imaging

Ants were briefly (for a few seconds) anesthetized with CO_2 and fixed in a plexiglas stage using soft dental wax (surgident periphery wax, Heraeus Kulzer, Germany). Compared to treatment with CO_2, anaesthetization by cooling on ice did not show any obvious differences in the responses to the odors tested. A small window was cut into the head capsule to access the ventral part of the brain and the site for dye application. A sharp glass electrode coated with few crystals of Fura-2 dextran (potassium salt, 10.000 MW, F3029, Molecular Probes, Eugene, USA) dissolved in 2% bovine serum albumin solution was inserted for several seconds into the lateral protocerebrum, dorsolaterally to the vertical lobe of the MBs, aiming for the m- and l-ACT (see Fig. 7 position 4). Following dye application, the brain was rinsed with ant-saline solution to remove excessive dye. The window in the head capsule was closed with the cut piece of cuticle, and the ants were released from the plexiglas stage for 4 hours.

During the staining period, the ants were allowed to move freely in a moistened plexiglas container before they were fixed again in the plexiglas stage as before. Antennae were immobilized with wax, and a larger window was cut into the head capsule to access the whole brain and the ALs. Glands and tracheae were carefully removed, and the esophagus was cut at the mouth parts and pulled out of the head capsule to prevent movement of the brain during data acquisition.

3.2. Calcium Imaging

Calcium-Imaging experiments were performed using an Olympus imaging system (Cell^R, Version 1.1-2.3) with an upright epifluorescent microscope (BX51WI; with the filter set UM2FUR) equipped with a LD 20x water- immersion lens (XLUMP, NA 0.95) and epifluorescent illumination was provided by a 150 W xenon light source (MT20, with excitation filters for 340 nm and 380 nm). The focal plane within the AL was adjusted to a depth of 35 µm below the surface of the AL using a piezo-driven nanofocusing system (PIFOC, PI, Germany). For each stimulus, a series of 24 double frames was recorded with an air cooled CCD-camera (model 8484-03G, Hamamatsu Photonics, Japan) at a sampling rate of 4 Hz. A 2 x 2 on chip binning resulted in an image pixel size of 0.645 µm x 0.645 µm. Exposure times ranged from 40-60 ms for the first frame at 340 nm and 20-30 ms for the second frame at 380 nm. Odor stimulation started at frame 10 and was terminated at frame 14 (1 s). Repeated odor stimuli were given at an inter stimulus time interval of at least 1 minute. Imaging data were analyzed by calculating the ratio of fluorescence intensity in the images taken at 340 and 380 nm excitation for each pair as: $R= F_{340} / F_{380}$, and subsequently applying an NxN filter (5x5 pixels with 5 iterations) to reduce noise. Autofluorescence and stained neurons caused inhomogeneous fluorescence images (background fluorescence), and by subtracting the average ratio-image using frames 1 to 9 from all ratio images, background was set to zero prior to odor stimulation. Filtered ratio-images with background subtraction are labeled as $\Delta(F_{340} / F_{380})$. Following odor stimulation, calcium signals were measured as changes in fluorescence $\Delta(F_{340} / F_{380})$ and considered as neuronal activity in response to a given odor stimulus when they exceeded 40% of the maximal response. The maximal response was measured across all different odors and concentrations tested in the preparation. Neuronal activities (activity areas) are represented as false-color-coded images using the average of frame 11 to 14 (during odor stimulation). In most cases, activity areas could not be assigned to single glomeruli because often their size was considerably larger than the size of single glomeruli. The circular shape and small size (10-40 µm) of other

activity areas were similar to the dimensions of single glomeruli and termed activity spots (see Figure 9 A-E).

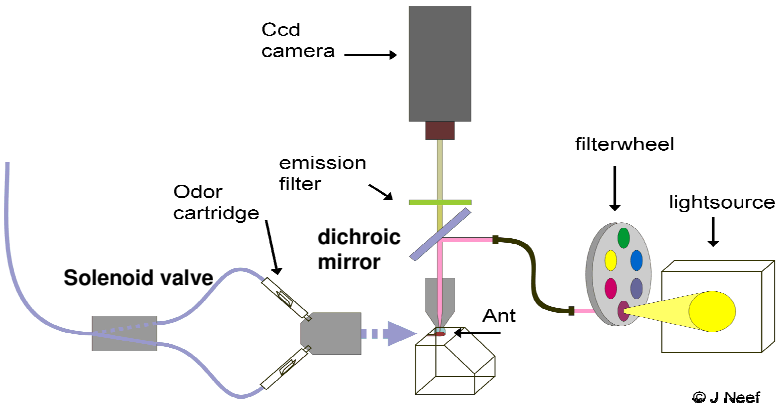

Fig. 8: Schematic drawing of the calcium imaging setup. The wavelength of the excitation light is controlled by a fast switching computer controlled filter wheel. The excitation light is reflected by a dichroic mirror onto the specimen. Emitted light passes the dichroic mirror and an emission filter and is recorded by an air cooled CCD-camera. For odor stimulation the air flow is switched from constant air stream to the odor cartridge via a computer controlled solenoid valve.

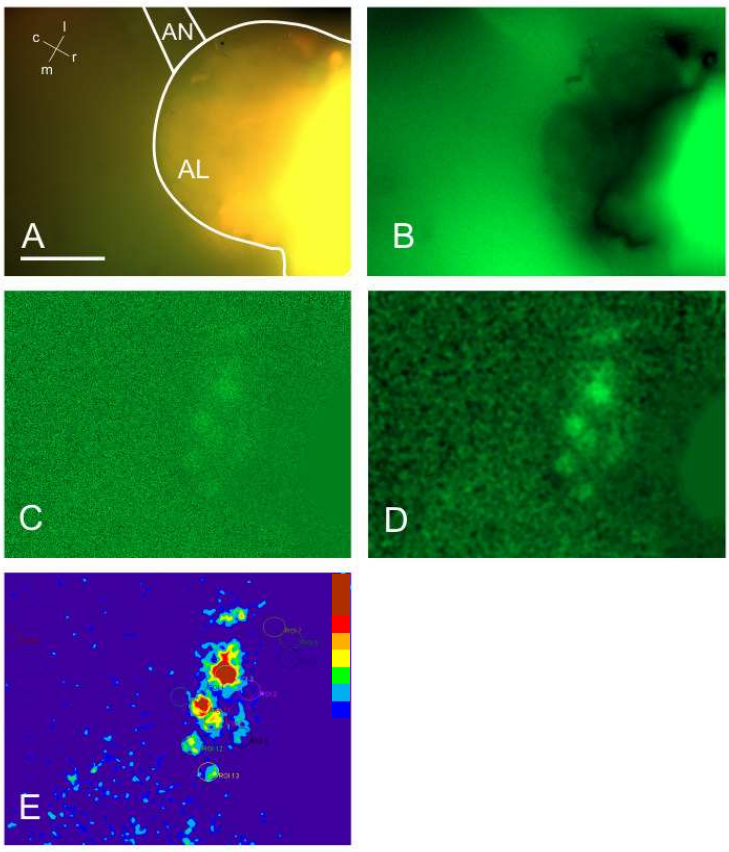

Fig. 9: Calcium imaging data processing procedure for visualization of neuronal activity within the AL. **A:** Fura2- raw fluorescence image of the AL. The position of the AN and the spatial directions are valid for all following false-color coded images. **B:** Ratio image of the fura2-raw fluorescence image. C: Ratio image after background correction. **D:** Noise reduced picture after application of a 5x5 filter with 5 iterations. **E:** false color coded view of the picture shown in D with regions of interest (ROI). AL, antennal lobe; AN, antennal nerve; r, rostral; c, caudal; m, medial; l, lateral. Scale=100µm.

3.3. Odor stimulation

A constant and moistened air stream of 1 l/min was produced by two independent flow controllers (VC-2LPM, Alicat Scientific, USA) both set to 0.5 l/min and connected to two solenoid valves controlled by the imaging software. The solenoid valves allowed switching of each of the two flow channels through a plastic cartridge (1 ml) containing a filter paper (1 cm^2). Only one of the two flow channels was used in the experiments. For odor stimulation, 5 µl of the odor diluted in mineral oil (Sigma Aldrich, Deisenhofen) was applied onto the filter paper, and the cartridge was placed into the olfactometer. Odor dilutions ranged from 10^{-1}-10^{-12}, and experiments always started with the lowest odor concentration. As control stimulus, 5 µl of mineral oil was applied onto the filter paper.

Eight different odors including two pheromones were used for stimulation. As general odors, citral, isoamylacetate (IAA), nerol, 1-hexanol, heptanal and 1-octanol (all from Sigma Aldrich, Deisenhofen) were used. As pheromones of *C.floridanus*, the alarm pheromone n-undecane (Sigma Aldrich, Deisenhofen) and the trail pheromone (releaser component) nerolic acid (Haak et al., 1996; Cardiff Chemicals, Cardiff, Great Britain) were used for odor stimulation (see Fig. 10).

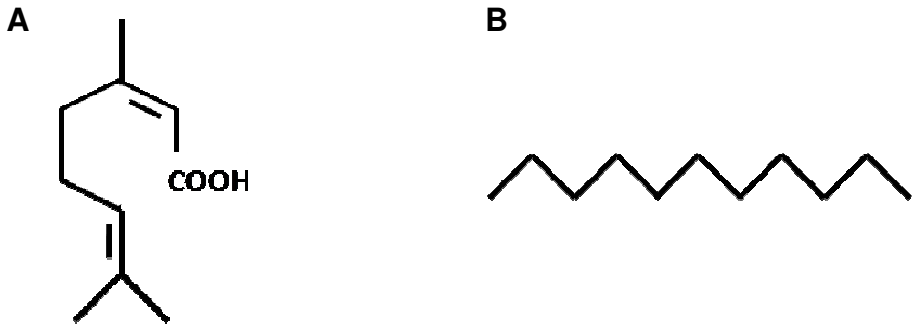

Fig. 10: Molecular structure of the tested pheromones nerolic acid and n-undecane. **A:** The releaser component of the trail pheromone in *C.floridanus*, nerolic acid (cis-3.7-Dimethyl-2.6-octadienoic acid; C10H16O2; MW 168.23) **B:** n-undecane (C11H24 MW 156.31) acts in various Camponotus species as a trigger of alarm behavior.

Activity areas were described for all odors, but not all odors were tested in each experiment. Odor representation across animals was investigated using heptanal, isoamylacetate, citral, and nerolic acid. The overlap of activity areas in response to different odors and the threshold concentration for each single odor were investigated using nerolic acid, n-undecane, citral, heptanal and octanol. The duration and the change of signal amplitude of the calcium signal across different odor concentrations were investigated for the nerolic acid, n-undecane, heptanal and octanol. For analysis of odor mixtures the four odors were first applied in a

single odor stimulation and then applied randomly as binary mixtures with the three other odors tested.

3.4. Experimental procedures and data analysis

The spatial overlap of activity areas in response to two odors was calculated as the percentage of an odor specific activity area that was activated by the other odor. All pixels within the AL with intensity values above threshold (40% of the maximal response) during stimulation with one odor were counted and compared with the odor specific activity areas elicited by one of the 4 other odors using the software AMIRA 3.1 (Mercury Computer Systems, Germany). Calcium signals in a total of 5 animals and in response to 5 different odors were used for this analysis.

The threshold odor concentrations were measured in 4 to 11 animals using the threshold criterion mentioned above. In order to analyze the dynamic range of the calcium responses at the different odor concentrations, at least two areas with the highest calcium-signal amplitudes (circular regions of interest, ROIs) were selected and normalized within each animal. The calcium-signal amplitude within the ROIs and the duration (number of frames) was measured across all tested odor concentrations (dilutions for duration analysis: 10^{-11}, 10^{-8}, 10^{-5} and 10^{-2} for nerolic acid and 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, 10^{-3} and 10^{-1} for the other three odors). To analyze whether signal amplitude and duration correlate with odor intensity, two ROIs of each preparation were separated according to either showing clear concentration independent calcium-signal amplitudes (cd-) or concentration dependent calcium-signal amplitudes (cd+). Signal durations within the resulting two classes of ROIs were compared using a Spearmans-rank correlation on pooled data of animals tested on Nerolic Acid. For all other odor data sets a Friedman Anova was applied. This was due to specimens where a clear characterization of ROI_{cd+} and ROI_{cd-} was not possible and only ROIs with either cd+ or cd- were present. These pooled data were thus dependent data and requested a different statistical test (here Friedman Anova).

In odor mixture experiments the Euclidian distances between single odor representation and single odor representation in binary mixtures were calculated for heptanal, octanol, n-undecane and nerolic acid. The Euclidian distances were calculated to analyze to what extend a single odor is represented in a binary mixture.

The Euclidian distance is defined as follows:

$$d_{ij} = \sqrt{\sum_{k=1}^{p} (X_{ik} - X_{jk})^2}$$

with *i* and *j* indicating odors, *p* the number of dimensions – here number of glomeruli investigated- and X_{ik} the response of glomerulus k to odor i and X_{jk} as the response of glomerulus k to odor j. Statistical analysis for each odor and its representation in a binary mixture with the three other odors tested was performed based on the Euclidian distance data sets using a Friedman ANOVA.

4. SOLUTIONS FOR IMMUNOCYTOCHEMISTRY AND PHYSIOLOGY

Ant Ringer

Solution A: dissolve in 900ml Aqua dest.
NaCL 7.4 gr
KCl 0.5 gr
$CaCl_2$ 0.22 gr
Solution B: dissolve in 100ml Aqua dest.
Na_2HPO_4 0.11 gr
KH_2PO_4 0.05 gr
when solution A and B have been put together, add
Tes 1.1 gr
Trehalose 1.2 gr
adjust pH to 7.0

Fixative

1% Glutaraldeyde in 0.1M PBS – pH 7.2
(Glutaraldehyde Solution Grade I, G-5882, 25%, Sigma Aldrich, Steinheim, Germany)

Phosophate Buffer PB

Na_2HPO_4	162 mM	22.94 gr/l
NaH_2PO_4	38 mM	5.25 gr/l

0.2M stock solution diluted with aqua dest. to 0.1M and adjusted to pH 7.2

PBS

NaCL	137.0 mM	40.03 gr/l
KCl	2.7 mM	1.0 gr/l
Na_2HPO_4	8.0 mM	7.2 gr/l
KH_2PO_4	1.4 mM	0.95 gr/l

1 volume of this solution plus 4 volumes H_2O dest. and adjusted to pH 7.2 with NaOH and HCl

Phosphate buffered saline with Triton X-100 (PBST)

0.2% Triton X-100 diluted in PBS (pH 7.2)

RESULTS

1. NEUROANATOMY

1.1. Caste- and sex-specific features of the antennal lobes

To identify caste- and sex-specific differences within the primary olfactory centers of *Camponotus floridanus*, I performed 3D-reconstructions of the complete brains and analyzed the organization of antennal-lobe (AL) glomeruli. Figure 11 shows typical examples of whole brain (left side) and AL reconstructions (right column). They are compared at the same scale for a large (major) and a small (minor) worker (Fig. 11A, B), a virgin queen (Fig. 11C), and a male of *C. floridanus* (Fig. 11D). Qualitative comparison of the brain reconstructions already showed that the female castes (Figure 11A-C) have relatively small optic ganglia and large ALs, whereas the male brain (Figure 11D) contains comparatively larger optic lobes, smaller ALs and relatively small mushroom bodies. This emphasizes the importance of olfactory cues for orientation of the female castes and the relative importance of the visual system in male ants (Hölldobler and Wilson, 1990; Gronenberg and Hölldobler, 1999). 3D-reconstructions of AL glomeruli revealed substantial differences among the female castes and males. The total volume of male ALs (Figure 11D) was smaller compared to the ALs of all female castes (Figure 11 A-C), and the AL contained a smaller number of glomeruli (Table 2). In major workers, between 434 and 464 glomeruli were found in a previous study based on six preparations (Zube et al., 2008). In the ALs of two preparations of minor workers we counted 478 and 480 glomeruli and in two preparations of virgin queens we found 476 and 483 glomeruli indicating that the total number of glomeruli is in a similar range in the female castes. The total AL glomerular volume, however, was different among minor and major workers and queens (Table 2). In three preparations of male ALs the total number of glomeruli was only 240, 250, and 254, and the AL glomerular volume was substantially smaller (Table 2). Calculation of the relative AL volume revealed a substantially smaller value in males compared to all female castes (mean relative AL volume of 13-14% in all female castes compared to 9% in males).

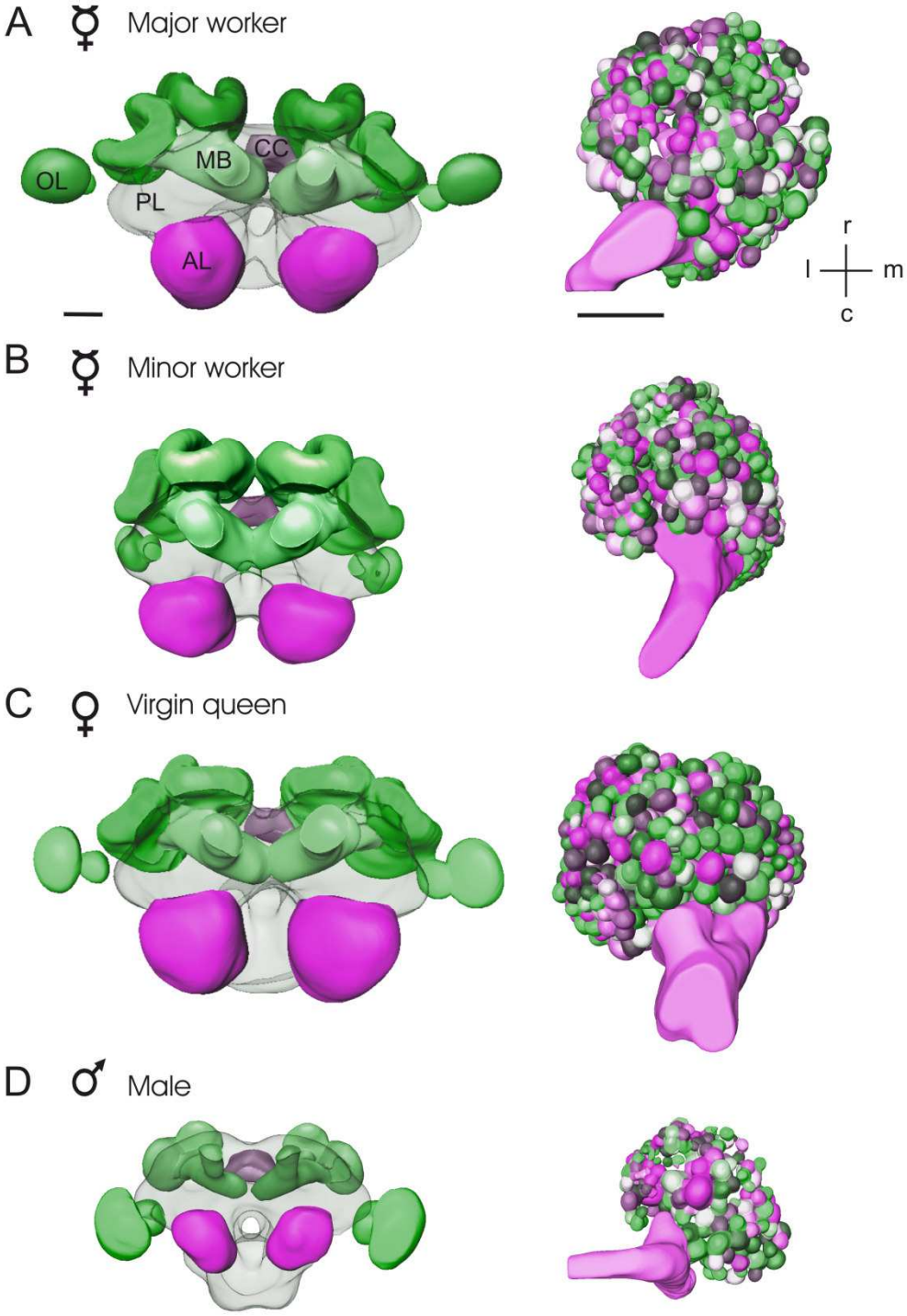

Fig. 11: 3D-reconstructions of the major brain neuropils (left column) and of the antennal lobe glomeruli (right column) from female castes and males of Camponotus floridanus. **A, B, C, D (left column):** 3D- reconstructions of the brain of a major worker (A), minor worker (B), virgin queen (C) and male (D) with major neuropils. The right side shows examples of 3D-reconstructions of all glomeruli in the antennal lobes. The different colors (green, magenta and white) and saturation levels of individual glomeruli were assigned randomly to enhance visibility and contrast. Central Complex, CC; mushroom body, MB; optic lobe, OL; lateral protocerebral lobe, PL; antennal lobe, AL. Directions: rostral, r; caudal, c; medial, m; lateral, l. Scale in A (also valid for B-D) = 100µm.

I identified one enlarged glomerulus at a similar location (~125µm focal depth) in the AL of all female castes and in males. Surprisingly, no specifically enlarged other glomeruli were found in the male AL. Figure 12 shows confocal images of the ALs of the female and male castes of *C.floridanus* at the focal plane of this enlarged glomerulus. The large glomerulus belonged to the T4 cluster according to the nomenclature by Zube et al. (2008) (see below). The glomerulus was located very close and lateral to the T4 at a position along about halfway on its course across the AL (Fig. 12A-D; arrows). Because of its relatively deep position within the AL, the glomerulus is hidden behind other glomeruli in the 3D views shown in Fig. 11 and 14. I calculated the mean volume of all glomeruli in the AL and determined the volumes of the largest glomeruli within each preparation. In 9 out of 13 preparations the T4-glomerulus was clearly the largest glomerulus when compared with the mean volume of all other glomeruli. In the remaining 4 preparations, glomeruli belonging to the T7 tract had the largest volume (Zube and Rössler, 2008, and see results below). In major workers, the volume of the T4 glomerulus was between 6 and 7.2 times larger than the average glomerular volume (applies to 3 out of 6 cases in which the T4 glomerulus was the largest). In minor workers the T4 glomerulus was 6.3 and 6.5 times larger (2 out of 2 cases in which the T4 glomerulus was the largest). In queens, the T4 glomerulus was 8.6 and 9.3 times larger (2 out of 2 cases in which the T4 glomerulus was the largest), and in males it was 9 and 14 times larger than average (2 out of 3 cases in which the T4 glomerulus was the largest). As mentioned above, large glomeruli were also found in the T7 cluster located at the dorsal part of the AL. In 4 preparations glomeruli of the T7 cluster contributed the largest glomeruli, but even in these cases the T4 glomerulus was clearly present and followed on a second or third position.

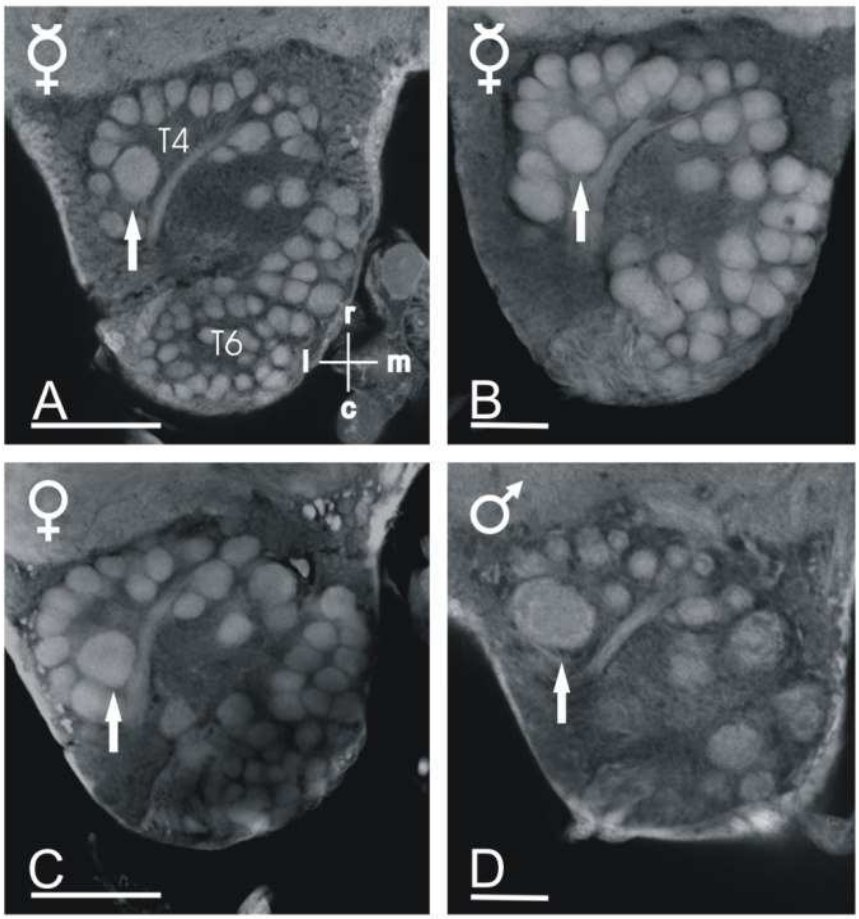

Fig. 12: Comparison of a specifically large glomerulus in the antennal lobe of the female castes and in males of *Camponotus floridanus.* Optical sections at a depth of ~125 µm show an enlarged glomerulus (arrows in A-D) within the T4 cluster of glomeruli, lateral to the T4 tract. This glomerulus was found to be present in the antennal lobe of all female castes and in males: **A.** major worker, **B.** minor worker, **C.** virgin queen, **D.** male. Directions: rostral, r; caudal, c; medial, m; lateral, l. T6 cluster of glomeruli, T6. Scale in A, C = 100µm; in B, D = 50µm.

1.2. Sensory tract innervations of antennal-lobe glomeruli

1.2.1. Major worker

The mass-labeled projections of ORN axons were analyzed to investigate the pattern of sensory-tract specific innervation of glomerular clusters in the AL of *C.floridanus* (n=6). A representative example is shown in Fig. 13, and the corresponding 3D-reconstruction is shown in Fig. 14A, B. In the AL of 6 preparations between 434 and 464 glomeruli were counted indicating some variability in the total number of glomeruli in large workers. The mean number of glomeruli was 452 $\pm$ 14. Tracing of the antennal nerve (AN) revealed that ORN axons separate (together with axons from motor neurons and mechanoreceptors that proceed further to the dorsal lobe, DL) forming seven distinct sensory tracts that enter the AL

(T1 - T7). Each tract innervated a characteristic cluster of glomeruli dividing the AL into seven glomerular subregions. In the following, these glomerular clusters are termed T1 - 7 (Fig. 13 and 14A, B). An overview of the number of glomeruli innervated by T1 - 7 together with their volumes and ACT associations are given in table 1. The tracts were identified by closely following their trajectories image by image in 3D image stacks. T1 proceeded at the ventral surface of the AL and innervated a small cluster of ~34 glomeruli in the ventral-rostral part of the AL (Fig. 13A, B and 14A; Table 1). This cluster was flanked laterally by the T2 and medially by the T3 cluster. The T2 cluster extended along the ventral-lateral side of the AL and consisted of ~56 glomeruli. The T3 cluster with 96 glomeruli spread along the ventral medial part of the AL. Since the clear separation of T3 and T5 glomeruli was most difficult, we used additional criteria to distinguish among the two clusters such as the smaller size of T3 glomeruli compared to T5 glomeruli. A group of ~78 large glomeruli comprised the T4 cluster at the dorsal–lateral side of the AL. The relatively large T4 proceeded transversally through the inner lateral part of the AL (Fig. 13C, D and 14B). Opposite to this cluster, in the dorsal-medial AL, ~36 glomeruli were innervated by T5 which proceeded transversally through the AL in a dorsal-medial direction. Next to the T5 cluster we found the largest group of glomeruli in the AL, the T6 cluster, consisting of ~128 comparatively small glomeruli (Table 1; Fig. 13B-D and 14B). The T6 proceeded along the dorsal-medial surface of the AL and entered its target glomeruli from the periphery. The smallest cluster of T7 glomeruli was located in the dorsal part of the AL, close to the DL and consisted of 6 comparatively large glomeruli. The volume of these glomeruli was calculated as 4-10 times larger than that of most other AL glomeruli, and their innervation was characterized by particularly brightly labeled bundles of axons (Fig. 13D thin dashed lines and 14B; Table 1). In all preparations, these glomeruli were stained brightly. The corresponding ORN axons reached the T7 glomeruli via several loose bundles which I term the T7 tract.

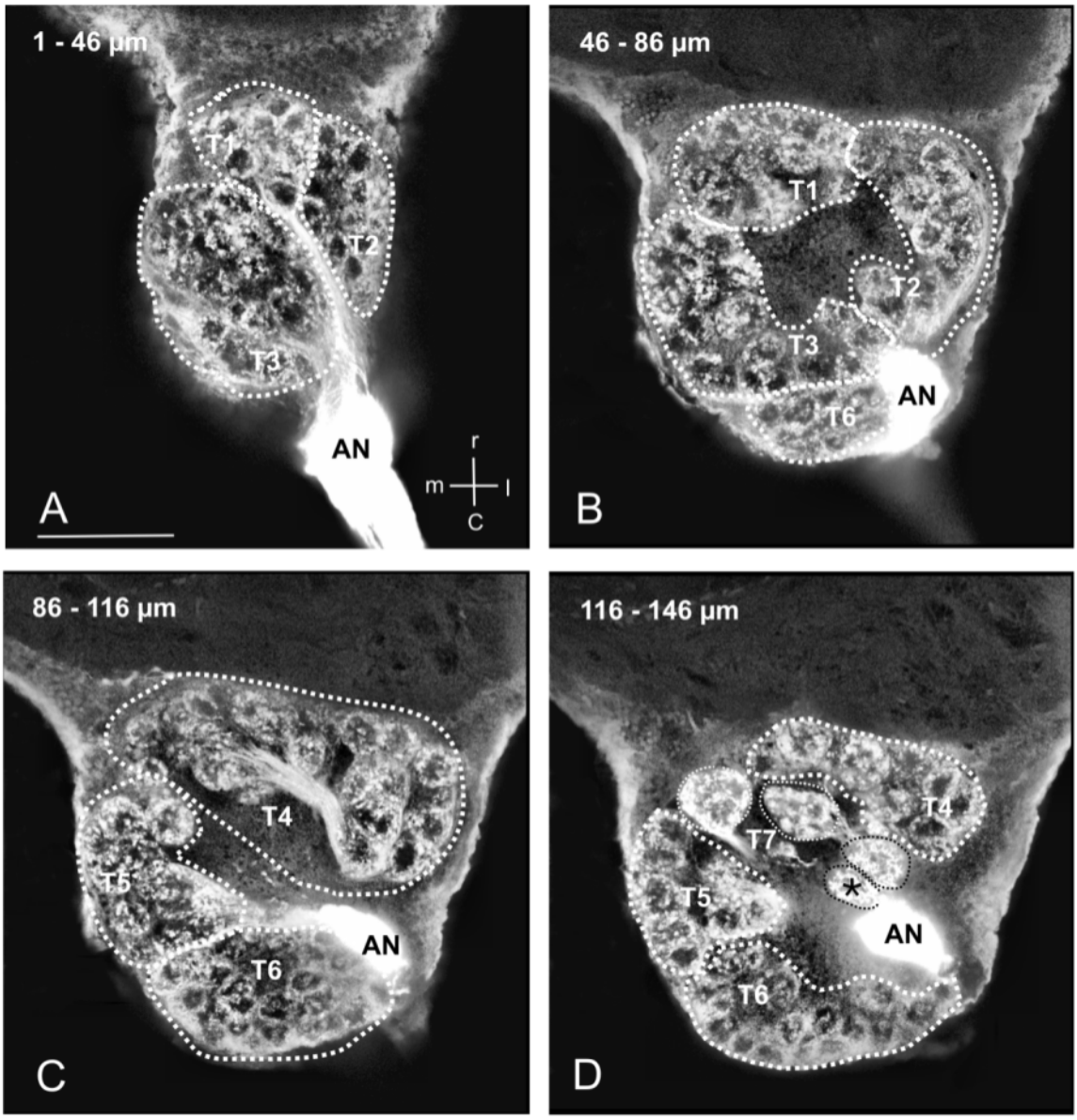

Fig. 13: Antennal sensory innervation of the antennal lobe (AL).
A-D: Series of confocal images of an AL with an anterogradely labeled antennal nerve showing the sensory innervation of AL glomeruli via 7 distinct antennal sensory tracts (T1-T7) and the associated cluster of glomeruli. Single optical sections at different depths (indicated in A-D) show the seven tracts and parts of the innervated glomerular clusters (for further details, see complete image stack in supplementary materials). The asterisk in D labels the single T7 glomerulus that is not innervated by m- or l-ACT PNs. Spatial directions and scale bar are indicated in A: rostral, r; caudal, c; medial, m; lateral, l. Scale bar = 100µm.

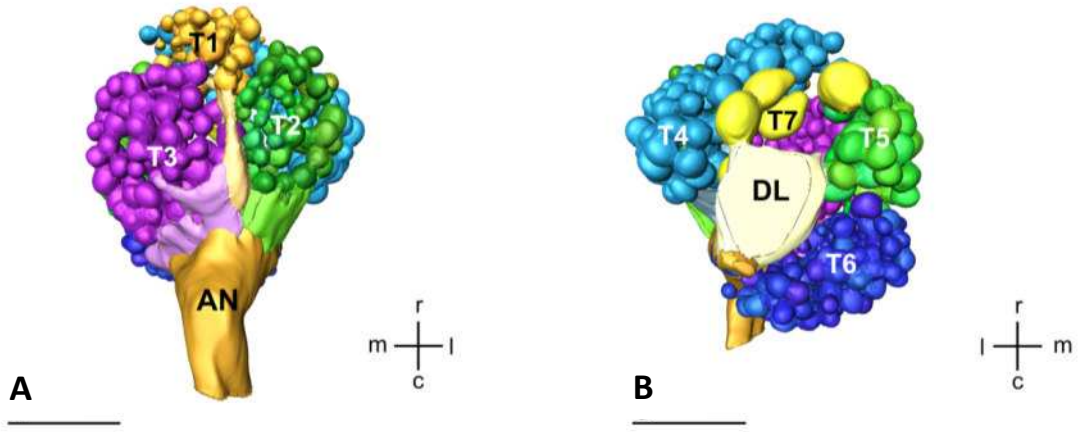

Fig. 14: Afferent innervation of the AL glomeruli in the brain of *Camponotus floridanus.*
A-B: 3D-reconstructions of the antennal-sensory tract specific innervation of glomerular clusters (T1-T7) based on 200 optical sections. A ventral and B dorsal view. The color code defines the different sensory tracts (T1-T7) and their innervated glomerular clusters. The T1-cluster (orange) contains ~34 glomeruli, the T2-cluster (green) ~56 glomeruli, the T3-cluster (magenta) ~96 glomeruli, the T4 cluster (light blue) ~78 glomeruli, the T5 cluster (light green) ~36 glomeruli, the T6 cluster (dark blue) is the largest cluster with ~128 glomeruli, and the T7 cluster (yellow) is the smallest cluster with the 6 largest glomeruli in the AL (see table 1). Directions: rostral, r; caudal, c; ventral, v; dorsal, d. medial, m; lateral, l. Scale = 100µm.

		T1	T2	T3	T4	T5	T6	T7	∑
A	∑ Glomeruli	34	56	96	78	36	128	6	434
B	∑ Volume [$\mu m^3 \times 10^3$]	91.6	159.5	402.8	423.6	255.0	331.4	133.2	1,797.1
C	Max Volume [$\mu m^3 \times 10^3$]	10.0	8.4	9.8	19.2	12.6	7.0	41.2	
D	Min Volume [$\mu m^3 \times 10^3$]	0.9	1.1	1.3	1.8	4.9	0.4	10.8	
E	Mean Volume [$\mu m^3 \times 10^3$]	2.7	2.8	4.2	5.4	7.1	2.6	22.2	
F	Output tract innervation	**l-ACT**	**l-ACT**	**l-ACT/ m-ACT**	**l-ACT**	**m-ACT**	**m-ACT**	**m-ACT** (5 out of 6)	

Table 1: Summary of the afferent and efferent connections of antennal lobe glomeruli in major workers.
A, B: Sum of the number and total volumes (in $\mu m^3 \times 10^3$) of all glomeruli belonging to a cluster supplied by the seven antennal sensory tracts T1 – T7. **C, D, E:** Maximum (Max), minimum (Min) and mean volume of glomeruli belonging to the different tract-specific clusters. **F:** Affiliation of the different clusters to the two main projection neuron output tracts (m- or l-ACT).

1.2.2. Males

The male AL contained only ~55% of the total number of glomeruli compared to the female castes (see results above). What are the consequences for the dual output connections to the MBs and LH in the male AL. In particular, I wanted to test whether the male AL contains a dual output pathway to the MBs and LH and whether the input and output connectivity of AL

glomeruli is organized similar to the female AL despite the much smaller number of glomeruli. I analyzed the projections of ORN axons from the antennal nerve in two preparations (Fig. 15A). Confocal image stacks were analyzed in 1µm steps to follow all labeled axonal tracts to their target regions within the AL. Individual tracts and their associated glomeruli were 3D-reconstructed and color coded (Fig. 15B, C). The results showed that upon entry to the AL, ORN axons segregate from the mechanosensory tracts that proceed further to the dorsal lobe and form six distinct sensory tracts innervating six defined glomerular clusters within the AL. I termed these glomerular clusters according to their innervating sensory tracts T1 - T7, described in detail in the worker caste (see above). I was able to identify all tracts and the associated glomerular clusters except for T5, which was not detectable in the male AL. Figure 15A shows a confocal image of an antennal nerve backfill at a focal plane showing four distinct glomerular clusters in the male AL. The 3D-reconstructions of all tract-specific glomeruli in Fig. 15B and C visualize the six glomerular clusters. The number of glomeruli innervated by the different tracts together with their volumes and output tract associations (see below) are presented in Table 2. Similar to the AL of workers, the T1 proceeded at the ventral surface of the AL and innervated a small cluster of ~25 glomeruli in the ventral-rostral part of the AL (Figure 15A, B; Table 2). Towards the medial side it was bordered by the T3 cluster (~56 glomeruli), and laterally by the T2 cluster (~50 glomeruli). The T2 cluster extended along the ventral-lateral side of the AL, and the T3 cluster proceeded towards the ventral-medial part of the AL (Fig. 15A, B). Ventral of the T1 and T2 cluster I found the largest cluster with ~69 glomeruli, the T4 cluster. It was located at the dorsal-lateral side of the AL and continued transversally through the inner lateral part of the AL (Figure 15C). Based on tract morphology and the location and shape of glomerular clusters in workers I did not detect any equivalent of the T5 tract and cluster in the male AL. The T6 and the associated glomeruli were clearly present in the male AL. The T6 cluster was located close to the T3 cluster, but with only ~52 glomeruli it contained less than half the number of glomeruli compared to the T6 cluster in female workers (Figure 13B-D). At the dorsal most part of the AL, very close to the dorsal lobe, I identified the T7 cluster as the smallest cluster comprising six relatively large glomeruli. Similar to the T7 glomeruli in the AL of female workers, the innervations of the T7 glomeruli in males exhibited a characteristic bright staining and several loosely organized but brightly stained axonal bundles, which I termed the T7.

		T1	T2	T3	T4	T5	T6	T7	∑
A	∑ glomeruli male	25	50	56	69	-	52	6	258
B	∑ glomeruli major worker	34	56	96	78	36	128	6	434
C	∑ glomerular volume								
	male x 10^3 [µm^3]	32.1	78.0	88.6	163.7	-	186.9	48.5	596.8
	worker x 10^3 [µm^3]	91.6	159.5	402.8	423.6	255.0	331.4	133.2	1,797.1
D	Output tract innervation:								
	in males	l-ACT	l-ACT	l-ACT	l-ACT	-	m-ACT	m-ACT	
	in major workers	l-ACT	l-ACT	l-ACT/ m-ACT	l-ACT	m-ACT	m-ACT	m-ACT	

		Major worker	Minor worker	Virgin queen	Male
E	AL glomerular volume x10^3 [µm3]	4489.6 3458.7	2497.6 1892.0	3903.1 3967.0	960.5 815.8
F	Relative AL volume [%]	13.78 ± 1.43	13.37 ± 0.72	13.19 ± 1.02	9.09 ± 0.92

Table 2: Comparison of glomerular clusters and their input-output innervation in the AL of females and males of *Camponotus floridanus.* **A:** Number of glomeruli belonging to the different sensory input tracts (T1-T7) in males (A) and in major workers(B). **C:** Total volume of glomeruli (in µm^3 x 10^3) in the male and worker AL belonging to the different clusters. **D:** Output tract innervations of the different glomerular clusters in the male AL. **E:** Comparison of the total glomerular volumes in of the AL in the female castes and in males (two examples in each case). **F:** Relative AL volumes (in relation to the remaining brain neuropil) for the female castes and in males (mean value of the entire ALs in % ± standard deviations; n=49).

1.3. Sensory output

1.3.1. Males

I successfully labeled the antenno-cerebral output tracts (ACTs) of the male AL in four preparations. In two cases the m-ACT was selectively stained retrogradely to visualize the dendritic and axonal innervation patterns of m-ACT projection neurons (PNs) in the AL, the MBs and the LH. Similar to the AL of workers, and despite a substantially smaller number of AL glomeruli, AL PNs in males projected via two prominent output tracts, the m- and l-ACT, to the MB and the lateral horn (LH) (Fig. 15D), and three small ml-ACTs (ml-ACT 1-3) projected directly to the lateral protocerebrum, especially the lateral horn (LH) (Fig. 15D). In virgin queens, too, a very similar organization of olfactory output tracts was present. Recent studies on workers of the honeybee and the ant *C. floridanus* (Kirschner et al. 2006; Zube et al., 2008) revealed a very characteristic hemispherical division of the AL with ~50% of the glomeruli innervated by m-ACT PNs and ~50% of the glomeruli in the other half of the AL innervated by l-ACT PNs. Selective staining of the m-ACT, in general, revealed a similar hemispherical organization despite the small total number of glomeruli and the obvious absence of T5

glomeruli in the male AL. Figure 15E shows an orthoslice of the dendritic innervation pattern of AL glomeruli by m-ACT PNs. Careful reconstructions and counting of glomeruli revealed that ~57 brightly stained glomeruli in the caudal-dorsal hemisphere of the AL were innervated by the m-ACT, and ~200 glomeruli in the rostral-ventral half of the AL had remained unstained in both preparations. I conclude that those glomeruli were innervated by l-ACT PNs. By combining the data obtained by antennal sensory-tract stainings and retrograde m-ACT labeling, I assigned the input-output connectivity of all glomeruli in the male AL (Table 2). These analyses revealed an exclusive affiliation of T1, T2, T3 and T4 glomeruli to the putative l-ACT hemisphere, and all glomeruli of the T6 and T7 cluster were exclusively innervated by m-ACT neurons (Table 2). Unlike the female AL, the T3 cluster exclusively belonged to the l-ACT portion of the AL and was not divided to either the m- or l-ACT as in workers (Zube et al., 2008). The results indicate that in contrast to the female AL, glomeruli in the AL of males are not equally divided into an m- and l-ACT hemisphere. About four times fewer glomeruli were innervated by m-ACT PNs compared to l-ACT associated glomeruli (58 m-ACT glomeruli vs. 200 l-ACT glomeruli; Table 2). The results suggest that males posses a dual PN output pathway to the MBs and lateral protocerebrum, but with unequal distribution of AL glomeruli connected to the m- and l-ACT. I further analyzed the pattern of m-ACT projections to the MB calyx. Axons from m-ACT PNs had a characteristic innervation pattern within the MB-calyx lip (Figure 15F). Boutons from m-ACT PNs were most densely packed in the peripheral region and less dense in the inner core and the intermediate layer of the lip region. The distribution of m-ACT PN boutons in the outer layer of the MB calyx, however, was broader and less distinct in males compared to the situation in female workers (Zube et al., 2008). Similar as in females terminal branches were absent in the collar region.

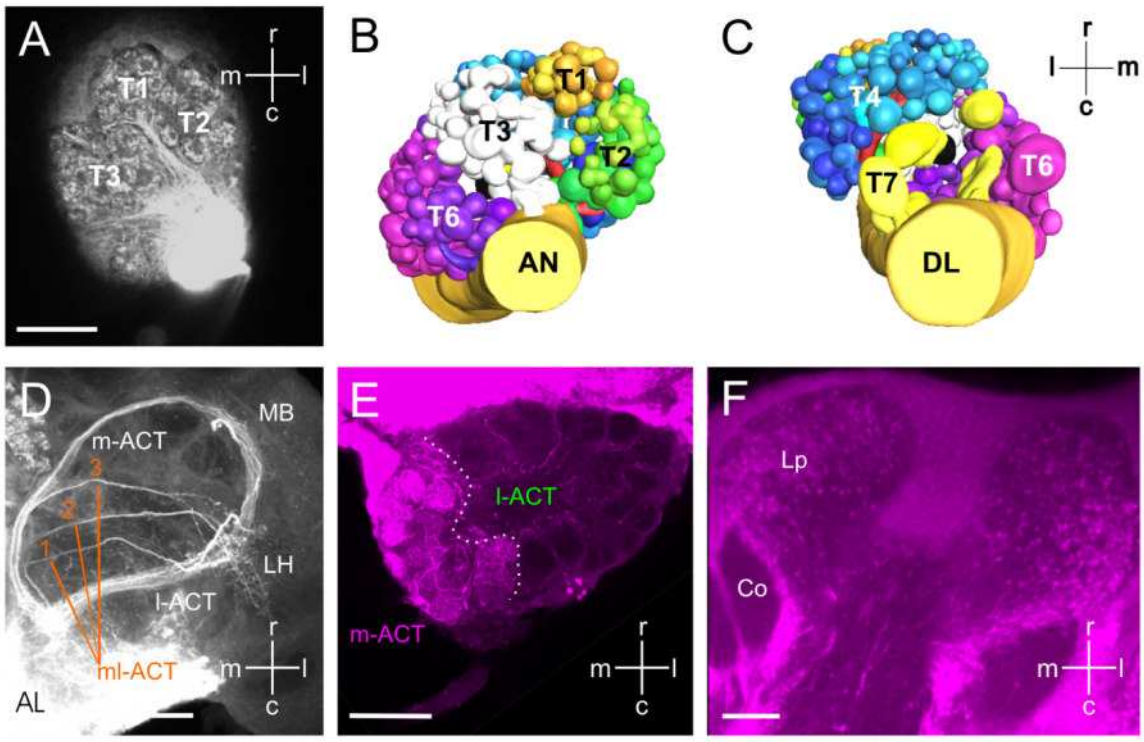

Fig. 15: Organization of the olfactory pathway in the brain of *Camponotus floridanus* males. **A:** Confocal image of the AL with the anterogradely labeled antennal nerve and innervation patterns of three antennal sensory tracts with their associated glomeruli (T1-T3). **B, C:** 3D-reconstructions of the antennal lobe and glomerular clusters innervated by antennal sensory input tracts (T1, T2, T3, T4, T6 and T7). **B:** ventral view (the spatial directions are the same as in A). **C:** dorsal view. **D:** Projection view of the AL output tracts and their projections to the mushroom bodies (MB) and lateral horn (LH). The medial and lateral antennocerebral tracts (m- and l-ACT) project to the MB and LH. Three smaller mediolateral tracts (ml-ACT 1-3) project to the LH only. **E:** Orthoslice of a retrograde mass staining of the m-ACT and its glomerular innervations within the AL. Stained glomeruli appear in magenta. Unlabeled glomeruli appear dark and are presumably innervated by projection neurons of the l-ACT. **F:** Anterograde labeling of the terminal branches of m-ACT projection neurons in the MB calyx show the highest density of boutons in the peripheral region of the calyx lip. Lip, lp; collar, co. Directions: rostral, r; caudal, c; medial, m; lateral, l. Scale A-E = 100µm, F = 20µm.

1.4. Sex-specific differences in serotonergic innervations

Previous results in the ponerine ant *Harpegnathos saltator* had shown that the AL and olfactory lip of the MB calyx are prominently innervated by serotonergic neurons (Hoyer et al., 2005). A recent study by Dacks et al. (2006) in *Camponotus laevigatus* indicated that in contrast to the complete innervations of all AL glomeruli in *Harpegnathos,* a group of glomeruli in the lower (caudal) half of the AL of *Camponotus laevigatus* was not innervated by serotonergic neurons. I wanted to test whether this is a special feature of the genus *Camponotus*, and whether I could assign these glomeruli to a particular cluster based on my 3D-analyses of AL glomeruli in *C. floridanus* (Zube et al., 2008). Finally, I wanted to test whether this pattern is similar across the female castes and males.

In the AL of female workers (n=12) and in virgin queens (n=2), the T6 glomeruli (innervated by m-ACT PNs) clearly lacked serotonergic innervations (Figure 16A, C). Careful analyses and comparison of staining patterns in sectioned material and in whole mount preparations revealed that all other glomeruli were innervated by serotonergic fibers. A similar pattern was observed in the AL of workers of second carpenter ant species (*Camponotus sericeiventris*) (data not shown). In workers of another formicine species (*Cataglyphis fortis*) all AL glomeruli appeared to be innervated by serotonergic processes (data not shown). In contrast to the female AL, all glomeruli in the male AL (n=5) including those belonging to the T6 cluster (Fig. 15B, C), were densely innervated by serotonergic fibers. This sex-specific difference in the AL glomeruli was reflected in a different serotonergic arborization pattern in the MB-calyx lip. In female workers and in queens, serotonergic fibers were absent in the outermost rim of the MB-calyx lip (Figure 16B, D), which was shown to be the main target area of axons from m-ACT PNs (Zube et al., 2008). In contrast to the female castes, serotonergic fibres in the MB calyx lip of males had arborizations across the entire lip area including the outermost m-ACT innervated rim (Fig. 16F and 15F).

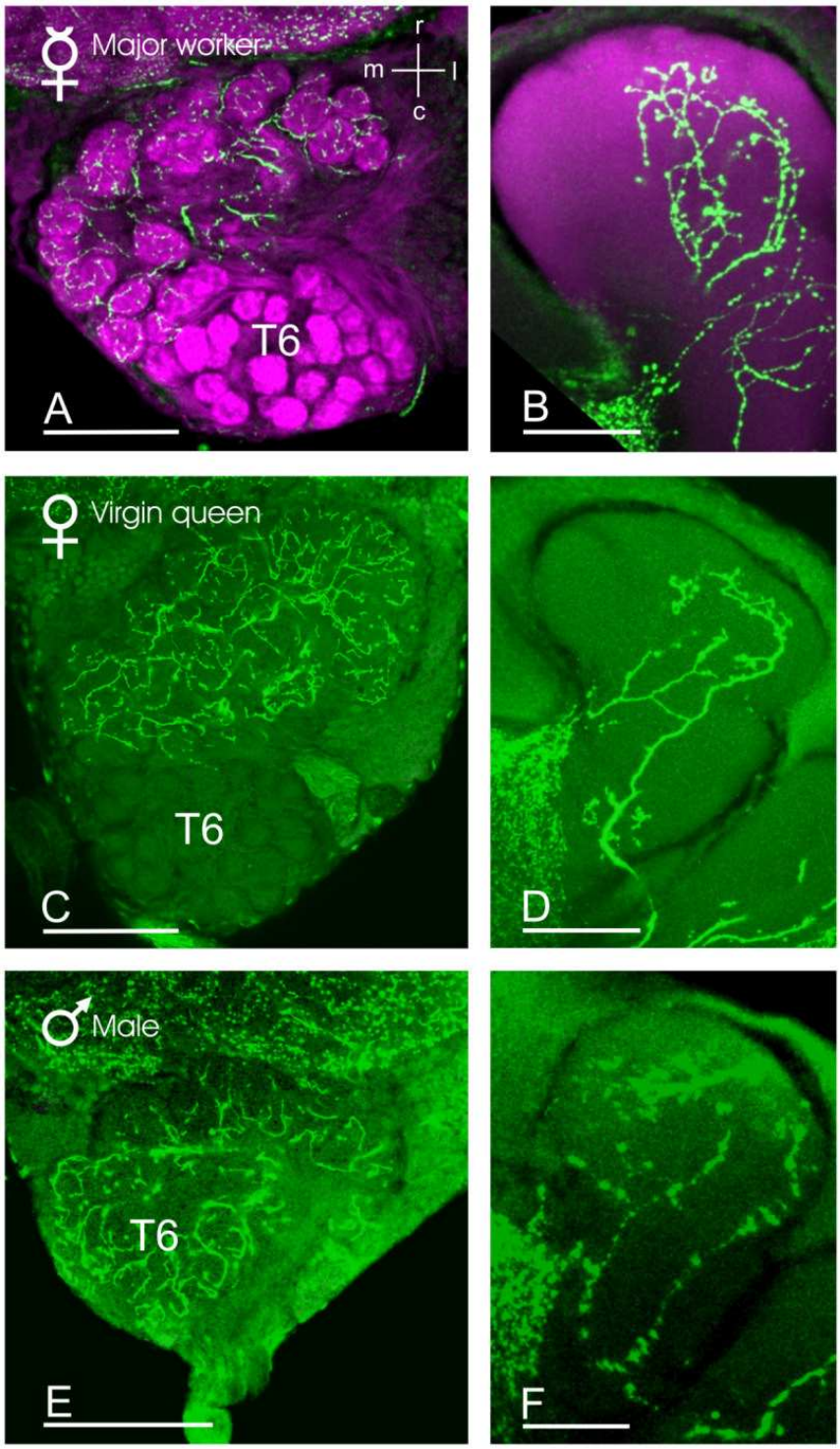

Fig. 16: Caste- and sex-specific differences in serotonergic innervation of the antennal lobe (**A, C, E**) and mushroom-body calyx (**B, D, F**) in *Camponotus floridanus* (f-actin-phallodin staining in magenta, serotonin-IR in green). The T6 cluster of glomeruli within the antennal lobe of female workers (**A)** and virgin queens (**C**) are not innervated by serotonergic processes. All other glomeruli show dense serotonergic innervations. In contrast to the female castes, the T6 cluster and all other glomeruli in the male antennal lobe are densly innervated by serotonergic fibers (**E**). Sex-specific differences were also present in the serotonergic innervation of the MB lip. In female workers and virgin queens the outermost rim of the lip region did not contain serotonergic fibres (**B,D**), whereas in males serotonergic arborizations in the lip region included the outer rim (**F**). Directions: rostral, r; caudal, c; medial, m; lateral, l. Scale bars A,C,E = 100µm, B,D,F = 20µm.

2. NEUROPHYSIOLOGY

2.1. Calcium imaging of odor responses in the ant antennal lobe

Intracellular calcium changes in AL projection neurons (PNs) were measured in response to different odors. Both, m- and l-ACT PNs were retrogradely filled with the calcium-sensitive dye Fura-2 dextran, and successful dye uptake resulted in a bright fluorescence of glomeruli and somata clusters in the AL. In all preparations tested, spontaneous neuronal activity (changes in calcium amplitude) could be observed in areas which were comparable to single glomeruli in shape and size (activity spots). During odor stimulation, changes in calcium responses were visible in larger areas of the AL (activity areas) or in activity spots. In most cases, it was not possible to assign activity areas to particular glomeruli or activity spots to a single glomerulus because optical resolution of the fluorescent images did not allow reliable discrimination of all glomeruli (~50) in the field of view. Therefore, odor representation in the AL in response to stimulation using activity areas was compared. Glomerulus diameter in the imaged area ranged between 15-20 µm (Fig. 2, 3; and volumes in Table 1). Some activity patterns covered larger areas within the AL indicating that in these cases groups of glomeruli rather than single glomeruli were activated. For measurements (see below), the regions of interest were set not larger than 20 µm to avoid measurement of such "group" effects.

2.2. Deep scan

Odor induced activity patterns to octanol were measured at different depths within the AL to investigate whether the odor signal changes over a depth range of 70µm. These measurements served as indicator of the proper choice of the 35µm focal plane during imaging experiments. At a depth of 35µm measured from the top soma layer the glomeruli were visible at the focal plane without fluorescence from soma.

In all experiments the focal range of 30-60µm delivered clear and strong odor induced calcium signals within single activity spots. The activity spots were clearly visible and were of approximate glomerular size (Fig. 17A, B). At further depths between 60-100µm signal clarity decreased and activity spots became blurry (Fig. 17C, D). Very rarely the appearance of additional activity spots during deep scans could be observed (cp. arrow Fig. 17C).

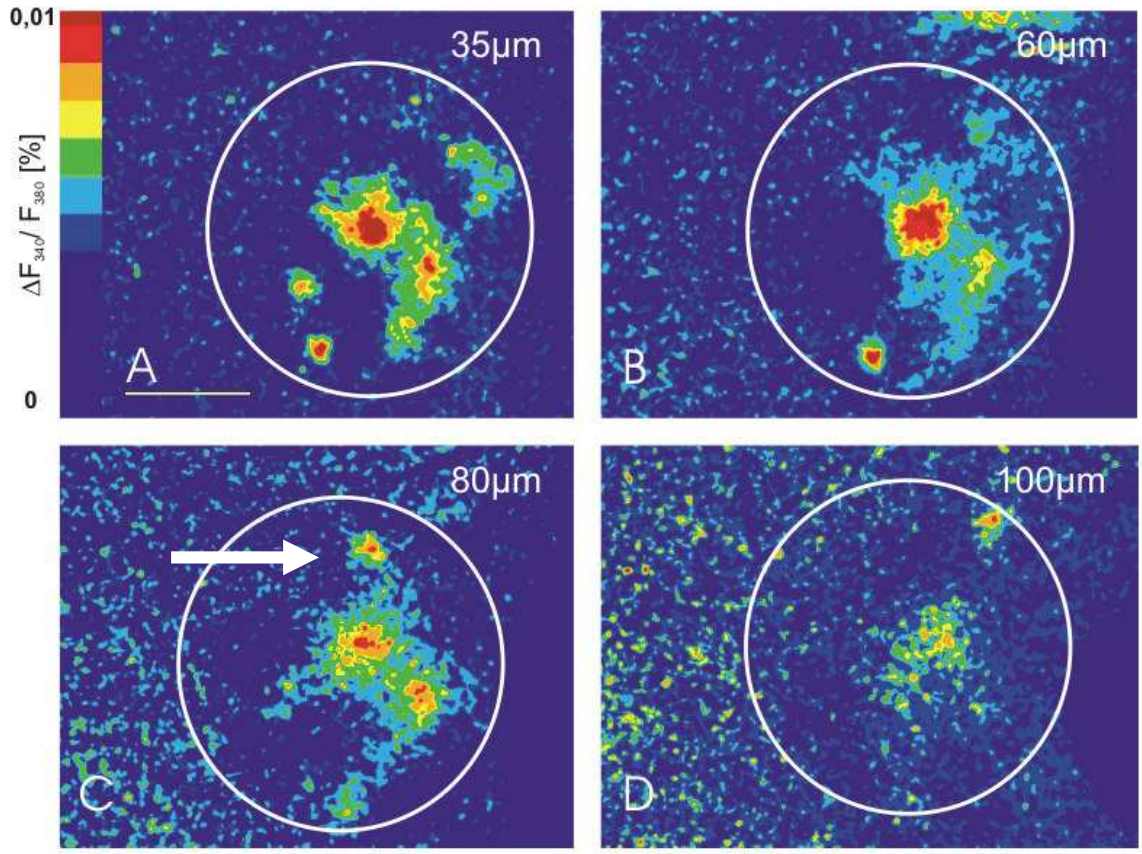

Fig. 17: False color coded activation pattern to stimulation with octanol at different focal depths. At a depth of 35µm single activity spots are clearly visible. With increasing depth activity spots disappear from the pattern and/or become less distinct. In very few cases additional but small activity spots were added to the pattern (arrow in C). At a depth of 100µm almost no signal was visible. Scale = 100µm.

2.3. Odor specific calcium signals

In response to stimulation, odor specific activity areas were found in 39 animals using 2-8 different odors at high concentration (dilution: 10^{-1}). Repeated odor stimulation with the same odor resulted in similar activity areas (data not shown). In response to some odors (citral, isoamylacetate, nerol and heptanal), the activity areas recorded in individual ants covered large parts of the AL, whereas responses to other odors (octanol, 1-hexanol, n-undecane and nerolic acid) were more confined in few activity spots (Fig. 18A-F). As an example, odor specific activity areas measured in one animal are shown as false-color-coded images. Pheromone stimulation resulted in qualitatively similar activity areas as compared to general odors (Fig. 18F and Fig. 18K, O, P). Odor specific activity areas were similar across individuals as shown for stimulation with heptanal (dilution: 10^{-1}), isoamylacetate (IAA; dilution: 10^{-1}), citral (dilution: 10^{-1}), and nerolic acid (dilution: 10^{-9}) in 2 ants, respectively (Fig. 18G-O).

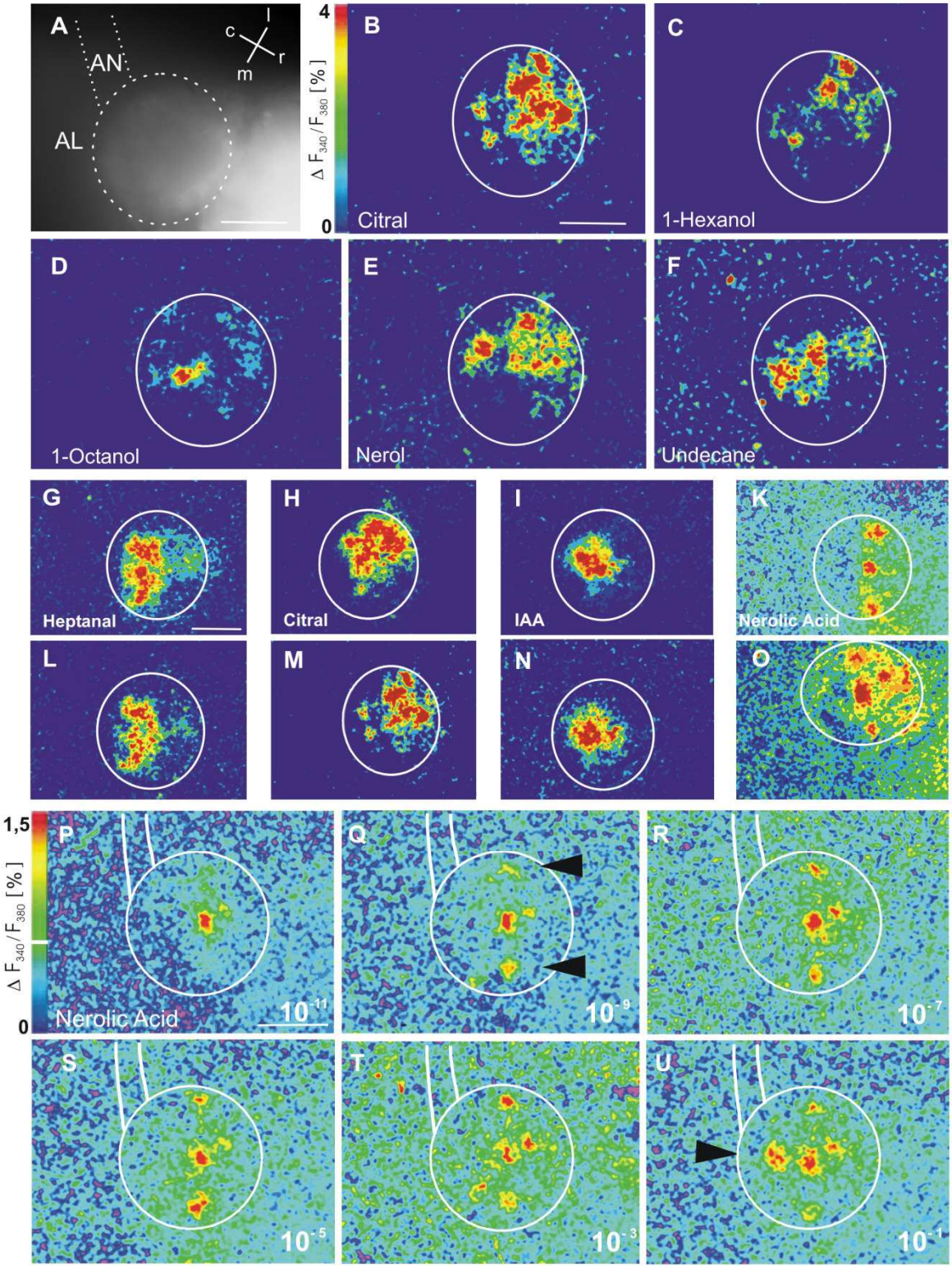

Fig. 18: Projection-neuron calcium responses in the antennal lobe
A: Fura-2 raw fluorescence image of the antennal lobe (AL). The position of the antennal nerve (AN) and the spatial directions are indicated. The spatial directions are valid for all false-color coded images. **B-F:** False-color coded images showing activity areas in the AL of *C.floridanus* in response to different odors. The calcium signals were recorded from PNs of the AL loaded with Fura-2 dextran. 500nl (dilution 10^{-1}) for all odors. The different odors elicit odor-specific activity areas within one ant. **G-O:** The same odors elicit similar activity areas in different ants. Examples showing activity areas in response to heptanal (dilution of 10^{-1}), isoamylacetate (IAA; dilution of

10^{-1}), citral (dilution of 10^{-1}), nerolic acid (dilution of 10^{-9}) in two different ants, respectively. **P-U:** Response patterns to the trail pheromone component nerolic acid presented at different concentrations. High concentration leads to the recruitment of an additional activation spot (arrow in P). The dilutions are given in the images. Note that the activity areas in response to general odors covered large parts of the AL (B,H,M, citral; E, nerol and G,L, heptanal) or were more confined with only few activity spots (**C**, 1-hexanol; **D**, 1-octanol). Pheromones (F, the alarm pheromone n-undecane; **K,O,P**, the trail pheromone component nerolic acid) elicited qualitative similar activity areas compared to general odors. False-colors are scaled in images **B-F** as shown in **B**, images **G-O** as shown in **B**, and images in **P-U** as shown in **P**. caudal, c; lateral, l; medial, m; rostral, r. Scale in **A** and **B** (also valid for **C-F**) = 100µm, in **G** (also valid for **H-O**) =100 µm, and in **P** (also valid for **Q-U**) = 100 µm.

2.4. Representation of pheromones and general odors in the ant antennal lobe

The activity areas in response to different odors overlapped both within the group of general odors as well as between general odors and pheromones (estimated areas ranged from very little overlap to up to ~70%). The activity areas of the two pheromones overlapped with each other in the range of ~10-70%.

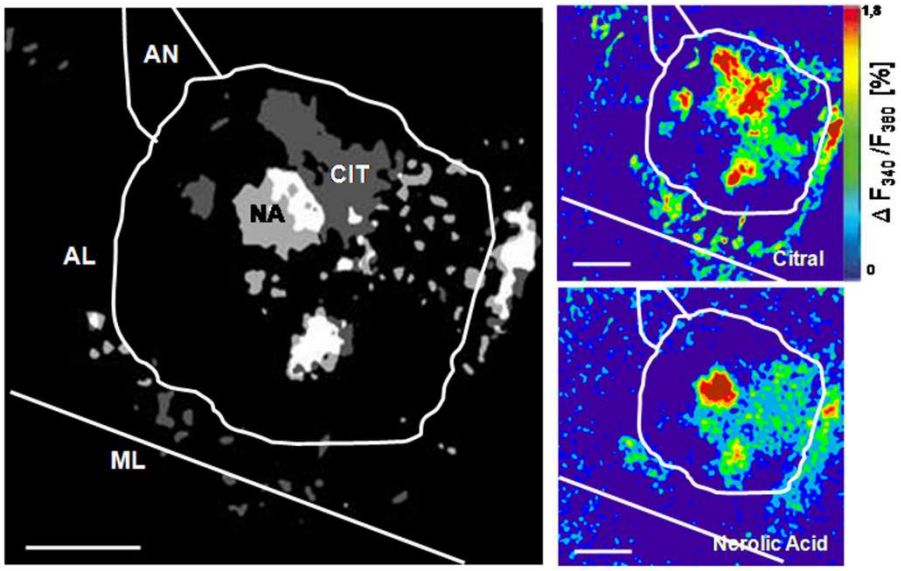

Fig. 19: The single activity areas in response to general odors and pheromones (see false color coded patterns) clearly overlapped. The grayscale picture shows a combination of both false color coded pictures with their overlap in white. citral (CIT), nerolic acid (NA). antennal nerve (AN), brain midline (ML). Scale =50µm.

2.5. Odor concentration threshold for calcium signals

To investigate the sensitivity of the olfactory system in *C.floridanus*, the lowest odor concentration that elicited consistent calcium patterns in the AL (Fig. 20) was measured. I found a very high sensitivity of PN calcium responses to both, general odors (1-hexanol, 1-octanol) and pheromones (nerolic acid, n-undecane). In at least 80% of the investigated animals, activity areas were found in response to very low odor concentrations (dilution: 10^{-11} or lower) of the 4 odors tested (Fig 20). I only tested one odor quality at

different concentrations in each animal (total n = 29), thus, all 4 response thresholds are independent of each other.

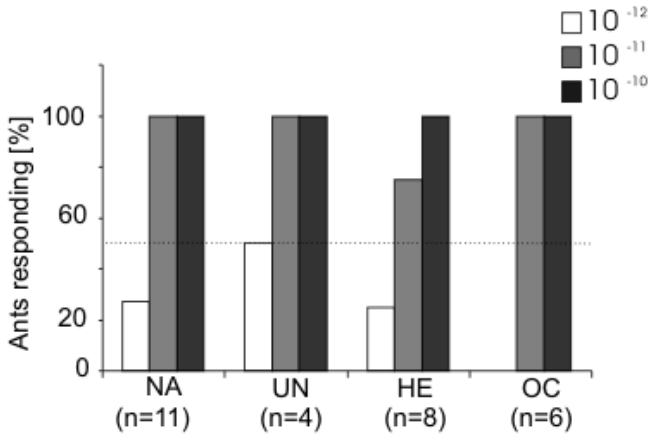

Fig. 20: Response-threshold odor concentration for pheromones (NA, nerolic acid; UN, n-undecane) and general odors (HE, 1-hexanol; OC, 1-octanol). Percentage of animals in which a calcium signal (activity areas) could be measured in response to different odor concentrations (dilutions: white bars, 10^{-12}; grey bars, 10^{-11}; black bars 10^{-10}). Loading the odor cartridge with 5 x 10^{-11} dilution of any of the four odors was sufficient to elicit a calcium response in at least 80% of the animals (n, number of animals tested).

2.6. Dependance of odor representation on odor concentration

2.6.1. Odor patterns

2.6.1.1. Nerolic Acid

The trail pheromone component nerolic acid was used as stimulus to investigate the variance of activity areas and the dynamic range of the calcium activation patterns across odor concentrations (11 log units). In all 7 animals investigated the spatial pattern of activity areas in response to nerolic acid was odor-concentration dependent only at very high and very low concentrations (Fig. 18P-U). The response threshold for nerolic acid was at a dilution of 10^{-11} with only a single activity spot. At a dilution of 10^{-9}, additional activity spots were found (arrows in Fig. 18Q), and this pattern remained largely stable over a concentration range of 7-8 log units (Fig. 18Q-T). At the highest odor concentration tested (dilution: 10^{-1}), a further activity spot added to the existing pattern (Fig 18U; arrow).

For the following experiments with the alarm pheromone n-undecane and the two general odors octanol and heptanal only six concentrations spanning a range of the former eleven log units were tested (10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, 10^{-3}, 10^{-1}). The acquisition of a whole data set with eleven concentrations in the nerolic acid experiments turned out to be extremely difficult with a final success rate of only 2% due to the high mortality of the ants during data acquisition. The number of concentrations tested was thus reduced to facilitate the

experimental procedure and to increase the probability of measuring a complete data set in one animal.

2.6.1.2. n-Undecane

The alarm pheromone component n-undecane was measured in seven animals. Six concentrations were tested spanning a concentration range of 11 log units (10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, 10^{-3}, 10^{-1}). In five out of seven preparations the n-undecane activity pattern appeared as a diffuse activity patch with a diameter of ca. 50-80µm in the center of the antennal lobe (Fig. 18). Single glomeruli were thus difficult to characterize. In one animal the activity pattern remained stable over all tested concentrations with no additional activity spots at higher concentrations. In 2 animals investigated, the activity pattern remained stable over seven log units from 10^{-11} to 10^{-5} (example in Fig. 21) and showed a concentration dependency at the highest concentrations of 10^{-3} and/or 10^{-1} where single glomeruli were added to the existing pattern (arrow in Fig. 21E).

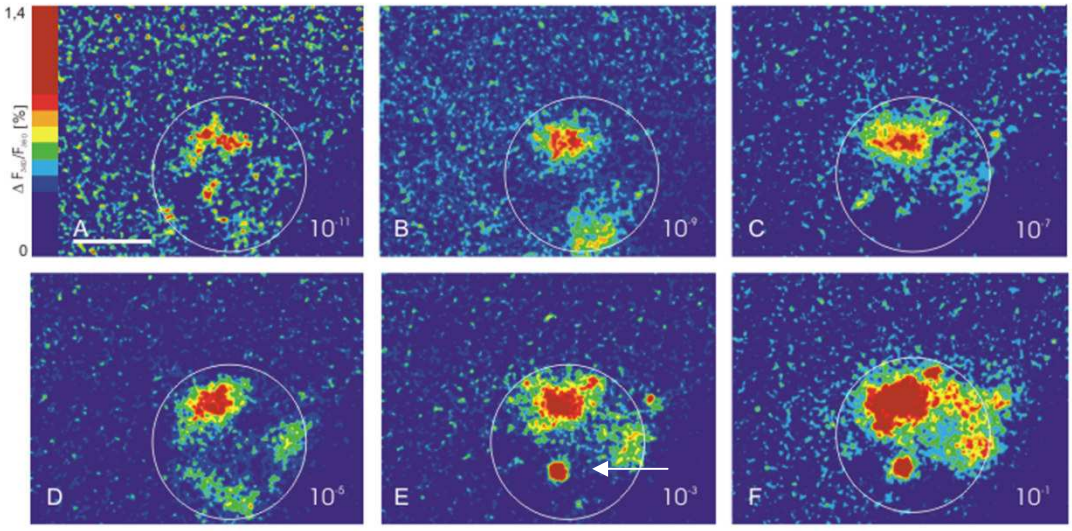

Fig. 21: Response patterns to the alarm pheromone component n-undecane. The pattern was stable over at least seven log units from 10^{-11} to 10^{-5}. At a concentration of 10^{-3} further glomeruli were activated (arrow) and the activation pattern expanded. Scale = 100µm.

In one animal the activity pattern at 10^{-11} showed three activity spots of which only one remained from 10^{-9} to 10^{-3}. At the highest concentration of 10^{-1} additional areas of activity appeared. A similar observation was made in another individual where the pattern remained stable between 10^{-11} and 10^{-9} (Fig. 22). At 10^{-7} one single activity spot did not appear anymore (arrow in Figure 22B) but at a concentration of 10^{-3} an additional spot showed up (arrow in Fig. 22E) which was not present at the highest concentration.

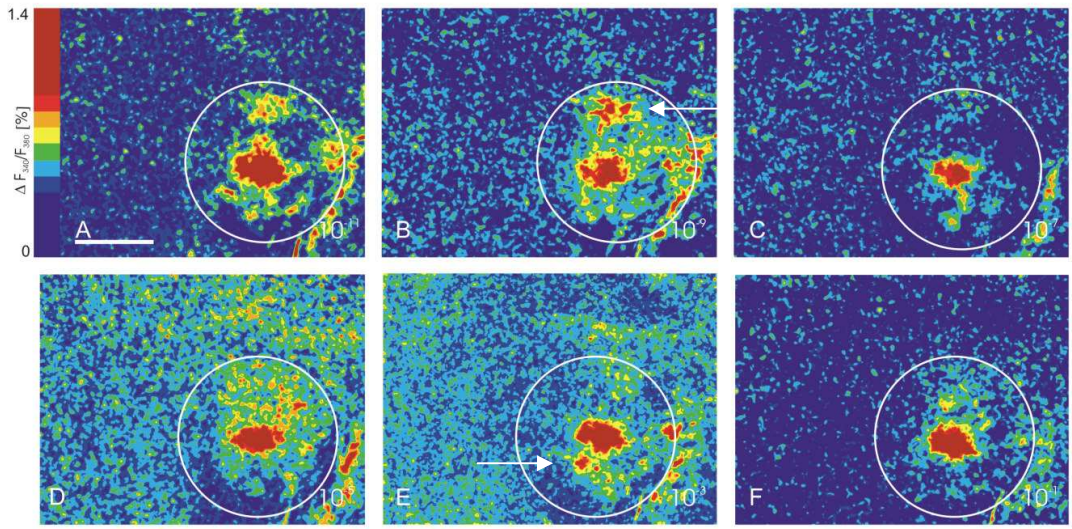

Fig. 22: False-color coded images showing activity areas in the AL of *C. floridanus* in response to n-undecane. The odor induced activity pattern remained stable between 10^{-11} and 10^{-9}. At 10^{-7} the pattern is reduced to a center activity spot until 10^{-3} where further spots are activated but not present at the highest concentration. Scale = 100µm.

In one preparation the activity pattern was at first monitored at 10^{-9} and consisted of one single activity spot. Two more activity spots appeared at 10^{-7} and the pattern remained stable until 10^{-1} where the signal intensity increased but no additional spots were added to the existent pattern. In one preparation the odor induced calcium signal was very weak and no specific activity patterns could be analyzed over all concentrations. In the majority of all cases the pattern remained stable over several log units, similar as in the trail pheromone nerolic acid.

2.6.1.3. Heptanal

Heptanal was successfully tested in 9 animals. For heptanal different concentration dependent and independent activity dynamics were monitored. In two cases the activity pattern remained stable over the whole concentration range up to 10^{-1} where additional glomeruli were added to the existing pattern.

In the remaining 7 animals the patterns changed with changing concentrations. Here some basic glomeruli could be characterized in each preparation but additional glomeruli were activated or deactivated with changing concentrations (examples from three different individuals are shown in Fig. 23I- III). No regularity regarding those glomeruli was observed. Five of those animals tested showed an enlargement of the activated areas within the AL at the highest concentration (10^{-1}) (cp. Fig. 23I-III).

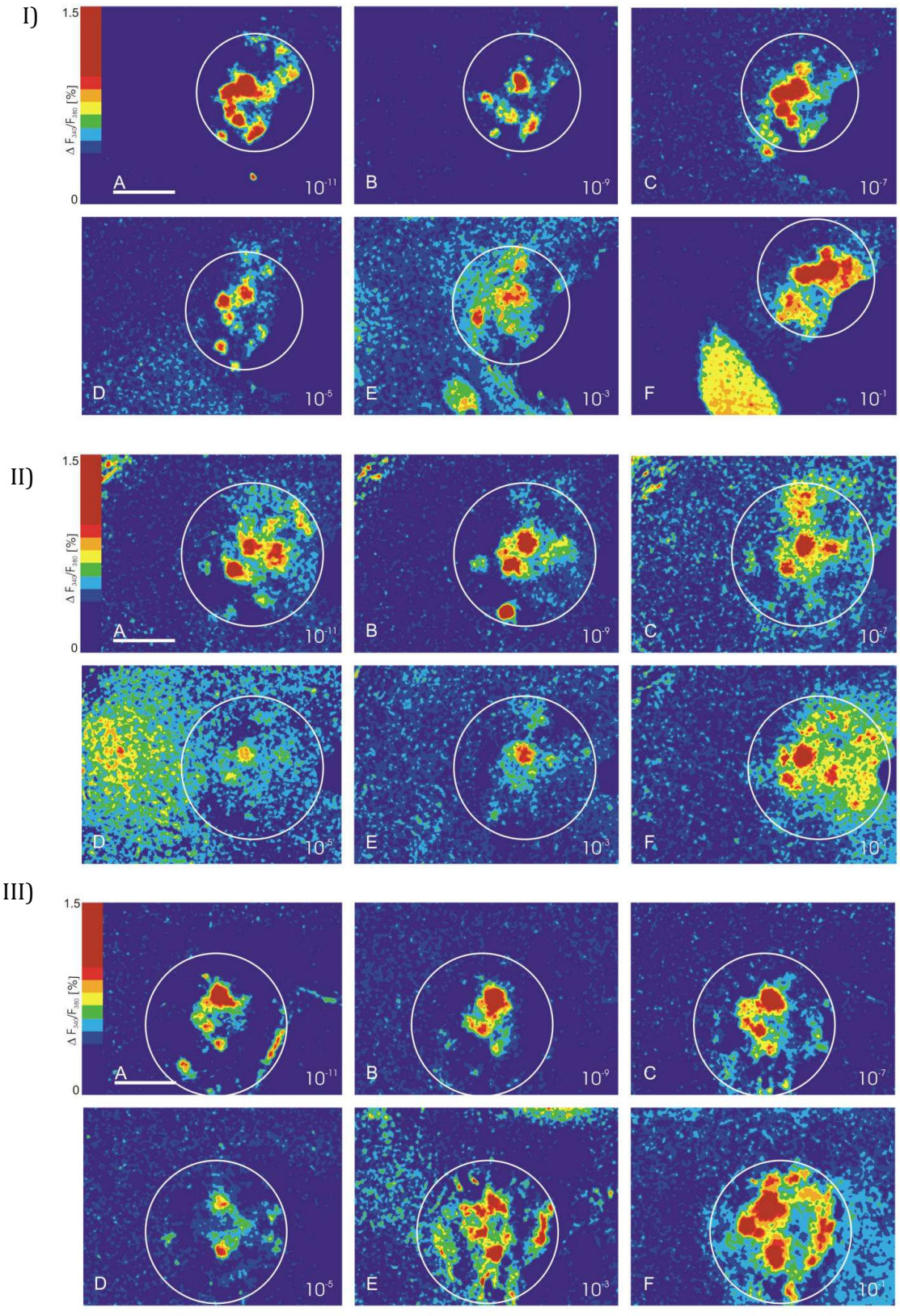

Fig. 23: False color coded activity patterns in the AL of three different ants to odor stimulation with heptanal. (I-III) In each preparation the odor induced pattern changed with increasing concentration. In all experiments one or two basic glomeruli could be defined which were constantly activated over all concentrations. Dependent on the odor concentration additional glomeruli were activated or deactivated during odor stimulation but no regularity could be monitored. Noticeably, in three preparations the patterns weakened at a concentration of 10^{-5}. Two examples are shown in II) and III). Scale = 100µm.

Another striking concentration dependent pattern dynamic was monitored in three preparations. At an odor concentration of 10^{-5} the activity pattern strongly weakened or even disappeared, so that almost no glomerular activity could be measured. During the following stimulation with 10^{-3} the pattern reappeared (see Fig. 23II and III).

2.6.1.4. Octanol

Octanol was successfully measured in nine preparations with odor dilutions of 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, 10^{-3} and 10^{-1}. In one animal the activation pattern remained stable over the whole concentration range. In two cases the pattern remained stable up to a concentration of 10^{-1} where additional glomeruli were activated (example see Fig. 24).

In the remaining six animals the activity pattern changed with changing concentrations. Depending on the concentration here activity spots were added or disappeared from the existing pattern (see three examples in Fig. 25 I-III). No regularity within these changing patterns could be observed. The most striking change of glomerular patterns appeared at the highest concentrations where single glomeruli were additionally added to the basic pattern and the area of activity was significantly enlarged. The weakened pattern at 10^{-5} which was monitored in heptanal was also present in octanol in one case (cp. Fig. 25III).

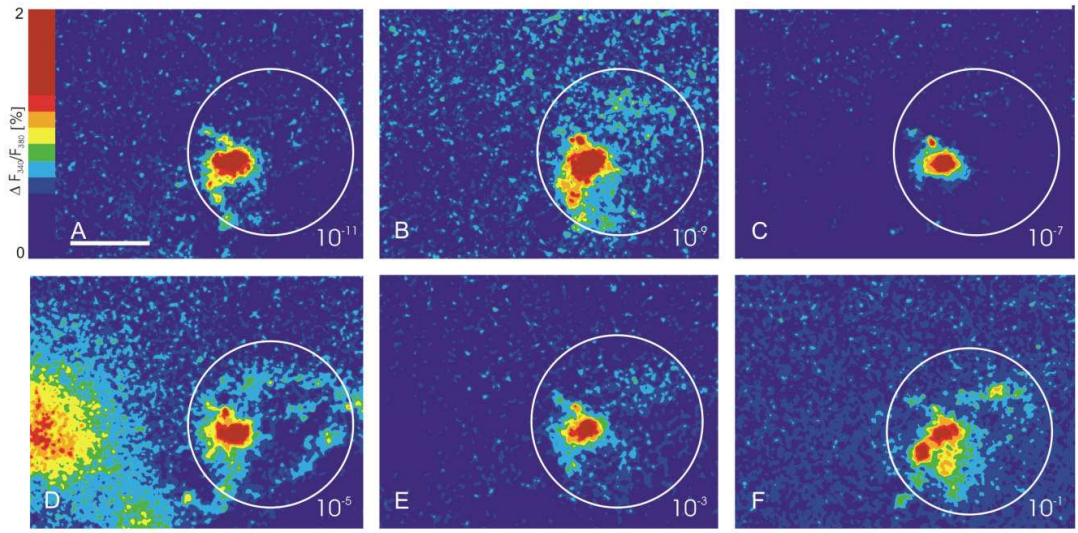

Fig. 24: False color coded glomerular pattern to odor stimulation with octanol. The pattern remained stable over the whole concentration range up to 10^{-1} where the pattern enlarged and further spots were activated. Scale = 100µm.

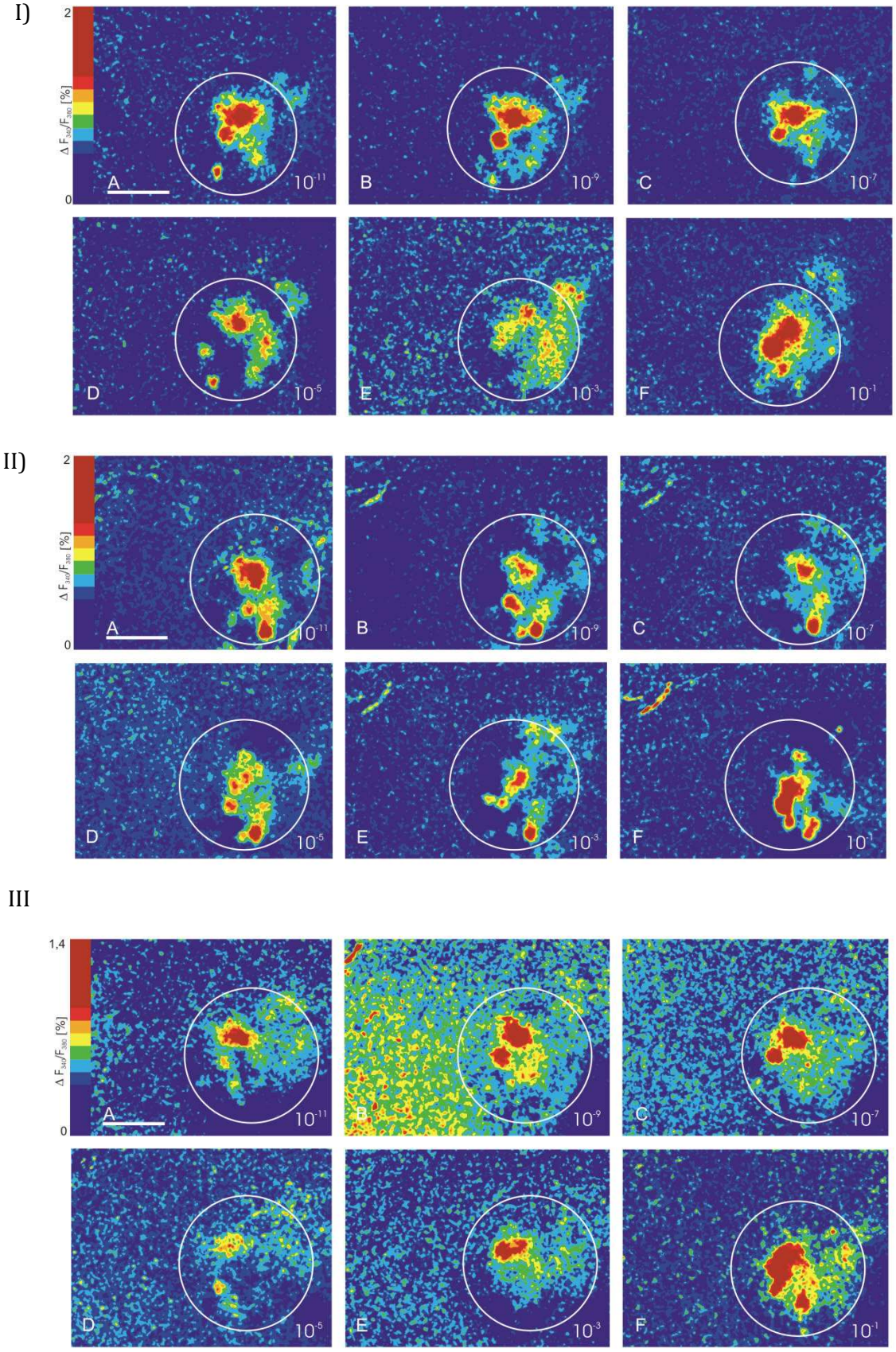

Fig. 25 I-III: False color coded profiles of AL regions activated during stimulation with octanol. The pattern consists in all cases of one or two basic glomeruli which appear at all concentrations. With changing concentration glomeruli are added to the central pattern with no obvious systematics. Scale = 100µm.

In summary, the comparison between the neuronal representation of pheromones and general odors applied at different concentrations revealed that the concentration dependence of the glomerular activation patterns appears to be odor specific.

2.6.2. Signal intensity

The amplitude of the calcium signals from activity spots was analyzed over different odor concentrations for all odors tested. Regions of interest (ROIs) were defined around activity spots, and response amplitudes were normalized within each animal (see materials and methods). The amplitude of the calcium signal within the ROIs was concentration dependent in some cases (ROI_{cd+}) and not concentration dependent in other cases (ROI_{cd-}).

2.6.2.1. Nerolic Acid

The pairs of ROIs, selected in each preparation of the 7 animals investigated were split to either ROI_{cd+} or ROI_{cd-} regions. This was possible in 5 animals shown in Fig. 26, in the remaining two animals, no clear concentration dependency was found.

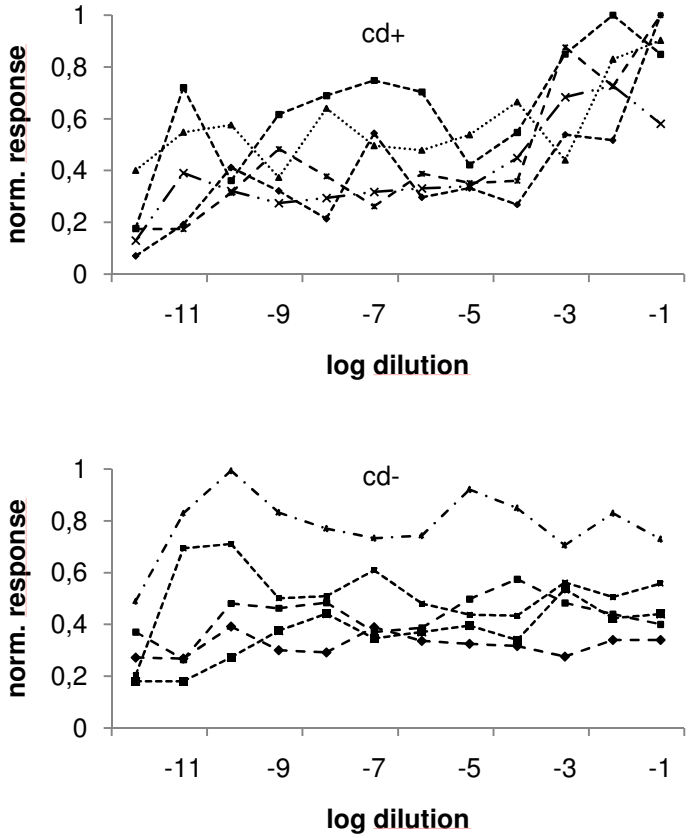

Fig. 26: Normalized amplitudes (see materials and methods) of the calcium responses to nerolic acid in five concentration dependent (cd+) and five concentration independant (cd-) ROIs within the antennal lobe of five animals across a range of odor concentrations (dilution: 10^{-12} - 10^{-1}).

2.6.2.2. n-Undecane

In the seven animals investigated a clear separation of either ROI_{cd+} or ROI_{cd-} regions was possible in four individuals shown in Fig. 27. In the remaining three animals only concentration independent regions of interest were found. The strong intensity decrease of two ROIs in the cd- diagram at a concentration of 10^{-9} was due to a strong tissue movement during the experiment. Both ROIs were measured within one animal.

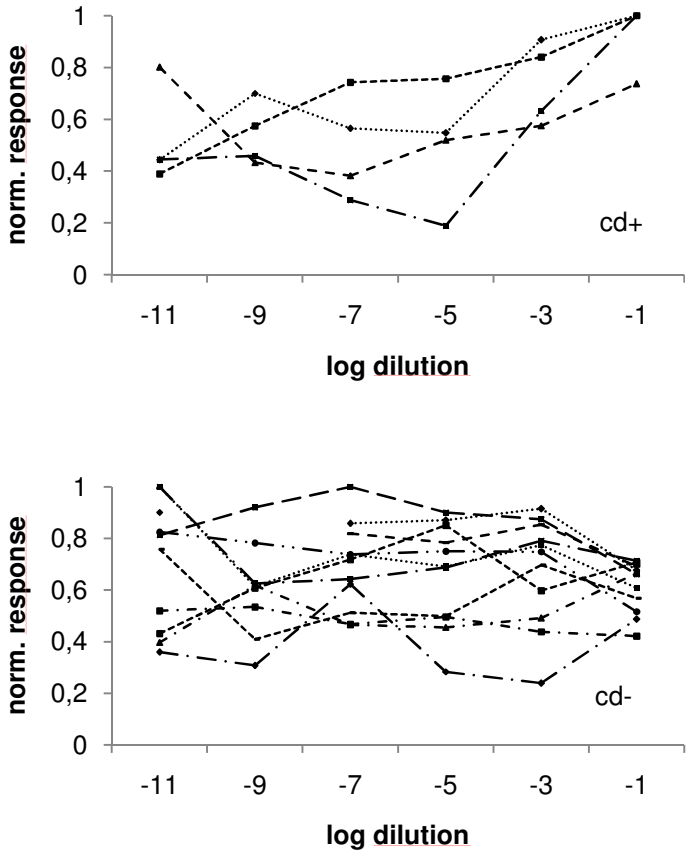

Fig. 27: Normalized amplitudes of the calcium responses to n-undecane in four concentration dependent (cd+) and eight concentration independant (cd-) ROIs within the antennal lobe of seven animals across a range of odor concentrations between 10^{-11} - 10^{-1}.

2.6.2.3. Heptanal

A clear definition of either ROI_{cd+} or ROI_{cd-} regions in the nine individuals measured could be monitored in two preparations (cp. Fig. 28). The remaining seven preparations only showed concentration dependent ROIs. Within concentration dependent ROIs two response dynamics were monitored. In seven ROIs response intensity did not increase notably within a concentration range of 10^{-11} to 10^{-5}. At 10^{-3} the signal amplitude increased and reached its maximum at a concentration of 10^{-1}. One ROI decreased at the highest concentration due to weakened overall response (see Fig. 28I). The second group of cd+ROIs is characterized by its different response dynamics over the whole concentration range (Fig. 28II). The term

concentration dependent ROI here is defined as a region of interest which shows different response dynamics over the concentration range tested and with no obligatory signal increase at highest concentrations.

Both cd- ROIs did not show striking concentration dependent response dynamics except for one single concentration in one ROI (Fig. 28III), where signal intensity increased at 10^{-9} and then remained almost stable for the following concentrations.

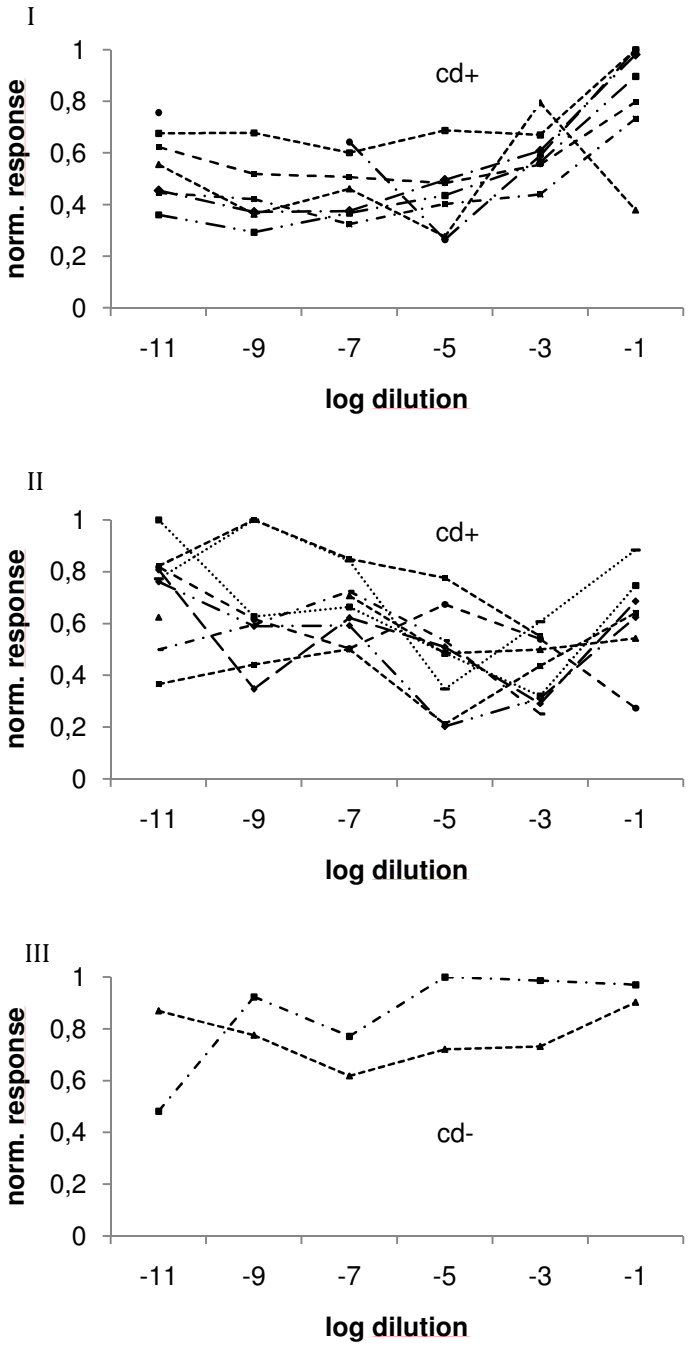

Fig. 28: Normalized amplitudes of the calcium responses to heptanal in 16 concentration dependent (cd+) and two concentration independant (cd-) ROIs within the antennal lobe of nine animals across a range of odor concentrations between 10^{-11} - 10^{-1}. For a better visualization the concentration dependent ROI dynamics were split on two graphs, I: The upper graph shows seven ROIs with small calcium signal dynamics over a concentration range from 10^{-11} to 10^{-5}. At 10^{-3} these ROIs show a strong increase in signal intensity. II: The lower cd+ graph shows nine ROIs which follow different response dynamics but do also increase in signal intensity at the highest concentration. III: In two calcium independent ROIs no significant increase in signal intensity at the highest concentrations was monitored.

2.6.2.4. Octanol

Pairs of ROIs showing either a ROI_{cd+} or ROI_{cd-} dynamic could be defined in four of nine investigated animals (see Fig. 29). In two cases only concentration independent regions were present, in two cases the ROIs showed only concentration dependent dynamics and in one animal no clear concentration dependency was found.

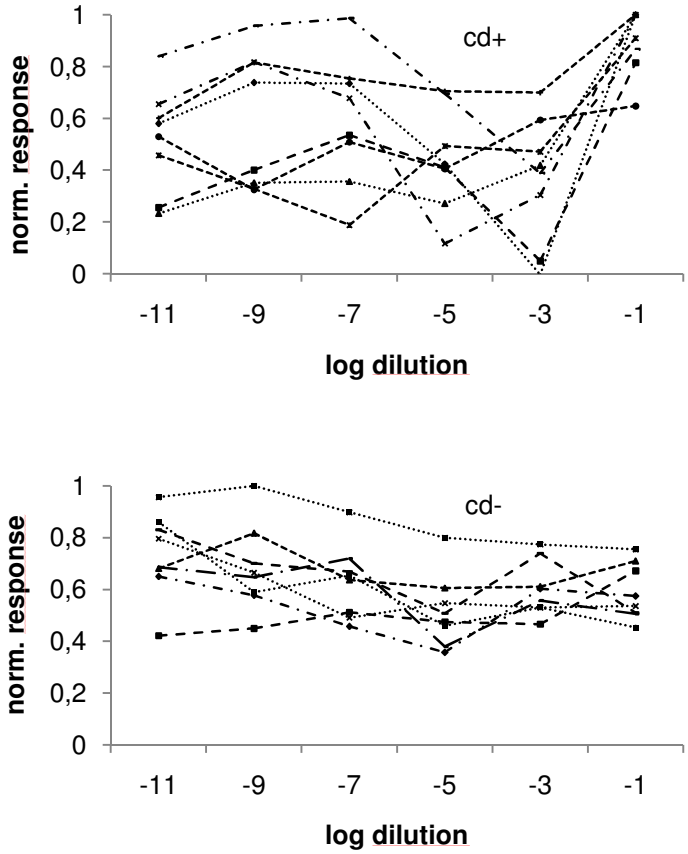

Fig. 29: Normalized amplitudes of the calcium responses to octanol in eight concentration dependent (cd+) and eight concentration independant (cd-) ROIs within the antennal lobe of eight animals across a range of odor concentrations (dilution: 10^{-12} - 10^{-1}).

2.6.3. Signal duration

2.6.3.1. Nerolic Acid

To compare the duration of the calcium signal, ROI_{cd+} and ROI_{cd-} regions were analyzed separately. As an example, the time courses of the response of one cd+ and one cd- to three odor concentrations are shown together with the control (response to solvent) in Fig. 30. The response dynamics for all measured ants were further analyzed. Increasing odor concentrations evoked a higher amplitude of the calcium signal in ROI_{cd+} regions and also a longer duration of the calcium signal (Spearman's rank correlation, $R = 0.69$, $p < 0.05$, slope = 0.36). Although increasing odor concentration did not result in a higher amplitude of the

calcium signals in ROI_{cd-} regions, the duration of the calcium signal was also concentration dependent (Spearman's rank correlation, R = 0.50, $p < 0.05$, slope = 0.33).

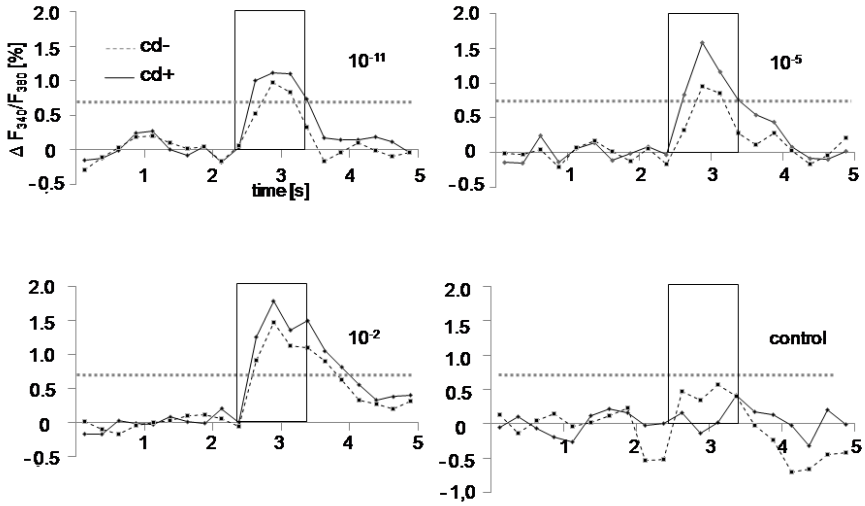

Fig. 30: **Intensity dependence of signal response duration to nerolic acid**
Temporal dynamics of the calcium responses to nerolic acid in one concentration dependent (cd+) and one concentration independent (cd-) ROI within the antennal lobe of one animal at three different odor concentrations (10^{-11}, 10^{-5} and 10^{-2}) and the control stimulus (solvent only). Threshold level of 40% is indicated by the horizontal dotted line. The response duration was longer at higher odor concentrations compared to lower concentrations.

2.6.3.2. n-Undecane, Heptanal and Octanol

In all three remaining odors significant concentration dependent signal duration was not clearly visible. The statistical analysis of both ROI_{cd+} and ROI_{cd-} regions revealed significant differences between the control stimuli and all concentration answers in all preparations but no significances between the concentration dependent signal durations of all tested odor concentrations. For each odor two examples are shown to demonstrate the variability of signal durations within the tested groups.

2.6.3.2.1. n-Undecane

Statistically analysis of all animals tested on n-undecane revealed no statistically longer duration of the calcium signal in ROI_{cd+} regions with increasing odor concentration (Friedman ANOVA = 21.2898, Dof = 7, $p > 0.05$). The same was observed for ROI_{cd-} activity spots (ROI_{cd-} Friedman ANOVA = 37.1907, Dof = 7, $p > 0.05$. The variance between the different animals and activity spots tested was very strong and is shown in two examples in Fig. 31I and II. Figure A shows two activity spots (ROI_{cd+}, ROI_{cd-}) measured in one animal which follow a

typical increase in signal duration over changing concentrations. In contrast, the activity spots in the second animal follow a different response dynamic with both spots having its intensity maximum at the lowest concentration. Stimuli with a higher concentration resulted in a decreased signal intensity and duration which increased with increasing concentrations in ROI_{cd+} and remained stable in ROI_{cd-}. In other experiments intermediate dynamics were monitored (data not shown).

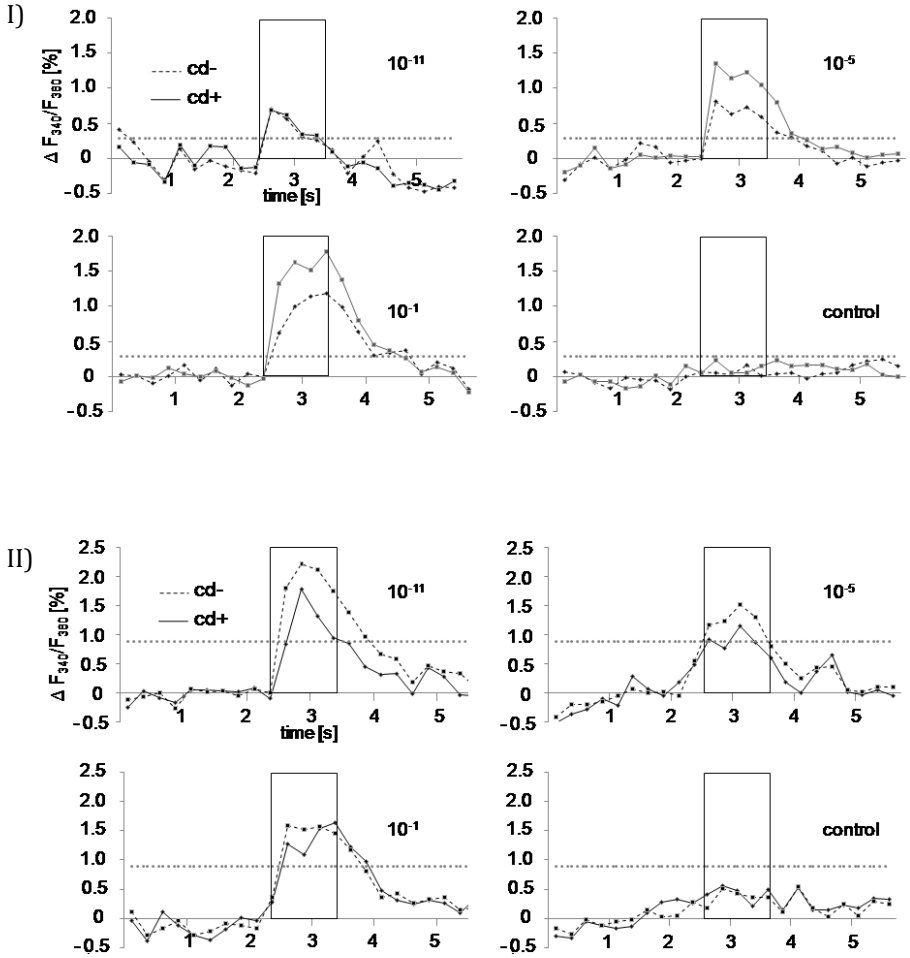

Fig. 31: **Intensity dependence of signal response duration to n-undecane**
Temporal dynamics of the calcium responses to n-undecane in one concentration dependent (cd+) and one concentration independent (cd-) ROI within the antennal lobe of two animals (I and II) at three different odor concentrations (10^{-11}, 10^{-5} and 10^{-2}) and the control stimulus (solvent only). Threshold level of 40% is indicated by the horizontal dotted line. The response duration was longer at higher odor concentrations compared to lower concentrations in I. In II the signal duration varied between the different concentrations.

2.6.3.2.2. Heptanal

In Heptanal statistical analysis revealed no significant increase in signal duration over the concentrations tested in ROI_{cd+} (Friedman ANOVA= 55.0378; Dof = 7, p >0.05). ROI_{cd-} activity spots were not statistically tested due the small sample size of two. Figure 22 shows two examples of two ants tested and the signal duration response dynamics of two activity spots. The response dynamics in Fig. 22I mirror that both ROIs follow their specific intensity response dynamics, but the signal duration changes over concentrations and does not follow a constant increase. The calcium dependent ROI does not increase its signal duration from lowest to highest concentration and even experiences a strong decrease at 10^{-5}. The calcium independent activity spot first shows a decrease and later a constant signal duration over the remaining concentrations. In Fig. 22II the signal duration apparently increases only at the highest concentration with no obvious changes during the less intense stimuli. This emphasizes the strong variability of signal duration within the heptanal experimental group.

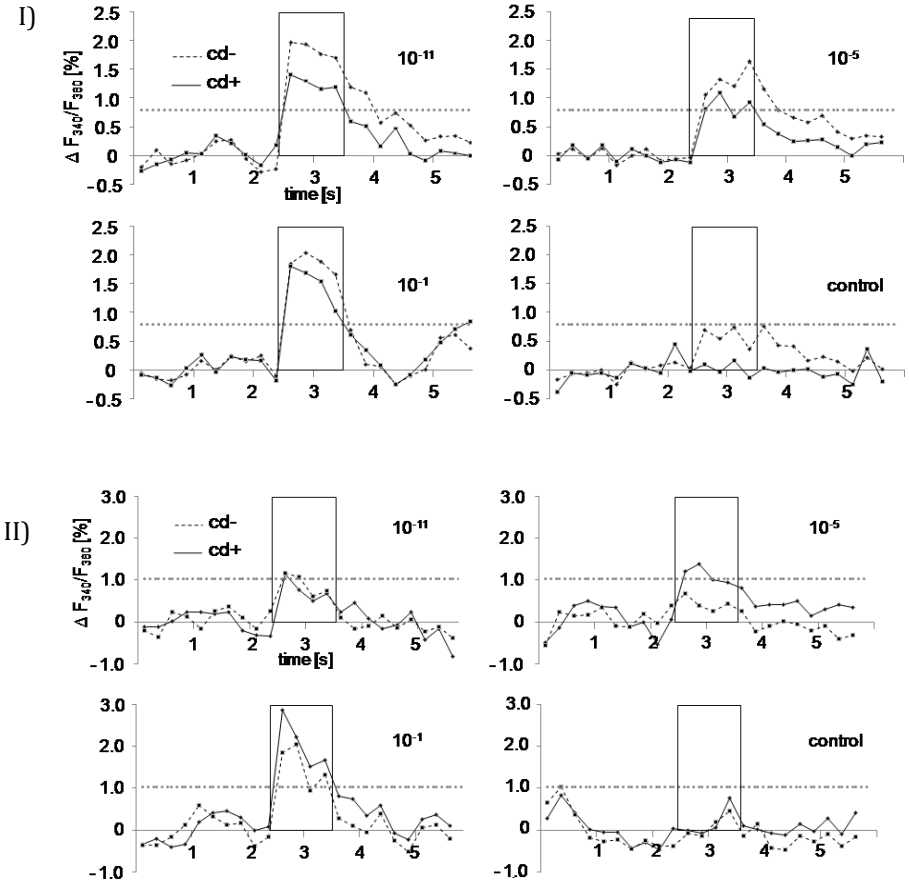

Fig. 32: **Intensity dependence of signal response duration to heptanal**
Temporal dynamics of the calcium responses to heptanal in one concentration dependent (cd+) and one concentration independent (cd-) ROI within the antennal lobe of two animals (I and II) at three different odor concentrations (10^{-11}, 10^{-5} and 10^{-2}) and the control stimulus (solvent only). Threshold level of 40% is indicated by the horizontal dotted line. The response duration varied in both animals from concentration to concentration.

2.6.3.2.3. Octanol

For octanol tested animals, the signal duration within ROI_{cd+} and ROI_{cd-} did not significantly increase from lowest to highest odor concentration (Friedman ANOVA ROI_{cd+}: 37.2537, Dof = 7, $p > 0.05$; ROI_{cd-}: 36.1472, Dof = 7, $p >= 0.05$). As demonstrated in the n-undecane and heptanal series, the octanol signal followed different signal duration dynamics. Figure 33I shows the response dynamics for ROI_{cd+} and ROI_{cd-} in one animal where the signal duration is prolonged with higher odor concentrations. In contrast, the signal dynamics in Figure 33II remain constant between highest and lowest concentration but experience a strong decrease at the medium concentration of 10^{-5}. Besides these two characteristic examples, some activity spots followed changing durations over all concentrations (data not shown) and made it very difficult to crystallize clear response dynamics from the available data.

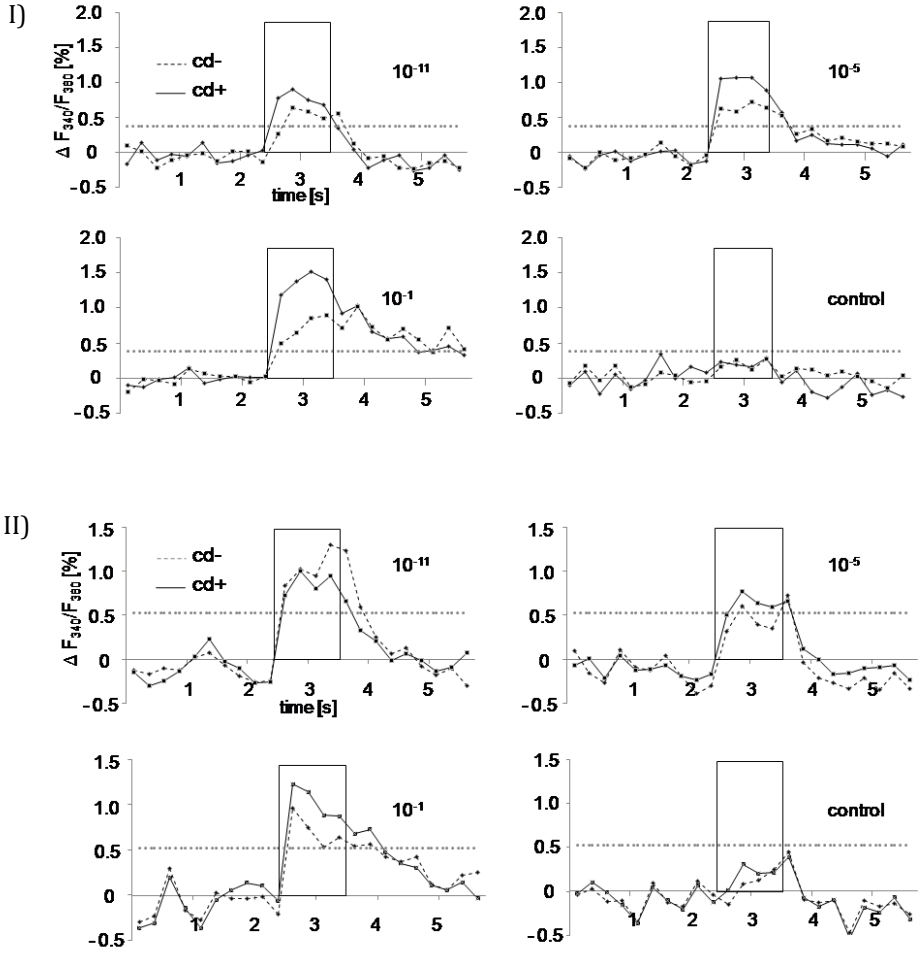

Fig. 33: **Intensity dependence of signal response duration to octanol**
Temporal dynamics of the calcium responses to octanol in one concentration dependent (cd+) and one concentration independent (cd-) ROI within the antennal lobe of two animals (I and II) at three different odor concentrations (10^{-11}, 10^{-5} and 10^{-2}) and the control stimulus (solvent only). Threshold level of 40% is indicated by the horizontal dotted line. The response duration was longer at higher odor concentrations compared to lower concentration in I, in II the response duration varied between concentrations.

2.7. Representation of single odors in odor mixtures

Since the natural odor world is dominated by mixtures of different kinds of single chemical stimuli, the question was addressed how, for example, binary mixtures find their reflection in the AL network. More precisely, it was of main interest whether behaviorally relevant odors such as pheromones receive a dominant representation during odor processing in the AL activation pattern. The responses to binary odor mixtures were measured in seven animals using the alarm pheromone n-undecane (UN), the trail pheromone component nerolic acid (NA) and the two general odors octanol (OC) and heptanal (HE). In a first attempt the glomerular response patterns to stimulations with each single odor were measured. Then, the mixtures of two odors each were applied randomly. Each single odor and the binary mixtures thereby elicited their own characteristic activation pattern (cp. Fig. 34). 170 animals were prepared for the mixture experiments, seven animals could be tested with all single odors and their binary mixtures. Only one animal out of seven showed very clear activation patterns with clearly visible activation spots during the whole experimental procedure (Fig. 34). In the remaining six animals the activation spots were either less distinct, the pattern was restricted to few small and hardly visible spots or the odor response intensity decreased during the experiment. For this reason and due to the small sample size the data analysis was restricted to simple pattern comparison amongst the different odors

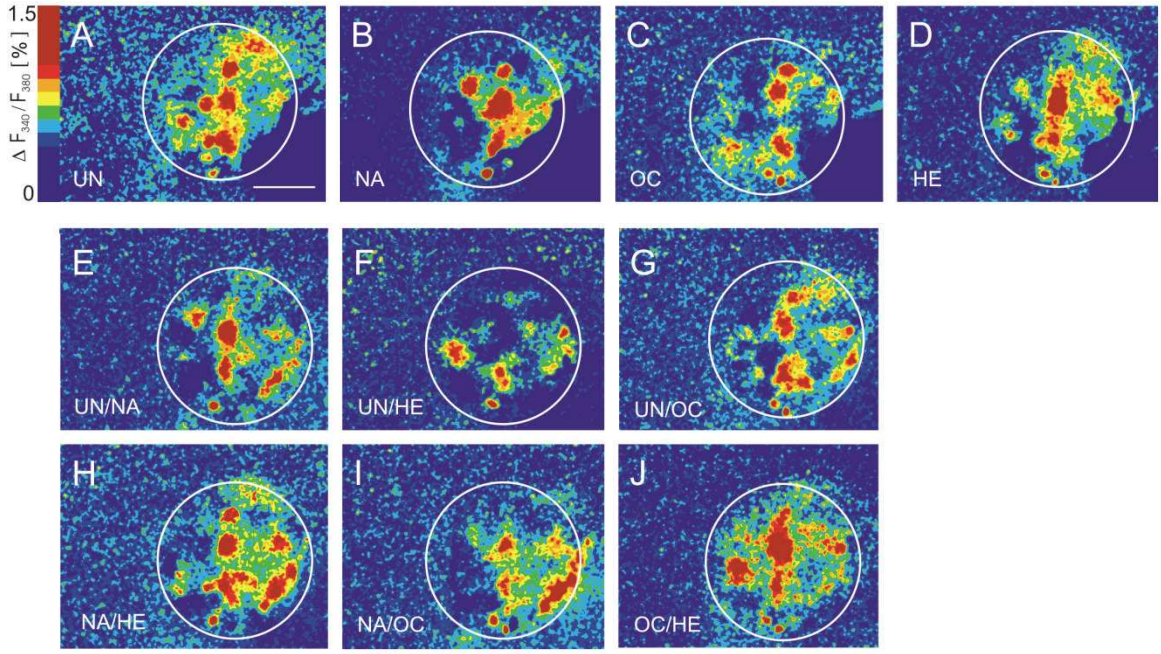

Fig. 34: **A-J:** False-color coded images showing activity areas in the AL of *C .floridanus* in response to different odors and their binary mixtures. The calcium signals were recorded from PNs of the AL loaded with Fura-2 dextran. 500 nl (dilution 10^{-5}) for all odors. **A-D:** glomerular activation patterns to single odor stimulation with n-undecane (UN), nerolic acid (NA), octanol (OC) and heptanal (HE). **E-J:** activation patterns to stimulation with binary mixtures of the single odors. Scale = 100µm.

By calculating the Euclidian distances for the single odors and their binary mixtures I tested whether the activation pattern of one single odor receives a stronger representation within the mixture than the second component. Compared over all animals tested no significantly stronger representation of one odor was given in a binary mixture except for the mixture of heptanal and octanol. Here the heptanal pattern was significantly stronger represented than the octanol pattern (Friedman ANOVA $p < 0.05$). It must be emphasized that the binary mixture of heptanal and octanol was a binary mixture of general odors. All other mixtures contained at least one pheromone.

DISCUSSION

This study represents a first comprehensive structure-function analysis within the central olfactory system of an ant's brain. The structural data revealed that the AL of *C. floridanus* contains a comparatively high number of olfactory glomeruli (up to 464 in the female caste and up to 250 in the male caste) supplied via seven distinct antennal sensory tracts in the female caste and six sensory tracts in males.

The T7 cluster of glomeruli and a prominent large glomerulus in the T4 cluster were found across all female castes and in males. Sex-specific differences were most prominent in the T6 cluster of glomeruli. In addition to a large difference in the number of glomeruli in the T6 cluster, serotonergic innervations of the associated glomeruli were present only in males and absent in all female castes.

The general organization and pattern of serotonergic innervation of AL glomeruli in *Camponotus floridanus* is rather similar across the female castes. Two aspects differ markedly in males: males posses a dual output pathway to the MB and LH despite a small number of glomeruli, but the proportion of glomeruli connected to the medial ACT is substantially smaller when compared to females (Fig. 11). This is in stark contrast to the precise hemispherical division of m- and l-ACT associated glomeruli in the female AL in the honeybee and *C. floridanus* (Kirschner et al., 2006; Zube et al., 2008). The sex-specific differences in connectivity and neuromodulatory innervations of the AL are likely to have important consequences for olfactory processing and behavior.

Functional calcium imaging studies of PN activity revealed reproducible glomerular activation patterns in response to pheromonal (alarm, trail) and general odors at about equally high sensitivity in female major workers. No obvious spatial segregation among pheromonal and non-pheromonal odor activation patterns was observed for the odors tested. Whereas the spatial response patterns to stimulation with trail pheromone (nerolic acid) were remarkably stable over a wide range of intensities (~8 log units), the response intensities, and especially response durations were dependent of odor intensities. For the alarm pheromone n-undecane and two general odors (heptanal, octanol) only in n-undecane a similar stability of odor response pattern was observed. Patterns associated with heptanal and octanol stimulation showed irregular dynamics with increasing odor intensity. Response intensities were in some activation spots concentration dependent and in some spots concentration independent. For none of the three odors concentration dependent signal duration dynamic could be observed.

Calculations of Euclidian distances in binary mixtures of the applied odors revealed no significant dominance of a single odor in the mixture, except for stimulation with octanol/heptanal. In all binary mixtures the single odor patterns were represented equally and pheromones and general odor patterns appear not to be spatially segregated in glomerular clusters within the AL.

1. NEUROANATOMY

1.1. Comparison with other Hymenoptera

The total number of AL glomeruli in *C. floridanus* is almost threefold higher than in the honeybee (up to 464 vs. 164), and glomeruli in the AL are innervated and organized into 7 clusters innervated by 7 antennal sensory tracts compared to 4 main tracts (T1-4) in the honeybee (Abel et al., 2001), or 6 input tracts when subdivisions of T3 (a-c) are counted as separate tracts as suggested by Kirschner et a. (2006). Since the calcium imaging data gives only information about the physiological properties of glomeruli belonging to clusters T1-3 on top of the AL, other methods that allow imaging of deeper layers of the AL, such as two photon confocal imaging or electrophysiology studies are required to gain information about the functional role of the division of the AL into m- and l-ACT hemispheres. It will be especially interesting to see whether a common picture may emerge from a physiological comparison between the honeybee and the ant. In addition to mapping different categories of odors, the function of a dual pathway could as well be to code different temporal aspects of the stimulus as suggested by Müller et al. (2002), or to extract different stimulus parameters. To study temporal aspects of odor coding, comparative electrophysiological studies of response profiles are needed.

In contrast to the results in moths or in honeybee drones (e.g. Hildebrand et al., 1997; Rössler et al. 1999a; Brockmann and Brückner, 2001; Sandoz 2006) and in ponerine ants (Hoyer et al., 2005) or leaf-cutting ants (Kleineidam et al., 2005) I did not find any sex-specific enlarged glomeruli or macroglomeruli in the AL of *C. floridanus* males that were not present in females. One enlarged glomerulus in the T4 cluster appeared in all four investigated castes and will be further discussed in chapter 1.2. . This, together with the fact that in the calcium imaging experiments the response patterns to stimulation with pheromonal and non-pheromonal substances were equally sensitive and not obviously segregated (or dominant in mixture responses) indicates that processing of pheromonal and non-pheromonal odors may be combinatorial in the ant AL (see further discussion of this aspect below).

Does the high number of glomeruli in the AL of *C. floridanus* indicate more sophisticated olfactory processing compared to e.g. the honeybee, the fly or moth? Comparison of the number of odorant receptor (OR) genes in recently sequenced insect genomes of *Drosophila melanogaster* (~62; Clyne et al., 1999; Vosshall et al., 2002; Robertson et al., 2003), the malaria mosquito, *Anopheles gambiae* (~79; Hill et al., 2002) and the honeybee (~170; Weinstock et al., 2006) reveal a rough correlation between the number of odorant receptor (OR) genes and the number of olfactory glomeruli in the AL (43 in *Drosphila*; Laissue et al., 1999; 61 in *Anopheles*; Ghaninia et al., 2007; 164 in *Apis*; Galizia et al. 1999b; Kirschner et al., 2006). Similar relationships were also found in vertebrate olfactory systems (Buck and Axel, 1991). If one assumes a similar correlation of OR numbers and AL glomeruli in *C. floridanus*, we expect a substantially higher number of ORs compared to the honeybee. To further prove this hypothesis would require to map OR expression to find out whether it may lead to novel glomeruli. The process of glomerulus induction itself, however, is not likely to be directly influenced by ORs because of their late expression in insects (Vosshall et al., 2002). *C. floridanus* has small optic ganglia compared to relatively large ALs (Fig. 11A). In contrast, the honeybee has elaborated optic ganglia and sophisticated visual capabilities (Gronenberg, 2001; Stach et al., 2004). In this context, evolution of a higher number of glomeruli in highly olfactory ants appears plausible. In the same line, neuroanatomical studies of the more primitive ponerine ant, *Harpegnathos saltator,* a highly visual predator, revealed a much lower number of ~178 glomeruli (Hoyer et al., 2005). An interesting hypothesis to follow up in the future is that the evolution of new OR genes may come along with the formation of new sensory tracts and clusters of glomeruli in the AL and/or the increase/decrease in the size of existing ones. Future comparison of more closely related ant species of the subfamily *Formicinae* and future genomic data may reveal further insight into the question.

Structural comparison of sensory-tract specific glomerular clusters in *C .floridanus* indicates some similarities (possibly homologies), but also clear differences compared to the situation in the honeybee (Kirschner et al., 2006). The T7 cluster of *C. floridanus* shares striking similarities with the T4 cluster in the honeybee: both possess 6 large glomeruli at the dorsal end of the AL with a unique sensory innervation pattern and with one or two glomeruli neither connected to the m-ACT nor to the l-ACT. If the two clusters turn out to be homologous, the perseverance of their anatomy throughout the *Apocrita* lineage would predict an important functional significance of these six glomeruli. Physiological, neurochemical and molecular characterization of this deep cluster, therefore, will be very elucidating.

The T3 cluster of *C. floridanus* shares similarities with the T3a cluster after Kirschner et al. (2006), especially its position at the ventral-medial AL and the division of its glomeruli to

both ACT-hemispheres. The best candidates for being homologous to the honeybee T1 cluster are T1, 2 and 4 in *C. floridanus*: all three together make up the rostral-lateral part of the AL, like in the honeybee if one considers the 45° shift of the ant AL, and they all belong to the l-ACT hemisphere. On the opposite side of the AL, the large T6 cluster in *C. floridanus* shares five anatomical features with the very small T3b cluster in the honeybee (after Kirschner et al., 2006): 1st a position at the medial-caudal AL flanking the AN, 2nd a sensory tract that approaches the glomeruli from the periphery of the AL, 3rd a clear separation from the other glomeruli clusters, 4th a composition of relatively small glomeruli, and 5th the affiliation to the m-ACT hemisphere. The most striking difference of the T6 cluster in *C. floridanus* compared to T3b cluster in the honeybee is the high number of ~128 glomeruli indicating that this could be a spot of strong evolutionary proliferation of glomeruli in the ant AL.

1.2. Caste- and sex-specific number and size of glomeruli

I found that the number of glomeruli in *C. floridanus* is similar across the female castes, but substantially smaller (by ~45%) in males (Table 1; Fig. 11). In the same line, previous investigations in the ponerine ant *Harpegnathos saltator* (Hoyer et al., 2005) and in the honeybee (Brockmann and Brückner, 2001; Sandoz, 2006) found that the number of AL glomeruli in males is substantially smaller compared to females. What are the possible functional consequences of a difference in the number and size of glomeruli for odor processing and behavior? For the number of glomeruli, genomic studies indicate a rough correlation with the number of odorant receptor genes (*Drosphila*: Clyne et al., 1999; Laissue et al., 1999; Vosshall et al., 2000; *Apis mellifera:* Weinstock et al., 2006; Kirschner et al., 2006; Galizia et al. 1999b; *Anopheles gambiae:* Hill et al., 2002; Ghaninia et al., 2007). Therefore, it would be very informative to find out whether the small number of glomeruli in males is related to a smaller number of functional odorant receptor genes in the genome. Calcium imaging experiments in honeybee drones have shown that the glomerular activation pattern in response to similar odors was different in drones compared to workers indicating that the number and position of glomeruli affects the glomerular representation of odors in the AL, which is likely to cause differences in odor perception (Sandoz, 2006). In contrast to the results in the honeybee (Brockmann and Brückner, 2001) and in *Harpegnathos saltator* (Hoyer et al., 2005) I did not find any sex-specific enlarged glomeruli or macroglomeruli in the AL of *C. floridanus* males that were absent in females. An enlarged glomerulus in the T4 cluster was found in all female castes and in males indicating that this glomerulus may be involved in processing odors that are equally important for both sexes. The same is true for all 6 glomeruli of the T7 cluster. The presence of the large T4 glomerulus in sterile workers

indicates that it is very unlikely to be involved in processing information about sex-pheromones. The absence of sex-specific macroglomeruli may indicate that sex pheromones in *C. floridanus* are processed in normal sized glomeruli as it was shown for other pheromonal odors such as alarm or trail pheromone (Zube et al., 2008). In two species of leaf cutting ants a macroglomerulus was found close to the entrance of the AL (Kleineidam et al., 2005). Physiological and behavioral studies indicate that the macroglomerulus in *Atta vollenweideri* may be involved in processing information about trail pheromone (Kleineidam et al., 2007). These studies show that representation of pheromones in enlarged glomeruli or macroglomeruli may differ substantially across ant species and other Hymenoptera. The presence of macroglomeruli may vary with certain requirements for odor processing such as the need for high sensitivity, gain, and improvement of signal-to-noise ratio, which all require that a high number of specialized ORNs on the antenna converge on the same glomerulus. Future imaging studies are needed to find out whether sex pheromones in *C. floridanus* are processed in normal sized glomeruli or in any specialized glomeruli. The similarities in the number and organization of glomeruli between minor and major workers may reflect that the worker castes *C. floridanus* express only subtle differences in olfactory related behavioral tasks (Gräf, 1995). However, possible consequences of variation in the absolute glomerular volumes as found among minor and major workers still are not known and need to be addressed in physiological experiments and behavior assays.

1.3. Sex-specific differences in the dual output pathway to the mushroom bodies

Previous studies in both the honeybee and *C. floridanus* workers found a very precise hemispherical division of AL glomeruli innervated by PNs of the m- and l-ACT (Kirschner et al., 2006; Kirschner, 2005). Interestingly this division is maintained despite a large difference in the total number of glomeruli and the number of sensory input tracts between the two species (4 input tracts and 164 glomeruli in the honeybee compared to 7 input tracts and up to ~450 glomeruli in *C. floridanus*; shown in this work). The present study shows that in *C. floridanus* males a four times higher number of AL glomeruli was connected to the l-ACT compared to glomeruli innervated by the m-ACT (Fig. 15E; Table 2). The numbers demonstrate that m-ACT associated glomeruli are reduced compared to the conditions in females. This may indicate that olfactory information processed in m-ACT associated glomeruli is less important in males. The small number of m-ACT associated glomeruli in males, therefore, may have important consequences for olfactory behavior. Males have a substantially reduced behavioral repertoire compared to females. Many olfaction related tasks such as foraging, brood care or nest building are solely performed by females, whereas

males use visual cues and mostly sex-pheromone detection during mating flights (Hölldobler and Wilson, 1990). This may indicate that m-ACT associated glomeruli are important for more sophisticated odor processing and in the context of olfactory learning and memory formation, tasks that are less needed in males.

In the honeybee, a recent study suggests that learning dependent plasticity may be absent in l-ACT PNs, and the authors hypothesize that plasticity may be encoded in responses of m-ACT PNs (Peele et al., 2005). A previous electrophysiological study in the honeybee indicated that m- and l-ACT PNs may encode similar odors, but with different tuning and temporal response properties (Müller et al., 2002). Responses of m-ACT PNs had longer latencies and were more specific compared to faster and less specific responses of l-ACT PNs to the same odors. Based on these findings one may suggest that two populations of olfactory glomeruli may receive similar input but process the information in different ways. Interestingly, there is evidence from the mouse olfactory system suggesting that glomeruli representing the same odorant receptor are symmetrically arranged in two separate domains of the olfactory bulb (Nagao et al., 2000). Following these lines, one may also speculate that the reduction of the m-ACT pathway in males of *C. floridanus* indicates that males possess a fast, but more unspecific and less plastic olfactory pathway. This would be in accordance with the reduced behavioral repertoire and less ambitious olfactory tasks performed by males, which may require less sophisticated olfactory processing. The presence of serotonergic innervation in the m-ACT associated T6 cluster of glomeruli may be another indication in the same direction (see below). Future imaging studies using multi-photon imaging to access the lower half of the AL combined with electrophysiological recordings of m- and l-ACT PNs as well as behavior studies are needed to substantiate these assumptions.

1.4. Differences in serotonergic innervations

Studies in various species of insects have shown that the ALs are prominently innervated by serotonergic neurons (e.g. Mercer et al. 1983; Kent et al., 1987; Sun et al. 1997; Bicker, 1999; Hoyer et al., 2005). In the moth *Manduca sexta* electrophysiological studies showed that serotonin enhances the sensitivity of AL PNs by increasing the spike duration via modulation of a potassium current (Kloppenburg and Hildebrand, 1995; Kloppenburg et al., 1999; Kloppenburg and Mercer 2008). Serotonin levels were further shown to modulate the sensitivity of male silkmoths in response to female pheromone (Gatellier et al., 2004). These studies indicate that serotonin plays a prominent role in modulating the sensitivity of glomerular odor processing. In most species investigated so far including the honeybee and the ponerine ant *Harpegnathos saltator* virtually all glomeruli in the AL were innervated by

serotonergic processes (e.g. Mercer et al. 1983; Schürmann and Klemm, 1984; Bicker, 1999; Hoyer et al., 2005). However, a recent study showed that in the closely related ant *Camponotus laevigatus* serotonergic innervation was absent in a group of glomeruli in the lower portion of the AL (Dacks et al., 2006). I found that this is also true for the female castes of *C. floridanus* and in workers of *C. sericeiventris*. In contrast to this all AL glomeruli of another formicine species (*Cataglyphis fortis*) appeared to be innervated by serotonergic fibers. Whether lack of serotonergic innervation in glomeruli of the lower AL is unique in carpenter ants, however, remains to be investigated in a more comprehensive phylogenetic study.

In *C. floridanus* I found that it is the m-ACT associated T6 cluster of glomeruli that was not innervated by serotonergic processes, whereas all other glomeruli had dense serotonergic innervations (Fig. 16A, C). In contrast to females, the T6 cluster of glomeruli in the male AL exhibited prominent serotonergic innervations, but with a reduced number of glomeruli compared to the female AL (Fig. 16E). In the same line, serotonergic processes in the MB-calyx lip differ between the sexes. This indicates that a subset of glomeruli in the female AL is not modulated by serotonin and may be under the influence of a different neuromodulator. The reduction of m-ACT associated glomeruli together with the presence of serotonergic innervation in males is very interesting in the light of a study by Mercer and Menzel (1982) showing that in the honeybee injection of serotonin reduced olfactory learning abilities. This may indicate that m-ACT associated glomeruli may express a higher degree of experience related plasticity compared to l-ACT related glomeruli in females, but not (or to a smaller extent) in males. This would mean that various aspects of odor processing and plasticity in the male AL are substantially different from the conditions in females. The differences among the female castes are more subtle, but may as well be present for other neuromodulators like dopamine, octopamine or nitric oxide. Furthemore, slight variations in glomerular position and size may as well have subtle influences on odor processing. Future investigations of odor processing and neuromodulatory influences in the T6 cluster will be most promising to further understand the neuronal basis of caste-specific behavior.

2. NEUROPHYSIOLOGY

2.1. Odor representation in the antennal lobe

Calcium imaging in the AL revealed odor induced activity of PNs. I employed the retrograde loading technique, originally developed in the honeybee by Sachse and Galizia (2002) for the first time successfully in the ant. Although both l- and m-ACT PNs were backfilled with our technique, our neuroanatomical analyses show that the field of view in calcium imaging experiments was restricted to the ventral part of the AL almost exclusively innervated by l-ACT PNs (with only few exceptions of m-ACT glomeruli in the T3 cluster; see Fig. 13A). Therefore, all further conclusions are mostly limited to l-ACT PNs.

Odor specific activation patterns in primary olfactory centers were described for a number of invertebrate and vertebrate species (e.g. Rodrigues, 1988; Friedrich and Korsching, 1997; Joerges et al., 1997; Rubin and Katz, 1999; Hansson et al., 2003; Carlsson et al., 2005), but only one earlier study in a closely related *Camponotus* species (Galizia et al., 1999). In my studies activation patterns in response to different odors were overlapping, indicating that some glomeruli contribute to the activation pattern elicited by different odors. I was further able to show that odor specific activity patterns are conserved between individuals, similar to findings in the honeybee (Galizia et al., 1999a).

Only in some cases, calcium signals from the PNs could be assigned to individual glomeruli (activity spots). Large activity regions indicate that the odor used for stimulation was represented in several adjacent glomeruli rather than in isolated single glomeruli of the AL. Studies in the honeybee indicate that adjacent glomeruli may have similar response profiles (Sachse et al., 1999). In our study, the size of cohesive activity regions covering several glomeruli remained constant over a large range of concentrations, and in most cases was only marginally smaller at odor concentration threshold. Are the large activity regions the result of scattered fluorescence from activated glomeruli out of focus, e.g. in deeper layers of the AL? Glomeruli in the AL of *C. floridanus* are clustered (Fig. 13 and 14), and some glomeruli may be above or below the focal plane during calcium imaging. Repeated stimulation with the same odor at different focal planes revealed large activity regions also in z-direction, and no confined activity spots were found up to a depth of 80 µm (data not shown). This indicates a large or even complete overlap of response profiles in at least some neighboring glomeruli. Other glomeruli seemed to have a more distinct response profile compared to their adjacent glomeruli. For example, with increasing concentration of nerolic acid, some activity spots appeared at low concentration and remained constant in size up to high odor concentrations. In this case, no glomeruli next to the activity spots were recruited to the activation pattern. In our study, I used only single-component odors, and it remains to be shown whether odor

mixtures may elicit a more distinct activation pattern across glomeruli within the same activity region by processing of odor information in the AL network.

The activity patterns in response to (non-sex) pheromones (or pheromone components) were distributed across the field of view, and there was no obvious qualitative difference compared to the activation patterns in response to general odors. Even very low pheromone concentrations elicited a distributed activation pattern in the AL, and no distinct cluster of pheromone specific glomeruli was found. Yamagata et al. (2006) using electrophysiological recording and staining of AL neurons described "alarm pheromone-sensitive" glomeruli in another *Camponotus* species that form a cluster in the most dorsal part of the AL. This particluar glomerular cluster was not accessible in my imaging experiments. Nevertheless, in contrast to this our calcium imaging experiments revealed a rather distributed representation of n-undecane in *C. floridanus*, and I conclude that for n-undecane as well as for nerolic acid pheromone-specific clusters of glomeruli do not exist in the AL of *C. floridanus* workers. This confirms and extends similar observations from a calcium imaging study in *Camponotus rufipes* (Galizia et al., 1999), and is consistent with the rather distributed representation of non-sex pheromones in glomeruli within the AL of the honeybee (Joerges et al., 1997; Galizia et al., 1999a). It remains to be shown, whether this arrangement is advantageous or even necessary to allow context specific behavioral responses to pheromones as described for ants (Hölldobler and Wilson, 1990; Knaden and Wehner, 2003). Differential neuromodulatory innervation of the AL (Dacks et al., 2006; Ziegler et al., 2007, and the present study) are likely to promote primary processing of pheromone information and certainly need further investigation.

2.2. Processing of odor intensity

The concentration thresholds I measured were at extremely low concentrations. Odor loading of the filter paper in the range of 0.05pg (equivalent to dilutions of 10^{-11}) was sufficient to elicit reliable calcium amplitudes in PNs, and concentration thresholds were similar for both, pheromones and general odors. Although it is difficult to compare data from different laboratories using different types of odor stimulation devices (e.g. variation in air flow velocity and volume), the odor sensitivity in ants seems to be 5 log units higher compared to what was described for the honeybee (Sachse and Galizia, 2003). In addition, in the honeybee the spatial activation pattern was shown to be concentration invariant only over a limited range of concentrations. My results in *C. floridanus* indicate that the spatial activation pattern in response to the trail pheromone component is largely invariant over a rather large concentration range of ~8 log units. Only at the lowest and highest concentrations, glomeruli disappeared or additional ones were recruited.

A very similar response dynamic was monitored in n-undecane, the second pheromone tested. Here the pattern was in most cases stable over at least 5 log units and only at lowest and highest concentrations glomeruli were either added or removed from the existing pattern. In contrast, for both general odors tested in most cases no pattern stability as for the pheromones was observed. The pattern varied between the different odor concentrations and showed a concentration invariance only within small concentration ranges. This indicates that the concentration dependence of odor induced activation patterns appears to be odor (group) specific. Whether these differences are persistent in other pheromones and other general odors remains to be tested in future experiments.

Concentration dependent activity patterns were also described in other species (Rubin and Katz, 1999; Johnson and Leon, 2000, Meister and Bonhoeffer, 2001, Fuß and Korsching, 2001; Wachowiak and Cohen, 2001). The main reason for the change in activity pattern at the level of the AL or olfactory bulb probably is that the receptive range of individual ORNs is concentration dependent and broadens with increasing odor concentrations (Firestein, 1993; Duchamp-Viret et al., 2000; de Bruyne et al., 2001). The perceived odor quality can depend on odor concentration, a phenomenon known in human psychophysics (Gross- Isserhoff et al., 1988) and from experiments in *Drosophila* (Rodrigues and Siddiqi, 1978; Stensmyr et al., 2003). In ants, concentration dependent responses may result e.g. in a well defined behavioral sequence when approaching a (alarm) pheromone source (active space hypothesis; Bradshaw and Howse, 1984; Hölldobler and Wilson, 1990). The idea that indeed the activity pattern found in the AL corresponds to perceptual measures recently received strong support from a study in the honeybee (Guerrieri et al., 2005). Interestingly, in response to trail pheromone I found activity spots only adding to the pattern at very high or low concentrations. Sampling of a chemical trail with antennation movements may cause large fluctuations at ORNs; therefore, it may be important to maintain a constant spatial pattern at the level of the PN output in order to stabilize odor quality perception. Higher concentrations of the trail pheromone on the other hand enhance the probability of a worker to follow a trail. Regarding the alarm pheromone active space model a similar processing theory can be applied to the alarm pheromone n-undecane. Here I found, similar to the trail pheromone, changes in the glomerular pattern in most cases only at very high and very low concentrations. Since the behavioral switch from attractiveness to aggression occurs at a certain high concentration and pheromone concentration varies during air release it might be of importance for an individual to retain clear information about odor quality in combination with information about intensity. Therefore, the maintenance of a constant quality perception

and, at the same time assessing odor intensity is important for trail following and aggressive behavior.

My results indicate that the response duration may be a reliable measure for coding odor intensity, at least in the trail pheromone nerolic acid. Concentration invariant activation patterns together with graded calcium-signal amplitudes, as also described in the honeybee, led to the assumption that the activity pattern is the important parameter for assessing odor quality while the peak activity of the PNs relates to odor intensity (Galizia and Menzel, 2001). For some activity regions, but not for all, I found graded calcium-signal amplitudes with increasing odor concentrations. In other cases, activity regions with almost constant calcium-signal amplitudes over a wide range of concentrations had considerably stronger amplitudes at the highest concentration than at any other concentration (see results chapter 2.6.2.). In case of high basal intracellular calcium concentration, weak neural responses may already lead to a saturation of the Fura-2 signal. In these cases the calcium amplitude would not increase with increasing response strength, but the duration would. However, the fact that even in concentration independend (cd-) cases I observed a slight increase in the amplitude only at the highest concentration indicates that the Fura-2 signal was not saturated.
In contrast to variations with respect to graded amplitudes, I found significantly graded response durations in case of the trail pheromone nerolic acid. This indicates that response duration might be an important parameter for assessing odor concentration.
For n-undecane, octanol and heptanal only in some cases graded response durations were measured with increasing odor concentration, confirming the results found in nerolic acid. In other cases the response durations varied over concentrations indicating that coding odor concentration may underlie different odor specific coding properties.
These different observations underline the complex odor coding mechanisms of the olfactory neuronal network. Odor coding properties may vary between single glomeruli and one has to keep in mind that the duration of the Fura-2 signal may be strongly influenced by both the strength (spike rate) and the duration of a neural response. For further detailed information I recommend further electrophysiological analyses at the single PN level to fully clarify this aspect in odor coding.

Odor information is relayed to higher brain regions via a combinatorial code of PN activation. Although it is not fully understood how odor quality and intensity are coded by the population of PNs, it seems plausible that the temporal structure of PN activity is important. Mixtures of odors lead to a synchronization of pheromone sensitive PNs in moths (Lei et al., 2002), and in the locust oscillatory synchronization of activity is considered as an important

feature for odor coding and recognition (Laurent, 2002). Prolonged activation of PNs in response to higher odor concentrations might enhance temporally coordinated PN activity, and temporal coordination may be important for both, odor quality and intensity coding (Lei et al., 2004). In this respect, spatial separation of m- and l-ACT PN pathways may be an important addition in the *Apocritan* lineage reflecting the evolution of advanced processing of a large variety of both chemical communication signals and environmental odors.

2.3. Processing of odor mixtures

Odor stimulation with most of the binary mixtures led to no significant results regarding the question whether a single odor component is represented dominantly in the glomerular activation pattern of a binary blend. Both odors were represented in the mixture, and mixture patterns were predictable from their single component, indicating that mixture interactions at the peripheral level are weak. Only the stimulation with the general odor blend of heptanal/octanol revealed a significantly higher representation of glomeruli activated by heptanal in the mixture pattern, which means that the mixture pattern was more similar to the heptanal pattern than to octanol. This result fits in a certain way with data from other studies which showed that although both components are represented within the mixture, one single component frequently appears to be represented dominantly, i.e. the glomerular activation dynamics fit even more to the strongest single component (Joerges et al., 1997; Tabor et al., 2004).

In all other binary mixture stimulations the general odor was combined with a pheromone and these data revealed no significant difference between the representations of both odors in their mixture. Both glomerular patterns were represented in the mixture which underlines my previous studies where I could show that there is no spatial segregation of pheromonal and general odor processing indicating that both odor classes are processed in a combinatorial way. Stating that their representation is equal in the mixture pattern, and from a view on the activation pattern solely, these data confirm previous studies showing that glomeruli from both odors contribute equally to the mixture response (Joerges et al., 1997; Tabor et al., 2004). For further information about how the pheromone processing differs from general odors within odor mixtures, at signal dynamics level or at the cellular level, remains to be investigated in more detail in the future. In this regard, predictions about how strongly activated a single glomerulus may be in a binary mixture were suggested and performed by Tabor and Colleagues (2004) as well as Silbering and Galizia (2007). In both studies a prediction on glomerular mixture responses was calculated from recordings at two different concentrations for each single component. Such detailed studies about mixture

representations within the ant olfactory system would require testing different concentrations for each mixture component plus testing the mixtures themselves within the same animal. With respect to the amount of work, these experiments and additional investigations of single glomeruli intensity profiles would require an additional study of its own and could not by analyzed in this study in sufficient detail.

3. CALCIUM IMAGING IN ANTS

3.1. Technical perspectives and future improvements

For a detailed characterization of the physiological aspects of odor processing within the AL I applied the calcium imaging technique and measured odor induced neuronal activity within AL glomeruli. From a technical perspective, calcium imaging bears some difficulties in ants. In the following I will discuss the technical difficulties associated with this technique in ants and provide suggestions for possible solutions in future research.

3.1.1. Tissue preparation

Since I measured PN responses in the AL glomeruli the established PN-tract staining protocol from the honeybee had to be adapted to the ant system. In this context it was very difficult to establish a standardized staining procedure due to the demanding dye application. Selective staining of single PN tracts was very critical due to missing reliable landmarks for dye insertion. In addition it was difficult to manually apply the dye at the correct depth of the PN-tracts within the brain. Although the tissue preparation was always performed very carefully the mortality of the ants after tissue treatment reached in some cases up to 50%. Furthermore some individuals showed abnormal locomotion behavior after tissue penetration indicating that during tissue treatment other brain regions were damaged. After the four hour dye diffusion period these individuals were usually too weak and not used for further experimentation.

3.1.2. Optical imaging procedure

During calcium imaging in many cases the stimulation with the control stimulus and even pure airstream led to a strong neuronal response in the AL of the tested ant. I assumed a contamination of the calcium imaging setup due to stimulation with high concentrations of the tested odors, but even after complete cleaning of the calcium imaging setup the responses were still present. This contamination problem was present several times but only over a period of a few weeks. One explanation left for this phenomenon could be that the “Hausluft”

used for a constant airstream was contaminated and the high sensitivity of the ant's olfactory system may cause a problem in this respect. To exclude that contamination of tubing etc. caused the contamination the setup was cleaned over night with a long term constant airstream protocol when the tubes had been changed to get the tubes as scent free as possible.

For the high mortality rate of the ants during the calcium imaging procedure I assume two explanations. On the one hand the UV-light exposure of the tissue during the experiment might be damaging for the neuropil and lead to increased neuronal death. However, imaging studies on the honeybee and other insects have used fura2-dextran before indicating that UV excitation may not be the cause of higher mortality. On the other hand the brain tissue might undergo increased cell death during the experimental procedure. To extend survival of brain tissue I used ringer solution with extra sucrose as energy source (in experiments where ringer without sucrose was used animals showed an even higher mortality), but no constant ringer flow. The latter should be changed in future experiments, but this will lead to an increase in tissue movement. Thus, tissue stabilizing media (such as kwik-seal) will have to be tested.

Fur further experiments I also recommend using a 2-photon microscope. With the standard calcium imaging setup only the upper part of the AL, and thus for the most part the l-ACT neurons, can be investigated due to the limitations in working distances of the microscope objectives. The 2-photon confocal microscope allows calcium imaging experiments at deeper layers of the AL and gives the opportunity to image glomeruli at the posterior part of the AL which are exclusively supplied via m-ACT neurons. In addition, 2-photon confocal calcium imaging allows measurements at a definite focal plane which is limited in CCD imaging.

3.1.3. Data analysis

Since the focal plane of the AL cannot be adjusted in exactly the same plane for all individuals due to the large number of glomeruli that are not clearly visible during the imaging experiment (and cannot be mapped as clearly as in the honeybee due to small glomerular size and the high number of glomeruli), each experiment and each ant had to be treated and analyzed individually. Hence, a comparable statistical analysis for single glomerulus odor processing was not possible across individuals. Here a future step would again be to use 2-photon confocal calcium imaging where a single focal plane can be measured and individual glomeruli can be visually identified at the same time. In *C. floridanus* I would recommend to use the focal plane where the enlarged glomerulus in the T4 cluster is visible. This glomerulus or larger tracts may serve as an excellent landmark for 2-photon experiments. Furthermore, two-photon imaging may give access to the very interesting T6 cluster.

4. SUMMARY

Besides their complex odor driven behavior social insects attract attention with the appearance of different castes within the colony. Queens, males and worker castes display caste-specific behaviors, and many species (such as leaf cutter ants) exhibit a strong polymorphism even among the worker caste. These caste-specific differences in behavior and morphology offer the great opportunity for comparative studies at the neuroanatomical and neurophysiological levels in order to analyze the neuronal substrate for olfactory guided caste-specific behavior.

In this study I investigated the olfactory system of the carpenter ant *Camponotus floridanus* with the main focus on the first odor processing neuropil, the antennal lobe (AL). My neuroanatomical analyses of the AL organization with its input- and output-connections to higher olfactory centers and its serotonergic innervation showed significant differences between the female and male caste. These differences are likely to contribute to caste- and sex-specific differences in olfactory behavior. Interestingly, the general organization of the olfactory pathway in *C. floridanus* showed high consistency with studies on the honeybee (Kirschner et al., 2006) indicating a common principle of neuronal connectivity in social Hymenoptera.

Using calcium imaging my physiological experiments focused on the representation of pheromonal and non-pheromonal odors within the AL of the major worker caste. In my PhD-thesis, I was able to show that processing of both odor groups is not spatially separated in the AL and that odor response thresholds ly at a similar and very low concentration level. Furthermore, my results indicate that the representation of odor intensity might be odor group dependent in terms of changes of activity patterns, signal intensity and signal duration. To summarize, this study contributes to a better understanding of common principles in odor processing in Hymenoptera and reveals caste-dependent plasticity in the organization and connectivity of olfactory centers in the ant brain. Furthermore, the new insights in structural and functional features of odor processing within the ant olfactory system will serve as an essential basis for future behavioral and physiological studies.

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ZUSAMMENFASSUNG

Für Ameisen spielt die olfaktorische Kommunikation und Orientierung eine zentrale Rolle hinsichtlich der Organisation des Ameisenstaates. Auffällig sind hierbei spezielle kasten- und geschlechtsspezifische Verhaltensweisen, die oft an eine strenge Arbeitsteilung geknüpft sind. Die Verwendung von chemischen Signalen übernimmt eine zentrale Aufgabe bei der Steuerung dieses kastenspezifischen Verhaltens. Ob sich kasten- und geschlechtsspezifische Verhaltensunterschiede auf neuronaler Ebene und besonders im olfaktorischen System der Ameise widerspiegeln ist die zentrale Frage meiner Arbeit. Im Speziellen stellte ich die Frage, ob sich in der olfaktorischen Bahn der Rossameise *Camponotus floridanus* strukturelle oder funktionelle Anpassungen an die Verarbeitung von Pheromonen und generellen Düften aufzeigen lassen.

Zur Analyse hinsichtlich neuroanatomischer Spezialisierungen wurde die olfaktorische Bahn im Gehirn von großen und kleinen Arbeiterinnen, Jungköniginnen und Männchen der Rossameise *C. floridanus* mittels Fluoreszenzmassenfärbungen, Immunzytochemie, konfokaler Laserscanningmikroskopie und 3D-Auswertung untersucht. Um die Verarbeitung von Pheromonen und generellen Düften im primären olfaktorischen Neuropil, dem Antennallobus (AL), auf physiologischer Ebene zu charakterisieren wurden olfaktorische Projektionsneurone mittels Calcium Imaging untersucht.

Obwohl sich das glomeruläre Gesamtvolumen der ALs zwischen Arbeiterinnenkasten und Jungköniginnen unterscheidet, lag die Gesamtzahl der Glomeruli im AL in einem ähnlichen Bereich. Der AL besteht in allen drei weiblichen Kasten aus bis zu 460 Glomeruli, die in sieben Clustern angeordnet sind und von sieben sensorischen Eingangstrakten innerviert werden. Der AL unterteilt sich in zwei Hemispheren, deren entsprechende Glomeruli von Projektionsneuronen innverviert werden, die vom AL über die Nervenbahn des "dual output pathway" in höhere Hirnregionen projizieren. Diese Nervenbahn besteht aus dem medialen (m) und lateralen (l) Antennocerebraltrakt (ACT) und verbindet den AL mit höheren Integrationszentren wie den Pilzkörpern (MB) und dem lateralen Horn (LH). M- und l-ACT unterscheiden sich in ihren Zielregionen im MB Calyx und dem LH. Drei weitere ACTs (mediolateral – ml) projizieren ausschließlich ins laterale Protocerebrum. Männchen besitzen ca. 45% weniger Glomeruli im Vergleich zur Weibchenkaste. Ihnen fehlt weiterhin einer der sieben sensorischen Eingangstrakte vollständig. Trotz der wesentlich geringeren Anzahl an Glomeruli, besitzen auch Männchen den "dual output pathway". Im Gegensatz zu den Weibchen ist allerdings nur eine geringe Anzahl an Glomeruli durch m-ACT Projektionsneurone innerviert. Ein weiterer Unterschied im AL von Männchen und Weibchen findet sich in den Glomeruli des sensorische Trakts Nummer sechs, die bei Weibchen

keinerlei serotonerge Innervierung aufweisen während beim Männchen der gesamte AL dichte serotonerge Verzweigungen besitzt. Es zeigt sich somit, dass die kastenspezifischen Unterschiede in der allgmeinen glomerulären Organisation des AL innerhalb der Weibchenkaste nur sehr fein sind. Im Gegensatz dazu sind die geschlechtsspezifischen Unterschiede in Anzahl, Konnektivität und neuromodulatorischer Innervierung von Glomeruli zwischen Weibchen- und Männchen wesentlich ausgeprägter was Unterschiede in olfaktorisch geprägten Verhaltensweisen begünstigen könnte.

Die Calcium Imaging Experimente zur Untersuchung der Verarbeitung von Pheromonen und generellen Düften im AL der Ameise zeigten, dass Duftantworten reproduzierbar und zwischen Individuen vergleichbar waren. Die Sensitivität des Calcium Signals lag für beide Duftgruppen in einem sehr niedrigen Bereich (Verdünnung 10^{-11}). Die Antortmuster beider Duftgruppen überlappten zum Teil, was die Annahme zuläßt, dass die Verarbeitung von Pheromonen und generellen Düften keiner räumlichen Trennung innerhalb des AL unterliegt. Die Intensität der Antwortmuster auf die Pheromonkomponenten (Spurpheromon: Nerolsäure; Alarmpheromon: n-Undecan) blieben in den meisten Fällen über einen weiten Konzentrationsbereich konstant (7-8 log Einheiten). Die Dauer der Calciumantwort nach Stimulation mit Nerolsäure verlängerte sich mit steigender Duftkonzentration. Dies läßt für das Spurpheromon den Schluß zu, dass die Duftqualität in einem konstanten Duftmuster (Konzentrationsinvarianz) repräsentiert und die Duftintensität über die Dauer des Calciumsignals abgebildet wird. Da die Antwortmuster auf generelle Düfte (Heptanal, Octanol) dagegen sehr viel stärker innerhalb des getesteten Konzentrationsbereichs varrieren ließ sich für n-Undecan und die beiden generellen Düfte eine solche Dynamik nur in einigen wenigen Fällen beobachtet.

Zusammenfassend ist diese Studie die erste strukturelle und funktionelle Studie des olfaktorischen Systems der Ameise. Die Ergebnisse tragen zu einem besseren Verständnis der neuronalen Adaptationen und Mechanismen hinsichtlich Duftverarbeitung im zentralen Nervensystem von Insekten bei. Außerdem liefert diese Studie eine wichtige Grundlage für zukünftige neuroanatomische und –physiologische Untersuchungen auf dem Gebiet der Neurobiologie der Insekten.

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APPENDIX

PUBLICATIONS

FULL PAPERS

Zube C and Rössler W. 2008. Caste-specific adaptation in the olfactory pathway of *Camponotus floridanus.* Arthropod Struct Dev doi: 10.1016/j.asd.2008.05.004.

Zube C, Kleineidam CJ, Kirschner S, Neef J and Rössler W. 2008. Organization of the olfactory pathway and odor processing in the antennal lobe of the ant *Camponotus floridanus.* J Comp Neurol 506:425-441.

*Kirschner S, Kleineidam CJ, **Zube C**, Rybak J, Grünewald B and Rössler W. 2006. The dual olfactory pathway in the honey bee *Apis mellifera* J Comp Neurol 499:933-952

*Barbara GS, **Zube C**, Rybak J, Gauthier M, Grünewald B. 2005. Acetylcholine, GABA and glutamate induce ionic currents in cultured antennal lobe neurons of the honeybee, *Apis mellifera* J Comp Physiol A 191:823-36.

*the results presented in this paper were obtained during my diploma thesis and a practical training

CONFERENCE PRESENTATIONS

C. Zube, S. Kirschner, J. Neef, C. Kleineidam and W. Rössler (2007) Organization of glomeruli and olfactory processing of trail pheromone in the ant, *Camponotus floridanus*. Xth European Symposium for Insect Taste and Olfaction, Roscoff, France (talk).

C. Zube, S. Kirschner, J. Neef, C. Kleineidam and W. Rössler (2007) Organization of glomeruli and olfactory processing of trail pheromone in the ant, *Camponotus floridanus*. Invited talk at the Dalhousie University, Prof. Ian Meinertzhagen, Halifax, Canada (talk).

C. Zube, S. Kirschner, J. Neef, C. Kleineidam and W. Rössler (2007) Organization of glomeruli and olfactory processing of trail pheromone in the ant, *Camponotus floridanus*. Proceedings of the 31st Göttingen Neurobiology Conference and the 7th Meeting of the German Neuroscience Society, Göttingen, Germany (poster).

C. Zube (2006) Processing of olfactory information in the ant antennal lobe. 99. Annual Meeting of the German Zoological Society, Münster, Germany (talk).

C. Ziegler, **C. Zube**, S. Kirschner and W. Rössler (2006) Neuromodulation in olfactory centers of the ant *Camponotus floridanus*: serotonin and NO. 99. Annual Meeting of the German Zoological Society, Münster, Germany (poster).

C. Zube, J. Neef, CJ. Kleineidam and W. Rössler (2006) Processing of olfactory information in the ant antennal lobe. 5th Forum of European Neuroscience, Vienna, Austria (poster).

C. Zube (2006) Processing of olfactory information in the ant antennal lobe. Neurobiologischer Doktoranden Workshop, Berlin, Germany (talk).

S. Kirschner, **C. Zube**, CJ. Kleineidam, B. Grünewald and W. Rössler (2005) Subsets of antennal lobe glomeruli project via different output-travts in the honeybee brain. Proceedings of the 30th Göttingen Neurobiology Conference and the 6th Meeting of the German Neuroscience Society, Göttingen, Germany (poster).

C. Zube, CJ. Kleineidam and W. Rössler (2003) Darstellung olfaktorischer Projektionsneurone in den höheren Verarbeitungszentren im Gehirn der Honigbiene *Apis mellifera*. Proceedings of the 18th Congr IUSSI (German section), Regensburg, Germany (poster).

G. Barbara, **C. Zube**, J. Rybak, M. Gauthier and B. Grünewald (2003) Ionotropic receptors of cultured honeybee antennal lobe neurons. Proceedings of the 29th Göttingen Neurobiology Conference and the 5th Meeting of the German Neuroscience Society, Göttingen, Germany (poster).

ERKLÄRUNG

Erklärung gemäß § 4 Absatz 3 der Promotionsordnung der Fakultät für Biologie der Bayerischen Julius-Maximilians-Universität zu Würzburg vom 15. März 1999.

Hiermit erkläre ich, die vorliegende Arbeit in allen Teilen selbständig und nur mit den angegebenen Hilfsquellen angefertigt zu haben.

Diese Dissertation hat weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.

Ich habe bisher außer den mit dem Zulassungsgesuch urkundlich vorgelegten Graden keine weiteren akademischen Grade erworben, noch zu erwerben versucht.

Würzburg, den 27.07.2008