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Pheromone-sensitive glomeruli in the primary olfactory centre of ants

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Tremendous evolutional success and the ecological dominance of social insects, including ants, termites and social bees, are due to their efficient social organizations and their underlying communication systems. Functional division into reproductive and sterile castes, cooperation in defending the nest, rearing the young and gathering food are all regulated by communication by means of various kinds of pheromones. No brain structures specifically involved in the processing of non-sexual pheromone have been physiologically identified in any social insects. By use of intracellular recording and staining techniques, we studied responses of projection neurons of the antennal lobe (primary olfactory centre) of ants to alarm pheromone, which plays predominant roles in colony defence. Among 23 alarm pheromone-sensitive projection neurons recorded and stained in this study, eight were uniglomerular projection neurons with dendrites in one glomerulus, a structural unit of the antennal lobe, and the remaining 15 were multiglomerular projection neurons with dendrites in multiple glomeruli. Notably, all alarm pheromone-sensitive uniglomerular projection neurons had dendrites in one of five 'alarm pheromone-sensitive (AS)' glomeruli that form a cluster in the dorsalmost part of the antennal lobe. All alarm pheromone-sensitive multiglomerular projection neurons had dendrites in some of the AS glomeruli as well as in glomeruli in the anterodorsal area of the antennal lobe. The results suggest that components of alarm pheromone are processed in a specific cluster of glomeruli in the antennal lobe of ants.

Keywords: social insects; alarm pheromone; antennal lobe; olfactory centre; pheromone communication

1. INTRODUCTION

Alarm pheromone plays predominant roles in colony defence in social insects (Blum 1985; Hölldobler 1995; Vander Meer & Alonso 1998). In the ant *Camponotus obscuripes*, for example, workers use products of the poison gland and Dufour's gland as components of alarm pheromone, among which formic acid and *n*-undecane play major roles in alarming nest-mates (Fujiwara-Tsujii *et al.* 2006). Workers that receive formic acid exhibit elusion from the odour source, while those that receive *n*-undecane exhibit attraction towards the odour sources. Thus, the ratio of pheromone components is one of the factors by which defence behaviour against a nest intruder is regulated.

In ants, there is evidence suggesting that pheromonal odours and non-pheromonal environmental odours are received by specific classes of olfactory receptor neurons on the antennae (Dumpert 1972; Löfqvist 1976; Ozaki *et al.* 2005). The axons of receptor neurons project to the primary olfactory centre, the antennal lobe. Like the vertebrate olfactory bulb, the insect antennal lobe is composed of many glomeruli (Hildebrand & Shepherd 1997). Each glomerulus receives many antennal afferents that synapse on a few output neurons, called projection neurons. Axons of projection neurons terminate in the protocerebrum, where sensory signals are processed to lead to behavioural decision (Strausfeld 1976; Mizunami *et al.* 1998; Okada *et al.* 1999, 2003).

No brain structures specifically involved in the processing of non-sexual pheromone have been physiologically characterized in social insects, though the antennal lobe of large workers of leaf-cutting ants has been found to contain an enlarged glomurulus and it is speculated that this glomerulus participates in the detection of trail pheromone (Kleineidam *et al.* 2005). In calcium-imaging studies of the antennal lobe of the ant *Camponotus rufipes* (Galizia *et al.* 1999*a*) and the honeybee (Galizia *et al.* 1999*b*; Sachse *et al.* 1999), it has been shown that glomeruli that exhibited responses to alarm pheromone also responded to non-pheromonal odours, and alarm pheromone is, therefore, thought to be coded by an across-glomeruli activity pattern. These studies, however, dealt only with glomeruli at the surface of the antennal lobe, due to limited accessibility of optical recording techniques to deeper glomeruli.

Here we physiologically and anatomically characterize alarm pheromone-sensitive projection neurons of the antennal lobe of ants, and we show that alarm pheromone signals are processed in a specific cluster of glomeruli.

2. MATERIAL AND METHODS

(a) *Animals and preparations*

Workers of the ant *C. obscuripes* maintained in the laboratory under conditions of a constant light (12 h) and dark (12 h) cycle and at a temperature of 25 °C were used. Each ant was anaesthetized by carbon dioxide for 5–10 min and then fixed on a stage with wax. The frontal (ventral) surface of the brain was exposed by removing a piece of cuticle between the compound eyes. The oesophagus and some jaw muscles were

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removed, and a glass rod was inserted into the oesophagus foramen for stabilization of the brain. The brain was immersed in saline solution used for a related species, *Camponotus floridanus* (Gronenberg *et al.* 1996).

(b) *Intracellular recording and staining*

Borosilicate glass capillaries were pulled on a laser puller (P-2000, Sutter Instruments) and were filled with 8% Lucifer Yellow (Sigma) dissolved in 1 M $LiCl_2$ at the tips, with d.c. resistances of 60–90 MΩ (after Nishino *et al.* 2003). Recordings were made in various areas of the right hemisphere, including the antennal lobe, the lateral horn and the area beneath the calyx of the mushroom body where lateral and median anteno-cerebral tracts pass. The electrical signal was amplified with an amplifier (MEZ-8100, Nihon Kohden) and displayed on an oscilloscope and a digital recorder (Omniace, NEC). Data were stored on a DAT recorder (RD120-T, TEAC). The recorded neuron was filled with Lucifer Yellow by applying a hyperpolarizing current (−0.3 to −2.0 nA). After dye injection, the ant was incubated at 4 °C for 3 h and fixed in 4% formaldehyde in Millonig's buffer overnight at 4 °C. Then the brain was dissected out, rinsed in saline, dehydrated in alcohol and cleared in methyl salicylate. The brain was observed by a confocal laser-scanning microscope (LSM510, Zeiss). The thickness of optical sections was 4 μm. Brain orientation was according to the neuroaxis.

(c) *Three-dimensional reconstruction of the antennal lobe*

For three-dimensional reconstruction of the antennal lobe, the brain of an ant was fixed in 4% formaldehyde solution, dehydrated in an ascending ethanol series and cleared in methyl salicylate. The antennal lobe was imaged dorsally by using a confocal microscope, with individual glomeruli visualized by autofluorescence. The antennal lobe was reconstructed from serial optical sections (*ca* 1.8 μm) using AMIRA software (TGS, Germany). In figure 2, glomeruli AS1–AS5 from which alarm pheromone-sensitive uniglomerular neurons originate are mapped into the reconstructed antennal lobe.

(d) *Odour stimulation*

To deliver odour-containing air to an antenna of an ant, a continuous airflow system (Nishino *et al.* 2003) was used. An air current ($5\,l\,min^{-1}$) was continuously applied to an antenna, and air passed through a cartridge that contained a filter paper soaked with 40 μl odourant solution ($1\,l\,min^{-1}$) could be added to the constant air current without changing the flow rate, by using a solenoid valve. We used *n*-undecane and formic acid as pheromone stimuli (Fujiwara-Tsujii *et al.* 2006). Formic acid or *n*-undecane was applied to the filter paper with dilution to 10% by water or liquid paraffin, respectively (designated as 10% formic acid or 10% *n*-undecane), or without dilution (designated as 100% formic acid or 100% *n*-undecane, or simply as formic acid or *n*-undecane). Alarm pheromone-sensitive neurons exhibited no responses to formic acid or *n*-undecane diluted to 1%, thereby indicating that the concentrations used in this study were moderate. Animals typically received 11 odour stimulations: 100% formic acid, 10% formic acid, 100% *n*-undecane, 10% *n*-undecane, vanilla essence (Kyoritsu Shokuhin, Tokyo, Japan), peppermint essence (Miyako Kosho, Tokyo), 1-hexanol, banana essence (Narizuka, Tokyo), apple essence (Asaoka, Tokyo), peach essence (Asaoka, Tokyo) and maple essence (Narizuka, Tokyo). Behavioural observations show that ants of this species are attracted by most of these non-pheromonal odours (vanilla, banana, apple, peach and maple; N. Yamagata, unpublished results). The 'latency' of the response in this study included a mechanical delay from activation of the solenoid valve to arrival of the odour-containing air to an antenna of *ca* 300 ms. The duration of odour stimulation was 500 ms or 1 s, and each odour was presented two to four times. The number of action potentials for each 100 ms bin was counted during the odour stimulation, to determine the mean and peak frequencies of action potentials. A sufficient inter-trial interval (more than 9 s) was given to avoid sensory adaptation. The residual air was continuously sucked out of the room through a vacuum system.

3. RESULTS

By using intracellular recording and dye-injection techniques (Yamagata *et al.* 2005), we succeeded in making recordings from and in staining 23 projection neurons of the antennal lobe in the ant *Camponotus obscuripes* that are sensitive to formic acid and/or *n*-undecane. These neurons were encountered only very rarely and thus are likely to be very few in number: we encountered a much larger number of neurons that respond to non-pheromonal odours but not to pheromone components, but these neurons were, in most part, excluded from the present examination. Eight of these alarm pheromone-sensitive neurons were uniglomerular projection neurons with dendrites in one glomerulus, and 15 were multiglomerular projection neurons with dendrites in multiple glomeruli.

All pheromone-sensitive uniglomerular projection neurons originated from one of five glomeruli that form a cluster in the dorsalmost part of the antennal lobe (figure 1). These glomeruli are referred to as 'alarm pheromone-sensitive (AS)' and are labelled AS1–AS5 in figures 1 and 2. Glomeruli of the antennal lobe, which were visualized by autofluorescence (figure 1*a*), could be grouped into two clusters, a ventral cluster and a dorsal cluster, as shown in three-dimensional reconstruction of the antennal lobe (figure 2). The AS glomeruli are located at the dorsalmost part (broken lines in figure 1*a*) of the ventral cluster, at a depth of *ca* 130 μm from the frontal surface of the antennal lobe. Individual AS glomeruli were reliably identifiable from preparation to preparation on the basis of (i) relative position of the glomerulus to other AS glomeruli, (ii) relative position of the glomerulus to the exit point of the antennal nerve and the lateral soma cluster and (iii) the unique size, i.e. AS1, AS4 and AS5 are larger than AS2 and AS3 (see figure 1*a*,*b*). This allowed us to map AS1–AS5 glomeruli into the reconstructed antennal lobe in figure 2.

Response properties of alarm pheromone-sensitive uniglomerular projection neurons ($n=8$) are summarized in table 1 and are also marked in figure 2. Uniglomerular projection neurons with dendrites in AS1 ($n=2$) or AS2 ($n=2$) responded to formic acid but not to *n*-undecane or any of the seven kinds of non-pheromonal odours tested (vanilla, peppermint, 1-hexanol, banana, apple, peach and maple odours). Uniglomerular projection neurons with dendrites in AS3 ($n=1$) responded to *n*-undecane but not

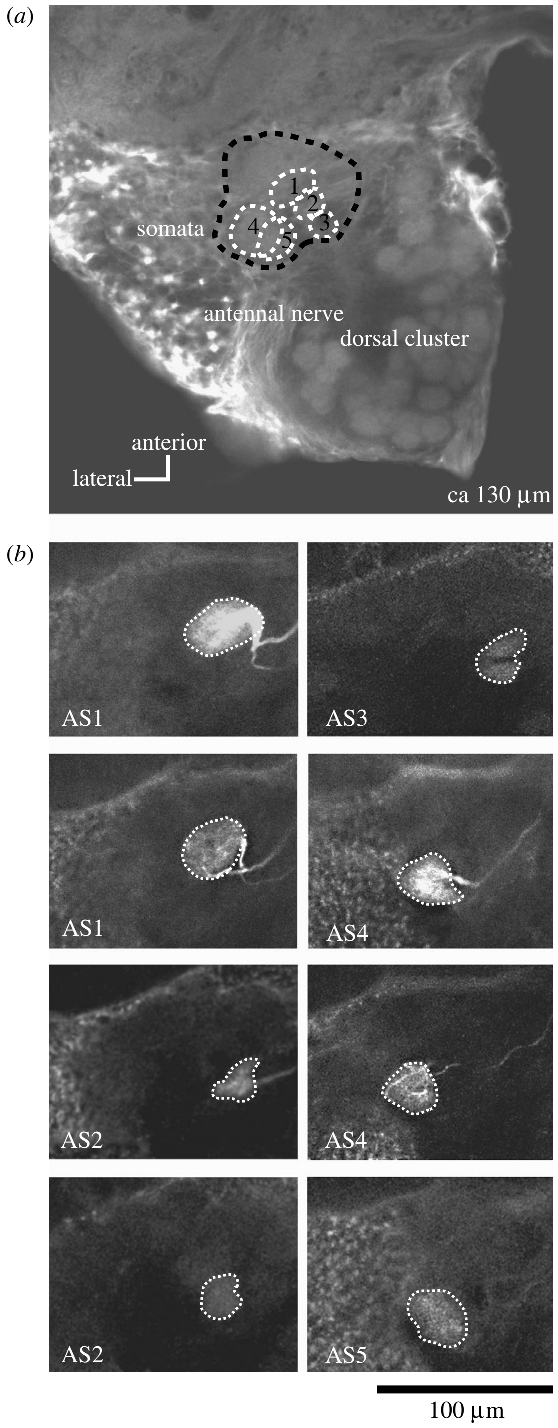

Figure 1. Confocal images of alarm pheromone-sensitive (AS) glomeruli in the antennal lobe of the ant. (*a*) Confocal image of unstained antennal lobe of an ant, demonstrating AS glomeruli (numbered 1–5; surrounded by white broken lines) in the dorsalmost part (broken lines) of the ventral glomerular cluster, at the depth of 130 μm from the frontal surface of antennal lobe. Individual AS glomeruli could be discriminated by autofluorescence. (*b*) Confocal images of dendrites of eight pheromone-sensitive uniglomerular projection neurons, demonstrating that they occupy one of five AS glomeruli. Two of these neurons had dendrites in AS1, two had dendrites in AS2, one had dendrites in AS3, two had dendrites in the AS4 and the remaining neuron had dendrites in AS5 glomerulus.

to formic acid or any of the seven kinds of non-pheromonal odours tested. Uniglomerular projection neurons with dendrites in AS4 ($n=2$) or AS5 ($n=1$) responded both to formic acid and *n*-undecane. AS5 projection neurons did not respond to any of the non-pheromonal odours tested, while an AS4 projection neuron responded to three of the seven kinds of non-pheromonal odours (1-hexanol, apple and banana odours).

Morphology of alarm pheromone-sensitive uniglomerular projection neurons is summarized in table 1, and a typical example of the morphology is shown in figure 3*a*. The soma of this neuron is located at the median cell cluster of the deutocerebrum. The main neurite gives rise to dense dendritic arborizations in AS1, and the axon ascends through the medial anteno-cerebral tract and passes beneath the calyces and gives rise to varicose terminal arborizations in the inner lip region (the lip region adjacent to the collar) of calyces of the mushroom body and in the lateral horn. Termination areas of the alarm pheromone-sensitive uniglomerular projection neurons are, in part, segregated out from those of alarm pheromone-insensitive uniglomerular projection neurons, although projection areas of alarm pheromone-sensitive uniglomerular projection neurons in the calyces have been shown to be fully overlapped with those of alarm pheromone-insensitive uniglomerular projection neurons, as will be reported elsewhere (N. Yamagata, H. Nishino & M. Mizunami, unpublished results).

The neuron depicted in figure 3*a* exhibited a low level of irregular spontaneous firing (figure 3*b*). Exposure to formic acid elicited excitatory responses with a latency of 476 ± 2 ms ($n=2$). Formic acid at a lower concentration (dilution of the odourant solution to 10%) induced a weak response with longer latency. *n*-Undecane, vanilla, maple, 1-hexanol, banana, peppermint, apple or peach odours did not evoke any responses. Averaged response latencies (which include a mechanical delay of *ca* 300 ms for the arrival of odour-containing air to the antenna, see §2) of pheromone-sensitive uniglomerular projection neurons were 535.8 ± 134.3 ms for formic acid ($n=6$) and 679.3 ± 119.6 ms for *n*-undecane ($n=3$). Mean and peak rate of action potentials of these neurons are 22.1 ± 6.4 and 54.2 ± 12.1 Hz for formic acid ($n=6$) and 17.9 ± 5.9 and 68.3 ± 8.8 Hz for *n*-undecane ($n=3$), respectively.

Morphology of alarm pheromone-sensitive multiglomerular projection neurons ($n=15$) is summarized in table 2. All of these neurons had dendrites in some of the AS glomeruli as well as in many other glomeruli in the anterodorsal area of the antennal lobe (figure 2, yellow region). Most of these neurons responded also to non-pheromonal odours. Axons of the majority of these neurons ascend through the medio-lateral anteno-cerebral tract, which is further divided into three subtracts as has been reported in honeybees (Abel *et al.* 2001). Terminal arborizations of these neurons are distributed in the dorsomedial area of the lateral horn, the median and dorsal protocerebrum, and the dorsal lobe. In contrast to uniglomerular projection neurons, none of multiglomerular projection neurons possess terminal arborizations in the calyces (table 2). In the lateral horn, termination fields of pheromone-sensitive multiglomerular projection neurons are largely segregated out from those of pheromone-sensitive uniglomerular projection neurons, with some overlap in the dorsomedial region, as we will report elsewhere.

Figure 4*a* shows the morphology of an alarm pheromone-sensitive multiglomerular projection neuron. The

Table 1. Physiology and morphology of eight alarm pheromone-sensitive uniglomerular projection neurons of the ant. (In the physiology column, + and − indicate that the neuron responded to that odour and did not respond, respectively. The ratio for the non-pheromonal odour indicates the number of odours to which the neuron responded per number of different kinds of odours tested. Neurons that exhibited only inhibitory responses to components of alarm pheromone were excluded. Medial soma belongs to medial soma cluster; the soma location was not specified. m-ACT, the axon of the neuron ascends through the medial tract. In the morphology column, + and − indicate that the axon of the neuron terminates in that area and does not terminate in that area, respectively. a lob, antennal lobe; ca, calyces of the mushroom body; l ho, lateral horn; v lob sat, satellite neuropil surrounding vertical lobe (or α lobe); d pr, dorsal protocerebrum; d lob, dorsal lobe.)

neuron no.		1	2	3	4	5	6	7	8
physiology	formic acid	+	+	+	+	−	+	+	+
	n-undecane	−	−	−	−	+	+	+	+
	non-pheromone	0/7	0/7	0/7	0/7	0/7	1/5	3/7	0/7
morphology	a lob	AS1	AS1	AS2	AS2	AS3	AS4	AS4	AS5
	soma location	medial	medial	medial	medial	medial	medial	medial	medial
	ascending tract	m-ACT	m-ACT	m-ACT	m-ACT	m-ACT	m-ACT	m-ACT	m-ACT
	ca	+	+	+	+	+	+	+	+
	l ho	+	+	+	+	+	+	+	+
	v lob sat	−	−	−	−	−	−	−	−
	d pr	−	−	−	−	−	−	−	−
	d lob	−	−	−	−	−	−	−	−

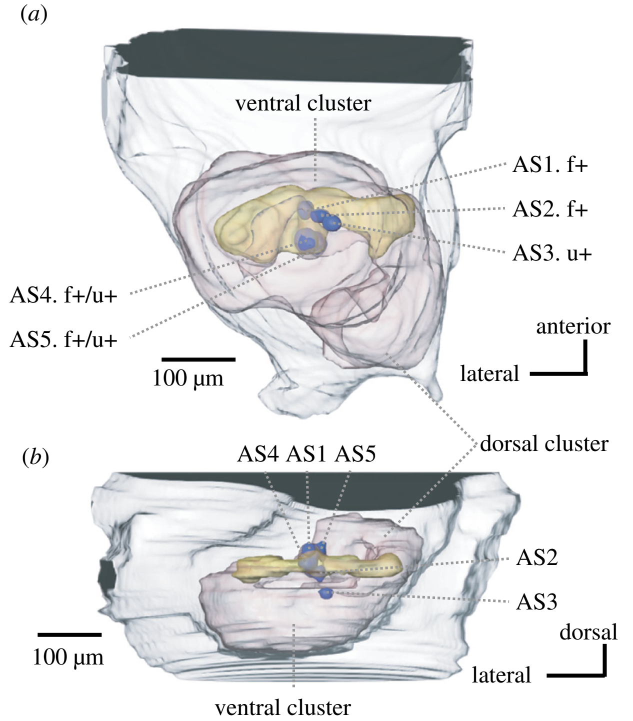

Figure 2. Three-dimensional reconstruction of the antennal lobe (red) of the ant, viewed (*a*) ventrally and (*b*) horizontally. Five glomeruli (blue spheroids, AS1–AS5) from which alarm pheromone-sensitive uniglomerular projection neurons originated are mapped into the reconstructed antennal lobe. f+ and u+ indicate that the projection neuron originating from that glomerulus was sensitive to formic acid and *n*-undecane, respectively. Alarm pheromone-sensitive multiglomerular projection neurons had dendrites in some of the AS glomeruli as well as in a number of glomeruli in the dorsalmost part (yellow) of the ventral glomerular cluster.

cell body of this neuron is located in the dorsal part of the deutocerebrum (figure 4*a*, arrowhead). The main neurite gives rise to many spiny dendrites that embrace or invade many glomeruli in the antero-dorsal cluster, and its axon ascends through the medio-lateral anteno-cerebral tract.

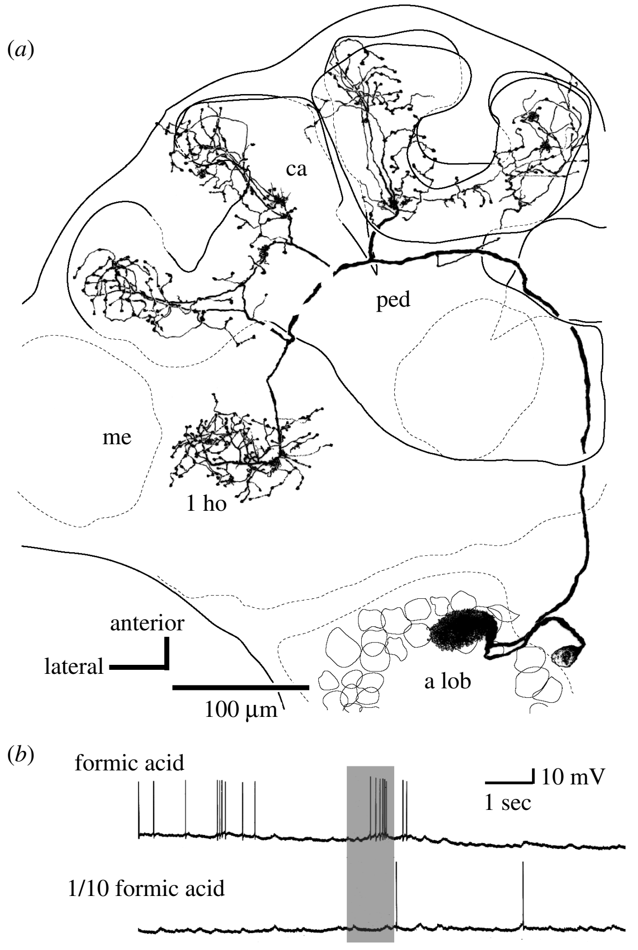

Figure 3. An alarm pheromone-sensitive uniglomerular projection neuron (number 1 neuron in table 1). (*a*) Reconstruction of an alarm pheromone-sensitive projection neuron from confocal sections viewed ventrally. The soma is located at the medial cell cluster of the deutocerebrum. The dendritic arbours cover the AS1 glomerulus in the antennal lobe (a lob). The axon ascends through the medial anteno-cerebral tract and terminates in the lip region of the calyces (ca) and the lateral horn (l ho). me, medulla; ped, pedunculus. (*b*) Responses of projection neurons to formic acid. 10% formic acid indicates that it was diluted to 1/10 by distilled water.

Table 2. Physiology and morphology of 15 alarm pheromone-sensitive multiglomerular projection neurons of the ant. (In the physiology column, + and − indicate that the neuron responded to that odour and did not respond, respectively. The ratio for the non-pheromonal odour indicates the number of odours to which the neuron responded per number of different kinds of odours tested. Neurons that exhibited only inhibitory responses to components of alarm pheromone were excluded. AS: the neuron had dendrites in AS glomeruli; medial, lateral and dorsal indicate that the soma belongs to medial, lateral and dorsal soma cluster, respectively. ? indicates that the soma location was not specified; m-ACT, l-ACT, ml-ACT and d-ACT indicate that the axon of the neuron ascends through the medial, lateral, medio-lateral and dorsal anteno-cerebral tract, respectively. In the morphology column, + and − indicate that the axon of the neuron terminates in that area and does not terminate in that area, respectively. a lob, antennal lobe; ca, calyces of the mushroom body; l ho, lateral horn; v lob sat, satellite neuropil surrounding vertical lobe (or α lobe); d pr, dorsal protocerebrum; d lob, dorsal lobe.)

neuron no.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
physiology	formic acid	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+
	n-undecane	+	+	+	+	+	+	+	+	+	−	+	−	+	−	+
	non-pheromone	0/7	1/7	1/7	5/7	0/7	1/5	1/7	3/7	1/7	2/7	0/7	0/5	1/7	0/5	0/7
morphology	a lob	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS
	soma location	medial	medial	medial	medial	lateral	lateral	dorsal	dorsal	dorsal	dorsal	?	dorsal	dorsal	medial	medial
	ascending tract	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	ml-ACT	d-ACT	m-ACT	m-/ml-ACT	m-/ml-ACT
	ca	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−
	l ho	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+
	v lob sat	−	−	+	−	−	+	+	+	+	−	−	−	−	+	+
	d pr	+	+	+	+	−	+	+	+	+	−	+	+	−	+	+
	d lob	−	−	−	−	−	+	−	+	−	−	−	+	−	+	+

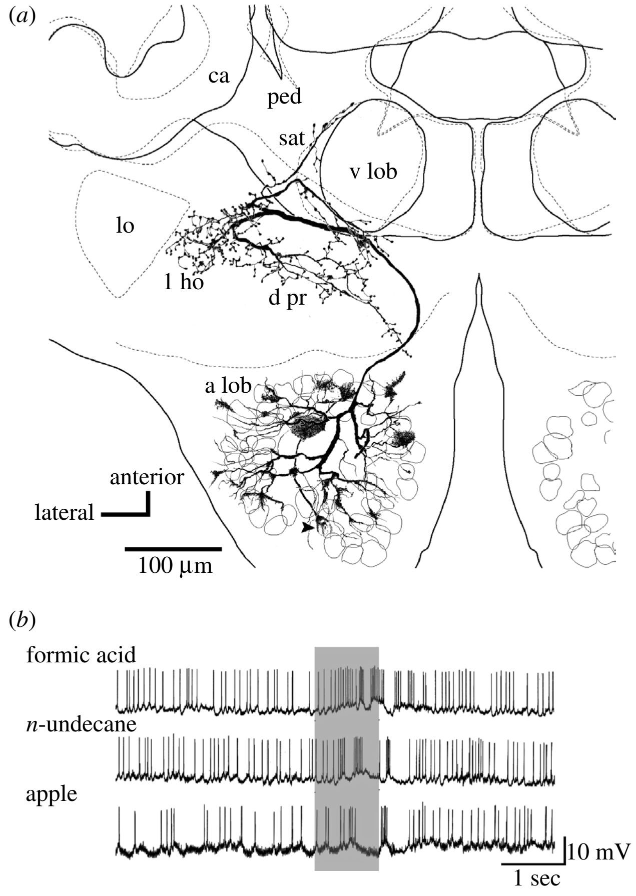

Figure 4. Alarm pheromone-sensitive multiglomerular projection neuron (number 7 neuron in table 2). (*a*) Reconstruction of the neuron from confocal sections viewed ventrally. The soma is located at the dorsal cell cluster of the deutocerebrum (arrowhead). The dendrites are distributed in some of the AS glomeruli as well as in glomeruli in the dorsalmost part of the antennal lobe (a lob). The dendrites are distributed in interglomerular regions as well as in the glomeruli. The axon runs through the medio-lateral anteno-cerebral tract and terminates in the lateral horn (l ho), satellite neuropil (sat) of the vertical lobe (v lob) of the mushroom body and the dorsal protocerebrum (d pr). lo, lobula; ped, pedunculus. (*b*) Responses to formic acid, *n*-undecane and apple odours. All of these odours evoked multi-physic responses.

Terminal arborizations with numerous blebs are distributed in the lateral horn, the satellite neuropil (Li & Strausfeld 1999) surrounding the vertical lobe (also referred to as the α lobe) of the mushroom body and the dorsal protocerebrum.

This neuron depicted in figure 4*a* responded to formic acid, *n*-undecane and apple odours among the nine kinds of odours tested (figure 4*b*). Presentations of formic acid and *n*-undecane evoked multi-phasic responses with latencies of 473±1 ($n=2$) and 546±68 ms ($n=2$), respectively. Apple odour elicited transient excitatory responses with a latency of 490±48 ms ($n=2$). Vanilla, maple, 1-hexanol, banana, peppermint and peach odours did not evoke responses.

4. DISCUSSION

Communication by means of pheromones plays major roles in maintenance of social organization in social insects (Blum 1985; Vander Meer & Alonso 1998). Although communication by means of non-sexual pheromone is not specific to social insects and is found in some solitary or semi-social insects (such as the use of aggregation pheromones by cockroaches), its unusual sophistication in social insects is evidenced by the development of a diverse array of exocrine glands devoted to social communication, 39 such glands existing in formicine ants and 21 existing in honeybees (Billen 1994).

In this study, we successfully obtained intracellular recordings from 23 alarm pheromone-sensitive projection neurons of the antennal lobe of the formicine ant *Camponotus obscuripes*. All pheromone-sensitive uniglomerular projection neurons ($n=8$ out of 23) had dendrites in one of five AS glomeruli in the dorsalmost part of the antennal lobe. Moreover, all pheromone-sensitive multiglomerular projection neurons ($n=15$) had dendrites in some of the AS glomeruli as well as in other glomeruli in the dorsalmost part of the antennal lobe. These findings suggest, for the first time, that alarm pheromone is processed in a specific cluster of glomeruli in the antennal lobe of ants.

Uniglomerular projection neurons with dendrites in AS1 and AS2 glomeruli responded to formic acid, and those with dendrites in the AS3 glomerulus responded to *n*-undecane. This is reminiscent of the findings that projection neurons in the antennal lobe of male cockroaches and moths that responded to each specific component of female sex pheromone had dendrites in each specific part of the macroglomerular complex (Boeckh & Ernst 1987; Kanzaki *et al.* 1989, 2003). Uniglomerular projection neurons with dendrites in the AS4 or AS5 glomerulus, however, responded to both alarm pheromone components, and signals about alarm pheromone components thus appear not to be segregated in these glomeruli. It should be noted that the results of the present study are not sufficient to reliably estimate the total number of AS glomeruli and to fully characterize functional subdivision among AS glomeruli, because the number of samples was limited. This reflects the difficulty in obtaining stable intracellular recordings from neurons of the ant brain, and further improvement of intracellular recording techniques will be necessary to gain a better understanding of alarm pheromone processing in the AS glomeruli.

Previously, Löfqvist (1976) speculated that the formicine ant *Formica rufa* possess different receptor neurons for each of the two alarm pheromone components, formic acid and *n*-undecane, since he observed that behavioural adaptation to repetitive exposure to one pheromone component did not affect behavioural response to the other pheromone component. Dumpert (1972) reported that some receptor neurons in the antenna specifically responded to *n*-undecane in the formicine ant *Lasiun fuliginosus*. Further study is needed to determine whether axons of alarm pheromone-sensitive receptor neurons specifically project to AS glomeruli.

In previous studies in ants and honeybees, glomeruli that specifically process non-sexual pheromonal signals were not identified, though Kleineidam *et al.* (2005) reported that the antennal lobe of large workers of leaf-cutting ants contains an enlarged glomerulus and speculated that it participates in the processing of trail pheromone. In a calcium-imaging study of the antennal lobe of the ant *Camponotus rufipes*, Galizia *et al.* (1999*a*) found that glomeruli that exhibited responses to *n*-undecane also exhibited responses to non-pheromonal odours in a part of the antennal lobe. Similar overlapping representation of alarm pheromone (isoamyl acetate) and non-pheromonal odours has been found in calcium-imaging studies of a part of the antennal lobe in honeybees (Galizia *et al.* 1999*b*; Sachse *et al.* 1999). Müller *et al.* (2002) performed intracellular recordings from projection neurons in honeybees and reported that projection neurons that responded to isoamyl acetate or other pheromonal odours also responded to a variety of non-pheromonal odours. Thus, it was deduced that non-sexual pheromones are coded by combinational patterns of glomerular activity in the antennal lobe (Galizia *et al.* 1999*a*,*b*). Our findings, however, suggest that specialized glomeruli in the antennal lobe were developed for pheromone processing in social insects.

Alarm pheromone signals are transmitted to the protocerebrum by two sets of neurons, uniglomerular and multiglomerular projection neurons. Most of the alarm pheromone-sensitive uniglomerular projection neurons did not respond to non-pheromonal odours tested. In contrast, many of the pheromone-sensitive multiglomerular projection neurons responded to non-pheromonal odours, in accordance with their dendritic morphology. Notably, termination areas of pheromone-sensitive uniglomerular projection neurons differed in large part from those of multiglomerular projection neurons: uniglomerular projection neurons terminate in the calyx of the mushroom body and the lateral horn, while multiglomerular projection neurons terminate in the lateral horn, satellite neuropil surrounding the vertical lobe and dorsal protocerebrum. We are currently studying how signals of these two types of projection neurons are further processed in the protocerebrum.

Specialized glomeruli for non-sexual pheromone processing in social insects may have evolved by elaboration of glomeruli to deal with non-sexual pheromones in solitary insects (Suh *et al.* 2004) or semi-social insects. Therefore, comparison of glomerular structures in social and non-social insects might lead to elucidation of the process of evolution of specialized glomeruli for pheromone processing. Moreover, since workers but not queens or males participate in colony defence, comparisons of glomerular structures in different castes (Kleineidam *et al.* 2005) will provide further insights into brain mechanisms underlying caste-specific behaviour in social insects.

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