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Central nervous processing of behaviourally relevant odours in solitary and gregarious fifth instar locusts, *Schistocerca gregaria*

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Abstract Physiological and morphological characteristics of antennal lobe neurons of solitary and gregarious fifth-instar nymphs of the desert locust, *Schistocerca gregaria,* were studied using intracellular recording and staining techniques. Physiological characteristics of antennal lobe neurons of both locust phases responding to stage-dependent aggregation pheromones, egg-laying attractants, a putative sex pheromone and plant-associated volatiles are described. Antennal lobe neurons showed excitatory, inhibitory, combined excitatory and inhibitory and delayed responses. In addition, one neuron showing an initial inhibition followed by an excitation and inhibition response was found. Pheromone-specific-, plant-specific- and pheromone-plant-generalist neurons were found in both locust phases. Antennal lobe neurons displayed stage- and phase-dependent differences in the processing of aggregation pheromone component input. Nymphal antennal lobe neurons showed stage-dependent response characteristics highly correlated with the preferential behavioural attraction to the nymphal aggregation pheromone. Phase-dependent differences were found in the response spectra and the sensitivity of the same neuron types. Neurons of solitary locusts responded significantly more frequently to some of the tested components than neurons of gregarious locusts. Furthermore, antennal lobe neurons of solitary locusts showed a higher sensitivity to most of the tested compounds.

Key words Olfaction · Locust, *Schistocerca gregaria* · Deutocerebrum · Electrophysiology

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Abbreviations *AL* antennal lobe · *LY* lucifer yellow · *PAN* phenylacetonitril · *PN* projection neuron · *RN* receptor neuron

Introduction

The desert locust, *Schistocerca gregaria*, is known to aggregate in large swarms and damage vegetation to a large extent in Africa and Asia. Locusts exhibit density-dependent phase polymorphism and are able to transform between two extreme phases, solitaria and gregaria. The locusts are hemimetabolic insects, and nymphs undergo five (gregarious and solitary) or six (solitary) instars (nymphal stages) before becoming adults. The aggregation behaviour shown by both nymphal and adult gregarious locusts is induced by visual, tactile and to a large extent by olfactory stimuli (see Byers 1991 for review). Olfaction also plays a major role in host-plant location (Chapman 1990) and mate location in solitary locusts (Inayatullah et al. 1994; Njagi and Torto 1998). In addition, Saini et al. (1995) and Rai et al. (1997) have identified semiochemicals emanating from froth of egg pods that attract gravid gregarious *S. gregaria*.

The existence of aggregation pheromones in the air surrounding gregarious *S. gregaria* has been shown in behavioural experiments (Fuzeau-Braesch et al. 1988; Heifetz et al. 1996; Obeng-Ofori et al. 1993, 1994a,b; Torto et al. 1994, 1996). Obeng-Ofori et al. (1994a) and Torto et al. (1994, 1996) revealed the presence of two aggregation pheromone complexes analysing volatiles from the faeces as well as from the air surrounding second- to fifth-instar nymphs and adults. Torto et al. (1994) identified six electrophysiologically active components in the volatiles of sexually mature male *S. gregaria*: phenylacetonitril (PAN), guaiacol, phenol, benzaldehyde, veratrole and anisole. However, behavioural aggregation occurred in response to a four-component adult aggregation pheromone consisting of 80% PAN, and low amounts of phenol, guaiacol and benzaldehyde. This pheromone evoked an aggregation

response in sexually immature and mature adults, whereas nymphs were behaviourally indifferent to the pheromone. In contrast to adults, both female and male nymphs have been shown to produce a nymphal pheromone (Obeng-Ofori et al. 1994a, Torto et al. 1996). Obeng-Ofori et al. (1994a) and Torto et al. (1996) identified a set of pheromone compounds emanating from second- to fifth-instar nymphs: guaiacol and phenol (faecal volatiles) and C_6 and C_8–C_{10} acids (hexanoic, octanoic, nonanoic and decanoic acid) and aldehydes (hexanal, nonanal and decanal) (nymphal volatiles). In addition, veratrole has been identified in the faecal volatiles of 5th-instar nymphs (Fuzeau-Braesch et al. 1988). Immature adults also produce faecal volatiles that may elicit aggregation behaviour in immature and mature adults, but not as effectively as the four-component pheromone (Obeng-Ofori et al. 1993, 1994a). In summary, the aggregation pheromone complexes seem to have an augmentative role in aggregating nymphs and mature adults and to keep immature adults cohesive when they transit from immature to mature adults. Njagi et al. (1996) also suggested that aggregation pheromones could evoke phase-independent responses in solitary individuals and play a major role in the arrestment and subsequent recruitment of solitary individuals into gregarious groups.

The rather clearcut change in pheromone production and behaviour that occurs between the nymphal and the adult stage of gregarious locusts provides us with the possibility to study developmental changes in olfactory integration. The antennal sensory equipment of hemimetabolic insects increases during postembryonic development. New annuli and new sensilla are added to the antenna in a specific pattern (Chapman and Greenwood 1986; Ochieng' et al. 1998). A behavioural switch could thus arise as a result of changes in the peripheral olfactory input between developmental stages. The numbers of olfactory sensilla also differ between solitary and gregarious locusts with solitary locusts having a higher number and more sensitive receptor neurons (RNs) than gregarious (Greenwood and Chapman 1984; Ochieng' 1997; Ochieng' et al. 1998). Phase-dependent differences in the behaviour of solitary and gregarious locusts may thus be a result of phase-dependent olfactory integration; gregarious locusts interact socially and are more active compared to isolated solitary locusts which are repelled by crowded locusts and show behavioural responses more consistent with a cryptic lifestyle (Roessingh et al. 1993).

Specifically responding RNs are housed in different sensillum types on the locust antenna (Hansson et al. 1996; Ochieng' 1997). Receptor neurons send their axons to the antennal lobe (AL) through the antennal nerve where they arborise in several glomeruli and synapse with AL neurons. Anton and Hansson (1996) described the physiological and morphological characteristics of projection neurons (PNs) responsible for processing aggregation pheromone components and plant volatiles in adult male and female gregarious *S. gregaria*. Projection neurons responded with varying specificity to behaviourally active aggregation pheromone components and mixtures, as well as to plant volatiles. Whereas locust PNs show clear action potentials local interneurons are non-spiking (Laurent and Davidowitz 1994) and have not been further studied.

In the present study, we used intracellular recording methods in order to determine how behaviourally relevant odours are processed in AL neurons in solitary and gregarious fifth-instar nymphs of the desert locust. A few of the physiologically characterised AL neurons were intracellularly stained to allow morphological comparison with AL neurons of adult desert locusts. Our results will help us to understand how developmental changes and phase-specific differences in the peripheral olfactory apparatus affect the central nervous processing of behaviourally relevant odours in this hemimetabolic insect.

Materials and methods

Animals

A gregarious desert locust culture was established in our laboratory from insects or eggs originating from the International Center of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya and from Blades Biological, Cowden Edenbridge, England. Solitary locusts, shipped as egg pods from ICIPE, were isolated after hatching in separate cages that were cleaned daily, in an isolated, well ventilated room maintained at 30 ± 1 °C in LD 12:12 h, 50% relative humidity. Wheat shoots and wheat bran were provided daily. Fifth instars (2–4 days after moult) were used in the experiments.

Preparation

After removing the legs with a pair of scissors, the locust was restrained in a plastic cylinder with only the head protruding. The head was secured with a piece of Parafilm. The brain was exposed by removing the frons and vertex of the head capsule with a sharp scalpel. Muscles, fat bodies, tracheae and the sheath overlaying the antennal lobes were removed with a pair of fine forceps. Due to the size of the preparation and to avoid movements, locusts were decapitated. The head and antennae was fixed on a wax layer in a small Petri dish with fine insect pins and superfused with insect Ringer solution set to pH 6.9. Antennae were positioned to ensure continuous ventilation by the airstream.

Stimulation

The antennae were continuously ventilated by a gentle (0.5 m s^{-1}) charcoal filtered and moistened airstream flowing through a glass tube ending about 10 mm from the antennae. The stimuli were presented by inserting a Pasteur pipette, containing a filter paper with or without odorant, in the glass tube about 100 mm from the antennae. A 0.5-s (4 ml s^{-1}) air pulse was sent through the Pasteur pipette by means of a stimulation controller (Syntech SFC-2/b). Stimuli were given randomly with a minimal interstimulation period of 10 s or when spontaneous action potential frequency, as measured from the oscilloscope, had been reestablished, starting with intermediate concentrations in order to characterise the neuron. Single neuron recordings used for data analysis lasted for 1–15 min.

Stimuli were diluted in decadic steps in paraffin oil (compounds 1, 4–13 in Fig. 1) or ethanol (compounds 2, 3 in Fig. 1) such that

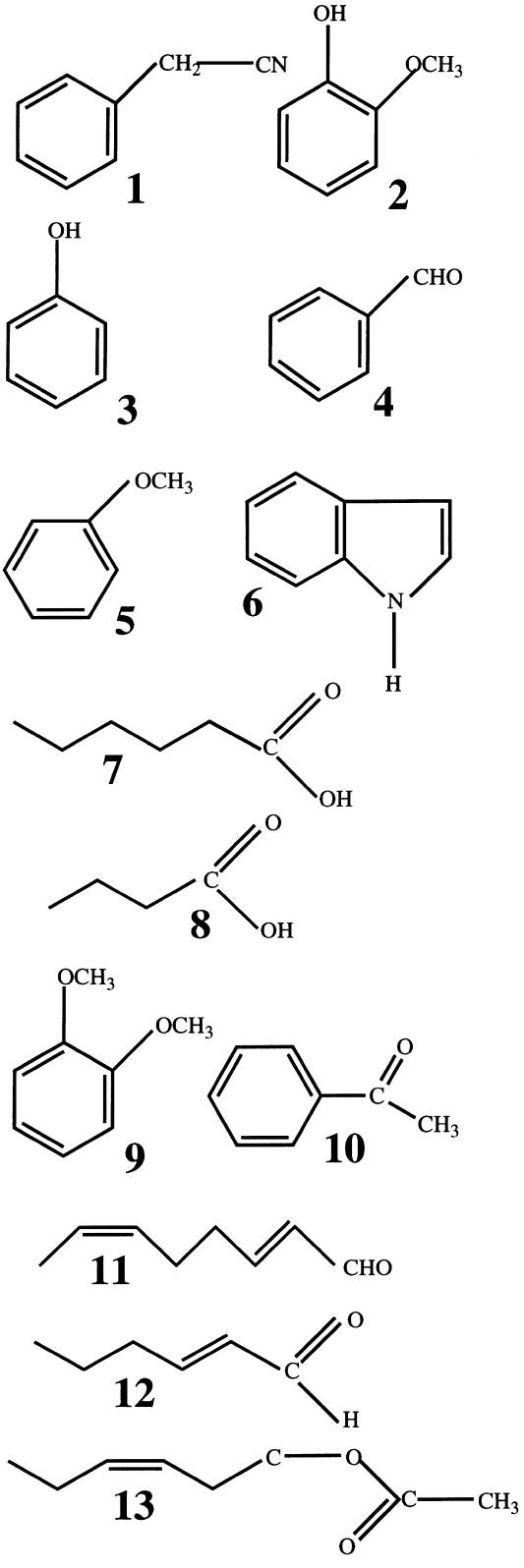


Fig. 1 Chemical structures of the stimuli used in the electrophysiological experiments: *1* phenylacetonitril (PAN); *2* guaiacol; *3* phenol; *4* benzaldehyde; *5* anisole; *6* indol; *7* hexanoic acid; *8* butyric acid; *9* veratrole; *10* acetophenone; *11* (*E*,*Z*)-2,6-nonadienal; *12* (*E*)-2 hexenal; *13* (*Z*)-3-hexenyl acetate. The nymphal blend contains stimuli 2 + 3 (60:40) and the adult blend contains 1 + 2 + 3 + 4 + 5 + 9 (80:3:3:5:4:5)

amounts of 1 μg, 10 μg, 100 μg (compounds 10, 11) and 1 mg (compounds 1–13) were present in 10 μl solvent on a filter paper. Compounds 1–9 are behaviourally active (compounds 1–4, 7 and 9) or potential (compounds 5, 6 and 8) aggregation pheromone components. Compounds 9 and 10 have been shown to be part of the oviposition aggregating pheromone of gregarious female locusts. Compound 11 is the major component of a preferred host plant *Tribulus terrestris* (Zygophyllaceae) and has been proposed as a sex pheromone emitted by female solitary locusts. Twelve and 13 are green-leaf volatiles. The two mixtures used, in the amounts 1 μg, 10 μg, 100 μg and 1 mg, resemble the adult male pheromone (adult blend) and the nymphal faecal odour (nymphal blend) (Fig. 1). All chemicals were purchased from Sigma and were at least 98% pure.

Intracellular recording and staining

Standard intracellular recording techniques were used (Christensen and Hildebrand 1987). The physiological data were stored on a video tape (Vetter) and visualised on a Tektronix digital oscilloscope or fed into an analysing computer programme (Data Wave, Data Wave Technologies) and printed from the programme. Action potentials (spikes) were counted manually from the storage oscilloscope. The number of spikes during 600 ms after the stimulus had reached the antenna minus the number of spikes during 600 ms before (representing the spontaneous activity of the cell) was noted as the number of net spikes. The time when the stimulus reached the antennae was defined by the time the earliest responses occurred (usually 100 ms after the trigger pulse). The net spikes in response to the blank stimulus were subtracted from the number of net spikes in response to an odour stimulus to quantify the response to a specific stimulus in one neuron.

A few physiologically characterised neurons were stained with lucifer yellow (LY, 2% aqueous solution, Sigma) by injecting a ca. 1-nA hyperpolarizing current through the LY-filled recording electrode for 10–15 min. The brain was then fixed for 1-h in a 2.5% buffered formaldehyde solution, dehydrated, and embedded in Spurr's resin. Sections (10 μm) were photographed on Fujichrome 400 colour slide film and the neurons were reconstructed from the slides.

Statistical analysis

The percentage of AL neurons of solitary and gregarious locusts that responded to the different stimuli (from all neurons in which the respective stimuli were tested) was calculated (Fig. 5). Differences in the percentage of AL neurons of solitary and gregarious locusts responding to each of the tested stimuli were analysed with the chi-square test using the Bonferroni method in order to limit the overall experiment-wise error rate.

Data from dose-dependent responses of physiological characterised neurons were square-root transformed and subsequently subjected to a one-way analysis of variance by phase followed by Fischer's PLSD test using SYSTAT (1992).

Results

General physiological characteristics of antennal lobe neurons

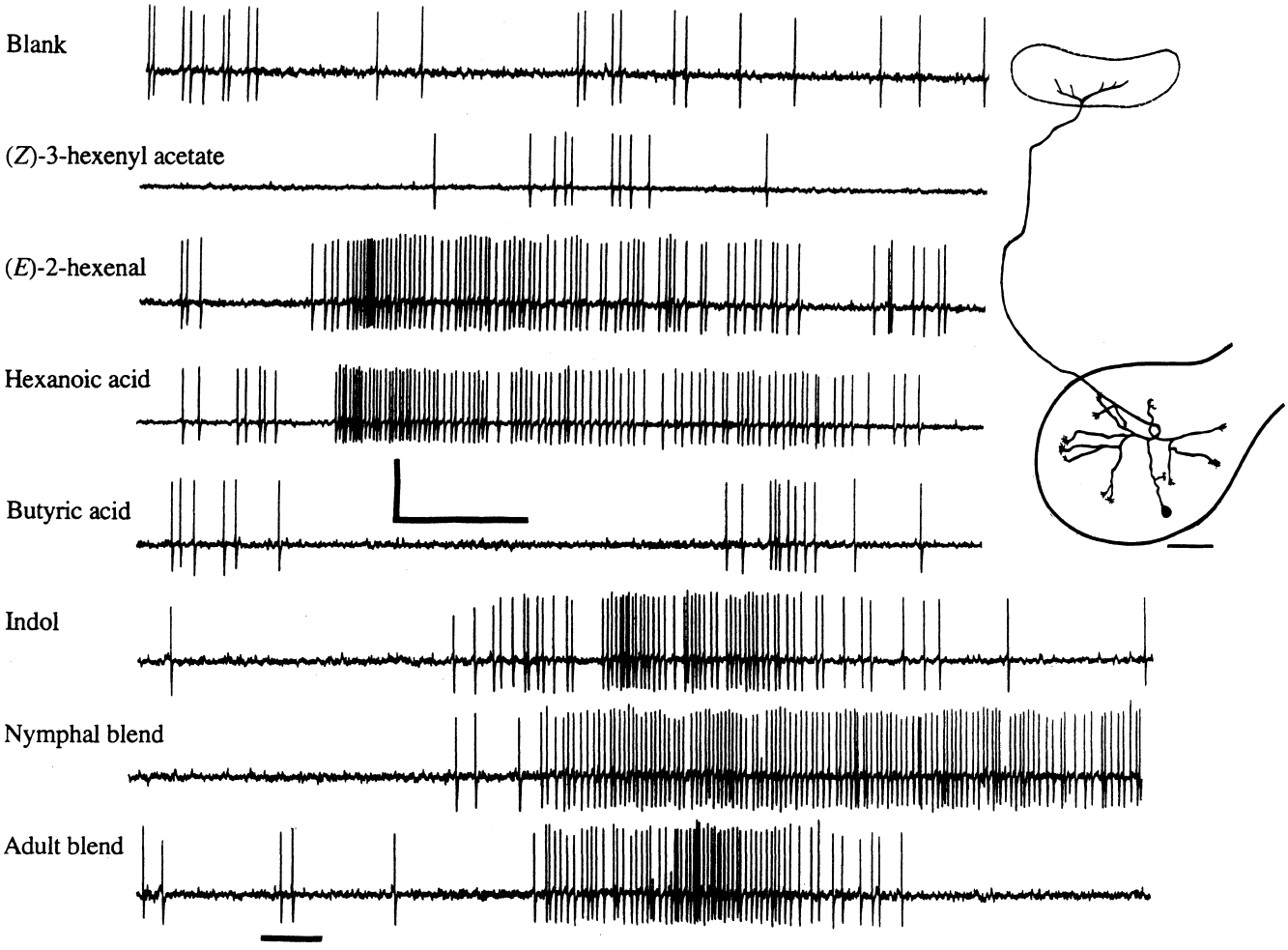
The physiological responses of 128 neurons in 53 solitary fifth-instar nymphs and 130 AL neurons in 71 gregarious fifth-instar nymphs were investigated. The AL neurons showed spontaneous activity between 0.1 and 15 Hz and the spike amplitude varied between 10 and 50 mV. Spontaneous activity was defined as the neural activity without stimulation, which was rather

constant for each individual neuron. The physiologically characterised AL neurons differed in their response to different odour stimuli. The most common response was an excitation (Fig. 2) (85 and 104 AL neurons in solitary and gregarious locusts, respectively), an increase in spike frequency, sometimes followed by an inhibitory period towards or after the end of the stimulus. The excitatory response lasted for 0.2–1 s, while the poststimulation inhibitory period lasted for 0.2 to several seconds. Inhibitory responses (Fig. 3) (24 and 12 AL neurons in solitary and gregarious locusts, respectively) were characterised by a decrease in action potential frequency, for a shorter or longer period, and a hyperpolarisation of the membrane potential. Some AL neurons responded with excitation to some stimuli and with inhibition to others (14 and 8 AL neurons in solitary and gregarious locusts, respectively) (Table 1, Fig. 6e). Another type of response was characterised by a poststimulation (delayed) excitatory response to some stimuli, 0.1–1.5 s after the end of the stimulation period (Fig. 2) while showing excitatory or inhibitory responses to other stimuli (4 and 6 AL neurons in solitary and gregarious locusts, respectively). In addition, one solitary locust AL neurons responded to most stimuli with an initial inhibition followed by an excitation and then by another inhibition period (Fig. 3).

Fig. 2 Physiological response of a pheromone-plant generalist neuron of a fifth-instar gregarious locust showing short delay (ca. 0.1 s) excitatory responses to hexanoic acid and (*E*)-2-hexenal and long delay (ca. 1.5 s) excitatory responses after the end of the stimulation period to indole and to the nymphal and to the adult pheromone blend. The stimulus amount used was 1 mg. *Bar below trace* indicates stimulation period (500 ms). *Horizontal scale bar* = 1 s; *Vertical scale bar* = 40 mV. *Inset* Morphology of a projection neuron. Reconstruction from 10 µm frontal sections with arborisation in outer glomeruli. The axon projection arborised in the calyces of the mushroom body (*hatched line*). The fill was not complete and could thus not be traced to the lateral protocerebrum. *Scale bar* = 100 µm. The antennal lobe (AL) is outlined by a *solid line*

Anatomical characteristics

All physiologically characterised and subsequently stained ($n = 8$) AL neurons were PNs. The PNs all had their cell bodies located in the frontal cell group of the AL. The primary neurite passed from the cell body to the central fibre core of the AL and formed an open ring,



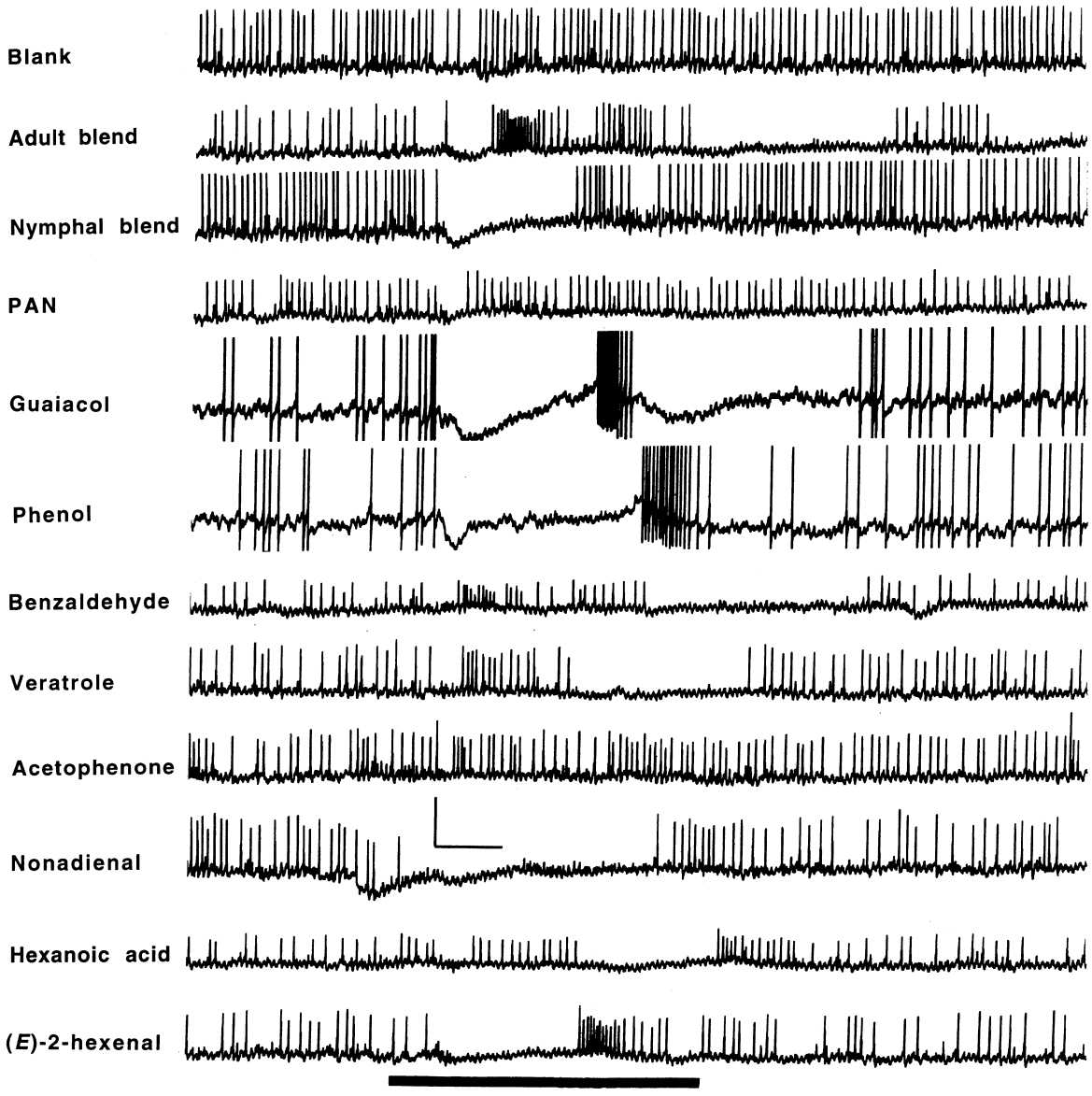


Fig. 3 Physiological response of a pheromone-plant generalist neuron of a fifth-instar solitary locust showing inhibitory response to the nymphal pheromone blend and nonadienal and a clear inhibitory-excitatory-inhibitory response to the adult pheromone blend, guaiacol, phenol and (*E*)-2-hexenal and a weaker inhibitory-excitatory-inhibitory response to PAN, benzaldehyde and veratrole. The stimulus amount used was 1 mg. The contact quality decreased during recording and for convenience the spikes were cut in the traces recorded from guaiacol and phenol. *Bar beneath trace* indicates stimulation period (500 ms). *Horizontal scale bar* = 100 ms; *vertical scale bar* = 10 mV

from which single branches projected symmetrically into the periphery of the lobe (inset Fig. 2). Each PN innervated between 10 and 20 glomeruli. Axon projections went through the tractus-olfactorio-globularis and arborized in the calyces of the mushroom body ($n = 5$). The fills were not complete and could thus not be traced to the lateral protocerebrum. PN morphology and AL structure did not differ from the results presented for adult locusts in Anton and Hansson (1996).

Response spectra of projection neurons

The percentage of PNs of solitary and gregarious locusts responding to the tested stimuli are shown in Fig. 4. The proportion of PNs that responded to the different components showed similar patterns in both solitary and gregarious locusts. In both phases PNs responded more frequently to some of the aggregation pheromone components, i.e. guaiacol and phenol as well as to the nymphal blend, oviposition-aggregating pheromone components, veratrole and acetophenone, and the putative sex pheromone (*E*,*Z*)-2,6-nonadienal than to other tested stimuli. In addition, a high proportion of PNs responded to PAN and the adult aggregation pheromone blend. An intermediate proportion of PNs responded to benzaldehyde, hexanoic acid, butyric acid, indol and the plant volatile (*E*)-2-hexenal. Few PNs responded to anisole and (*Z*)-3-hexenyl acetate. Projection neurons of solitary locusts responded significantly more frequently

Table 1 Characteristics and abundance of **a** pheromone-component-specific and **b** blend-specific neurons in gregarious and solitary fifth-instar nymphal desert locusts. In addition, examples of response spectra of pheromone-generalist neurons (**c**) are given. Physiological responses are characterised as 0: no response, (+) excitatory- and (−) inhibitory response

	Stimuli															No. of neurons	
	Adult blend	Nymphal blend	PAN	Guaiacol	Phenol	Benzal-dehyde	Anisole	Indol	Hexanoic acid	Butyric acid	Veratrole	Aceto-phenone	(*E*,*Z*)-2,6-non adienal	(*E*)-2-hexenal	(*Z*)-3-hexenyl acetate	Solitary	Gregar-ious
Response spectrum																	
a	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	3	0
	−	0	−	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	1
	0	−	0	−	0	0	0	0	0	0	0	0	0	0	0	1	0
	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	3
	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	1	3
	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	1	1
	0	0	0	0	0	0	0	0	0	−	0	0	0	0	0	1	0
	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	1	1
	0	0	0	0	0	0	0	0	0	0	0	−	0	0	0	2	0
	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	5	6
	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	7	8
	0	0	0	0	0	0	0	0	0	0	0	0	−	0	0	0	2
b	0	+	0	0	0	0	0	0	0	0	0	+	0	0	0	1	1
	0	−	0	0	0	0	0	0	0	0	0	−	0	0	0	1	0
	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	1	1
	+	0	0	0	0	0	0	0	0	0		+	0	0	0	0	1
	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
c	+	+	0	+	+	0	0	0	0	0	+	+	+	0	0	12	10
	−	−	0	−	−	0	0	0	0	0	−	−	−	0	0	5	4
	+	+	0	+	+	0	0	0	0	0	+	+	0	0	0	6	7
	+	+	0	+	+	0	0	0	0	0	+	0	+	0	0	2	1
	−	−	0	−	−	0	0	0	0	0	−	0	+	0	0	1	0
	+	+	0	+	+	0	0	0	0	0	0	+	0	0	0	1	1
	+	+	0	+	+	0	0	0	0	0	+	0	0	0	0	9	7
	+	−	0	−	+	0	0	0	0	0	0	+	+	0	0	1	0
	−	−	0	−	−	0	0	0	0	0	−	−	0	0	0	1	3
	−	−	0	−	−	0	0	0	0	0	−	0	−	0	0	2	2
	−	−	0	+	+	0	0	0	0	0	0	+	0	0	0	1	0
	0	+	0	+	+	0	0	0	+	+	+	0	0	0	0	2	1
	0	+	0	+	+	0	0	0	0	0	+	0	0	0	0	12	9
	0	−	0	−	−	0	0	0	0	0	−	0	0	0	0	3	4
	0	+	0	+	+	0	0	+	0	0	0	0	+	0	0	1	0
	0	+	0	+	+	0	0	+	0	+	+	−	+	0	0	1	0
	0	+	0	+	−	−	0	0	+	0	0	−	+	0	0	1	0
	0	+	+	+	0	+	0	0	0	0	+	0	0	0	0	1	1
	0	0	+	0	0	0	0	0	0	+	0	0	+	0	0	1	0
	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	1	0
	0	0	0	0	0	0	0	0	0	0	0	+	−	0	0	1	0
	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	1	2
	0	0	0	0	0	0	0	0	0	0	0	−	−	0	0	1	3
	−	0	−	0	0	0	0	0	0	0	0	−	0	0	0	1	0
	0	−	0	−	0	0	0	0	0	0	−	0	0	0	0	1	0

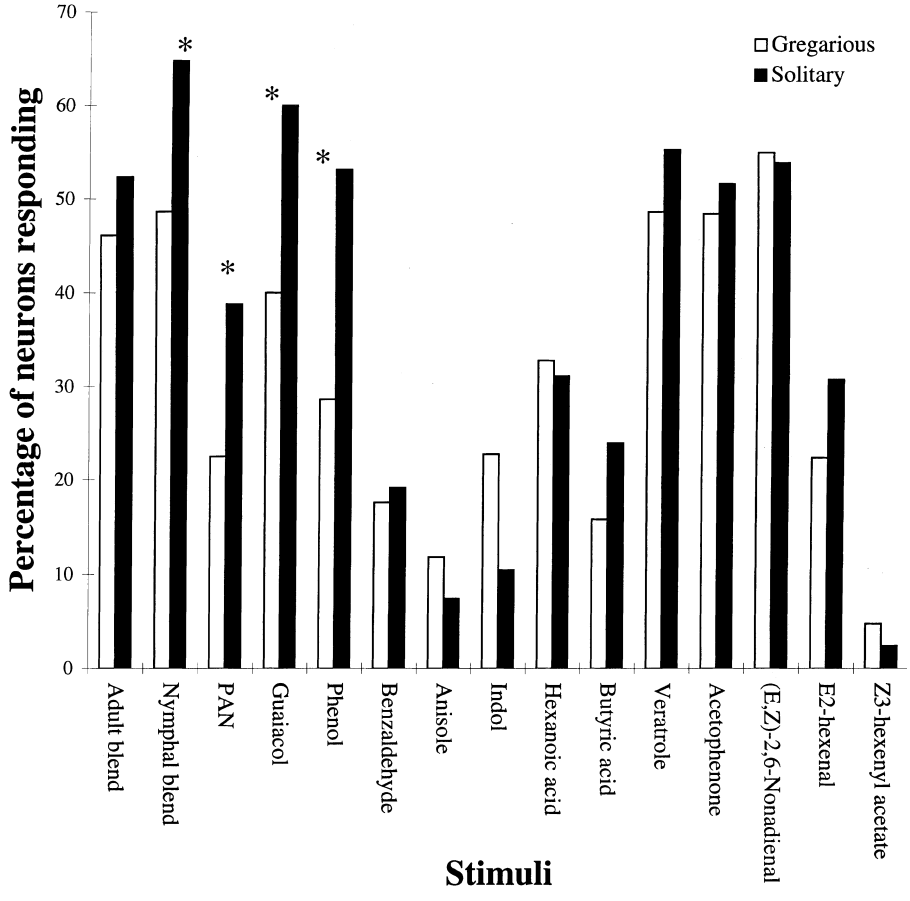


Fig. 4 Percentage of projection neurons responding to the tested components in solitary and gregarious fifth-instar locusts. A high percentage of projection neurons responded to guaiacol, phenol and the nymphal blend as well as to veratrole, acetophenone and (*E*,*Z*)-2,6-nonadienal. *Asterisks* indicate significant difference between proportions in the solitary and the gregarious phase (*$P < 0.05$)

to PAN ($x^2 = 8.92$, $df = 1$, $P < 0.05$), guaiacol ($x^2 = 9.49$, $df = 1$, $P < 0.05$), phenol ($x^2 = 11.20$, $df = 1$, $P < 0.05$) and the nymphal blend ($x^2 = 11.35$, $df = 1$, $P < 0.05$) than neurons of gregarious locusts (Fig. 4). A similar but statistically not significant trend was observed for the adult blend, veratrole, (*E*)-2-hexenal and butyric acid, and a reversed relationship was established for indol (Fig. 4).

According to their individual response spectra to different odour stimuli, three different physiological types of PNs could be distinguished: pheromone-specific neurons, plant-specific neurons and pheromone-plant generalist neurons. All physiological types of PNs were found in both solitary and gregarious locusts.

Pheromone-specific neurons

Pheromone-specific neurons responded to behaviourally relevant and putative oviposition-aggregating pheromone-, sex pheromone-, and aggregation pheromone components and blends. According to their specificity these neurons were further divided into component-specific-, blend-specific- and generalist neurons.

Pheromone-component-specific neurons in solitary (23 neurons) and gregarious (25) locusts responded to aggregation- and oviposition-aggregating-pheromone components and (*E*,*Z*)-2,6-nonadienal (Table 1a). Neurons responding to acetophenone and (*E*,*Z*)-2,6-nonadienal were more abundant than neurons responding to other pheromone components in both solitary and gregarious locusts.

Blend-specific neurons (4 neurons in solitary and 3 neurons in gregarious locusts) (Table 1b) responded to behaviourally active blends of aggregation pheromone components, but not to single components present in these blends. These neurons responded in addition to acetophenone or (*E*,*Z*)-2,6-nonadienal. Response characteristics of a blend-specific neuron are given in Fig. 6a.

Pheromone-generalist neurons (77 neurons in solitary and 81 neurons in gregarious locusts) responded to a large number of pheromone components but more frequently to components present in the nymphal blend, guaiacol and phenol, than to the major components present in the adult blend, PAN and benzaldehyde, in both locust phases (Fig 5). Additionally, pheromone-generalist neurons responded frequently to the oviposition-aggregating-pheromone components, veratrole and acetophenone, as well as to the putative sex pheromone (*E*,*Z*)-2,6-nonadienal but hardly ever to other tested pheromone components (Fig. 6). Response characteristics of pheromone-generalist neurons are given in Fig. 6. Both excitatory (Fig. 6b,c) and inhibitory (Fig. 6d) as well as mixed responses (Fig. 6e) were found in both

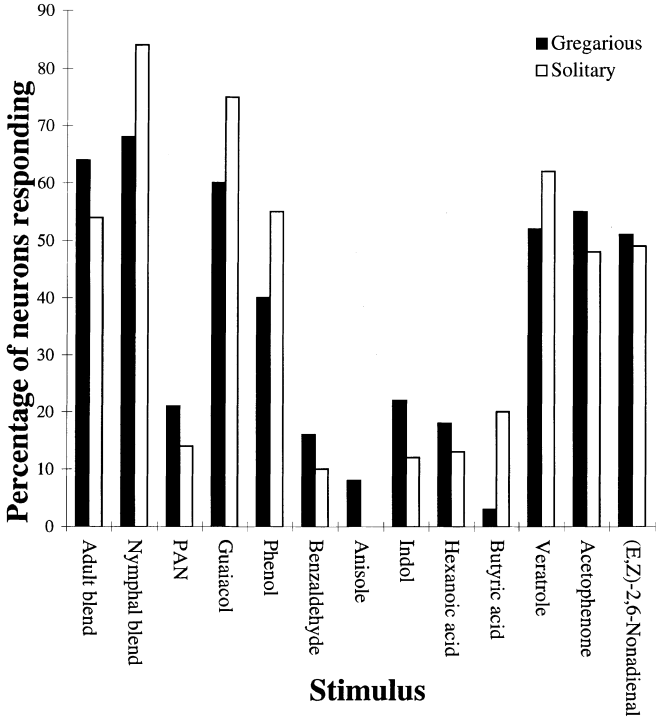


Fig. 5 Response spectra of pheromone-generalist neurons of solitary ($n = 76$) and gregarious ($n = 81$) fifth-instar locusts showing the integration pattern of species-specific attractants. Pheromone-generalist neurons of both solitary and gregarious locusts responded frequently to the adult- and nymphal-aggregation pheromone blends and guaiacol and phenol present in the nymphal blend. Furthermore, these neurons also responded more frequently to oviposition-aggregating pheromone components and the putative sex pheromone than to other tested pheromone components. The differences between the phases are not significant

solitary and gregarious locusts. Examples of response spectra of pheromone-generalist neurons, representing 90% of the neurons for solitary and 68% of the neurons for gregarious individuals, are shown in Table 1c.

Plant-specific neurons

Plant-specific neurons (2 and 3 in solitary and gregarious locusts, respectively) were either component-specific or responded to both (*E*)-2-hexenal and (*Z*)-3-hexenylacetate (Table 2a).

Pheromone-plant generalist neurons

Pheromone-plant generalist neurons (23 and 18 in solitary and gregarious locusts, respectively) responded to a wide variety of pheromone components and plant volatiles (Table 2b). These neurons were either inhibited, excited or differentially affected, i.e. mixed inhibitory and excitatory, delayed (Fig. 2) and inhibitory-excitatory-inhibitory (Fig. 3) responses were found in neurons of both solitary and gregarious locusts.

Fig. 6a–e Peristimulus time histograms showing characteristic responses of pheromone-specific neurons (not responding to plant volatiles). **a** Blend specific neuron responding to nymphal faecal volatiles (nymphal blend) and not to its two constituents guaiacol and phenol. Pheromone-generalist neurons showing excitatory (**b**, **c**) and inhibitory (**d**) as well as mixed excitatory and inhibitory (**e**) responses to a subset of aggregation pheromone components and blends hereof as well as to a few other tested components. Recordings **a**, **c** and **d** were recorded from gregarious locusts and **b** and **e** from solitary locusts. Physiological responses are characterised as +(4–9 net spikes), ++ (10–19 net spikes), +++ (> 19 net spikes), – (inhibition), *nt* not tested. The concentration used was 1 mg. *Bars beneath histograms* indicate stimulation period (500 ms)

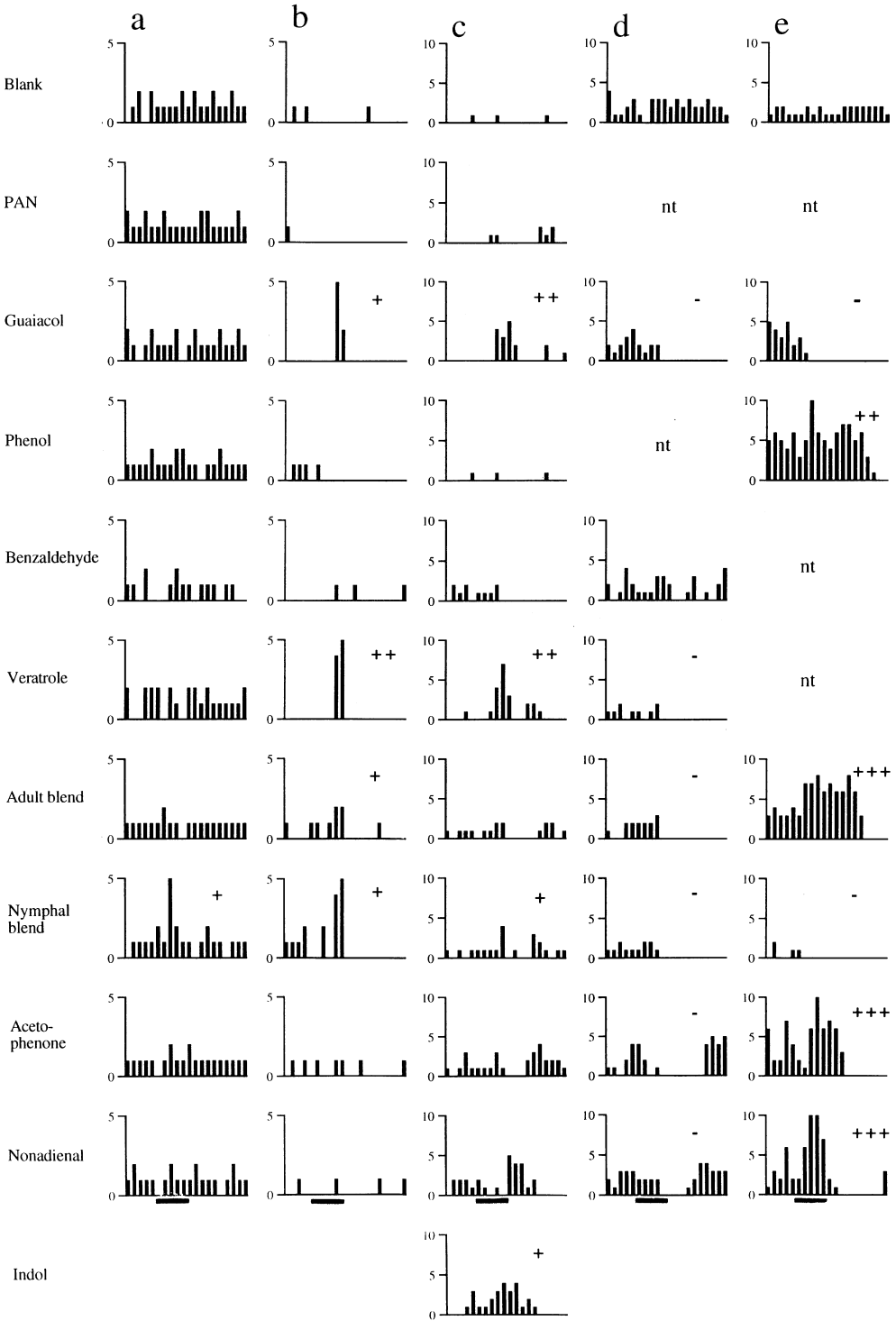
Dose-dependent responses

Most PNs of both solitary and gregarious locusts showed a dose-dependent response to the tested stimuli. Projection neurons of solitary locusts generally showed a higher sensitivity to the tested components (Fig. 7). Acetophenone ($H = 61.00$, $df = 1$, $P = 0.002$), nonadienal ($H = 39.50$, $df = 1$, $P = 0.043$) and the nymphal blend ($H = 50.00$, $df = 1$, $P = 0.006$) elicited significantly higher responses in PNs of solitary locusts than in gregarious locusts, at a stimulus load of 10 µg (Fig. 7). Additionally, nonadienal elicited a significantly higher response in neurons of solitary locusts than in gregarious locusts when tested at a load of 1 µg ($H = 0.027$, $df = 1$, $P = 0.027$) (Fig. 7b). No apparent changes in response specificity or in physiological characteristics were observed with decreasing or increasing stimulus loading. However, a few neurons showed slightly decreasing responses at higher concentrations, which clearly did not result from an increase in the spontaneous activity.

Discussion

In our study we have shown that some aspects of central processing of behaviourally relevant odours in fifth-instar nymphs of the desert locusts are phase dependent. A comparison of our data with results obtained in adult gregarious locusts (Anton and Hansson 1996) also shows that central processing of odours changes during development and correlates well with behavioural results obtained from different developmental stages (Obeng-Ofori et al. 1993, 1994a, b; Torto et al. 1994, 1996; Njagi et al. 1996). The observed phase- and stage-dependent differences in central processing may be explained by differential sensory input.

Fifth-instar solitary and gregarious nymphal PNs responded more frequently to components present in nymphal faeces than to the major component present in the adult aggregation pheromone. Comparing the proportion of PNs of fifth-instar gregarious nymphs to PNs of adult gregarious locusts (Anton and Hansson 1996; unpublished data) that respond to aggregation pheromone components or blends, stage-dependent differen-



ces are found (Table 3). Projection neurons of adult gregarious locusts respond significantly more frequently to PAN ($H = 7.20$, $df = 1$, $P < 0.05$), the major component of the adult aggregation pheromone, than PNs of fifth-instar gregarious nymphs. A similar but not significant trend is found for benzaldehyde. This result may be due to the significantly higher number of olfactory sensilla and RNs responding to aggregation pheromone components of adult locusts compared to fifth-instar nymphs (Ochieng' et al. 1998; Ochieng' and Hansson 1997a,b). Furthermore, differences in the response spectra of the physiologically characterised neurons were found. Pheromone-generalist neurons of adult gregarious locusts responded more frequently to PAN (56%) and benzaldehyde (40%) (data calculated from Anton and Hansson 1996; unpublished data) than

Table 2 Characteristics and abundance of **a** plant-specific and **b** pheromone-plant-generalist neurons in gregarious and solitary-fifth-instar desert locusts. Physiological responses are characterised as 0: no response, (+) excitatory- and (−) inhibitory-response

	Stimuli															No. of neurons	
	Adult blend	Nymphal blend	PAN	Guaiacol	Phenol	Benzal-dehyde	Anisole	Indol	Hexanoic acid	Butyric acid	Veratrole	Aceto-phenone	(*E*,*Z*)-2,6-non adienal	(*E*)-2-hexenal	(*Z*)-3-hexenyl acetate	Solitary No. of neurons	Grega-rious
a	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	1	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	2
b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	3
	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	2	0
	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	0	1
	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	1	0
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0	2	0
	−	−	−	−	−	−	−	0	−	−	−	−	−	−	0	1	0
	−	−	−	−	−	0	−	−	−	0	−	−	−	−	0	1	0
	−	+	−	−	−	−	−	0	0	0	−	−	−	−	0	1	0
	+	+	+	0	−	0	0	0	+	0	+	−	−	+	0	1	0
	−	−	−	−	−	0	0	0	−	0	−	−	0	−	0	1	0
	0	−	+	+	−	0	0	0	−	0	0	+	−	−	0	1	0
	−	+	−	−	−	0	0	0	+	0	−	−	−	−	0	1	0
	−	+	−	−	−	−	0	0	0	0	−	−	+	−	0	1	0
	+	+	+	+	0	+	+	+	0	0	+	+	+	+	0	0	2
	+	+	0	+	+	0	0	+	+	0	+	+	0	+	0	1	2
	+	+	+	+	+	+	0	0	0	0	+	+	+	+	0	1	0
	−	−	−	−	0	0	0	0	−	−	−	−	−	−	0	1	1
	+	0	+	0	0	0	0	0	+	+	0	0	+	+	0	1	0
	−	−	0	0	0	0	0	0	−	0	0	−	−	−	0	1	0
	0	0	0	0	0	0	0	0	+	+	0	+	+	+	+	0	1
	0	0	0	0	0	0	0	0	+	+	0	0	+	+	0	0	1
	0	0	0	0	0	0	0	0	−	−	0	0	−	−	0	0	1
	0	0	0	0	0	0	0	0	+	0	0	0	0	+	0	0	4
	0	0	0	0	0	0	0	0	+	0	0	0	+	+	0	1	0
	0	0	0	0	0	0	0	0	0	+	0	0	+	0	+	0	1
	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	1

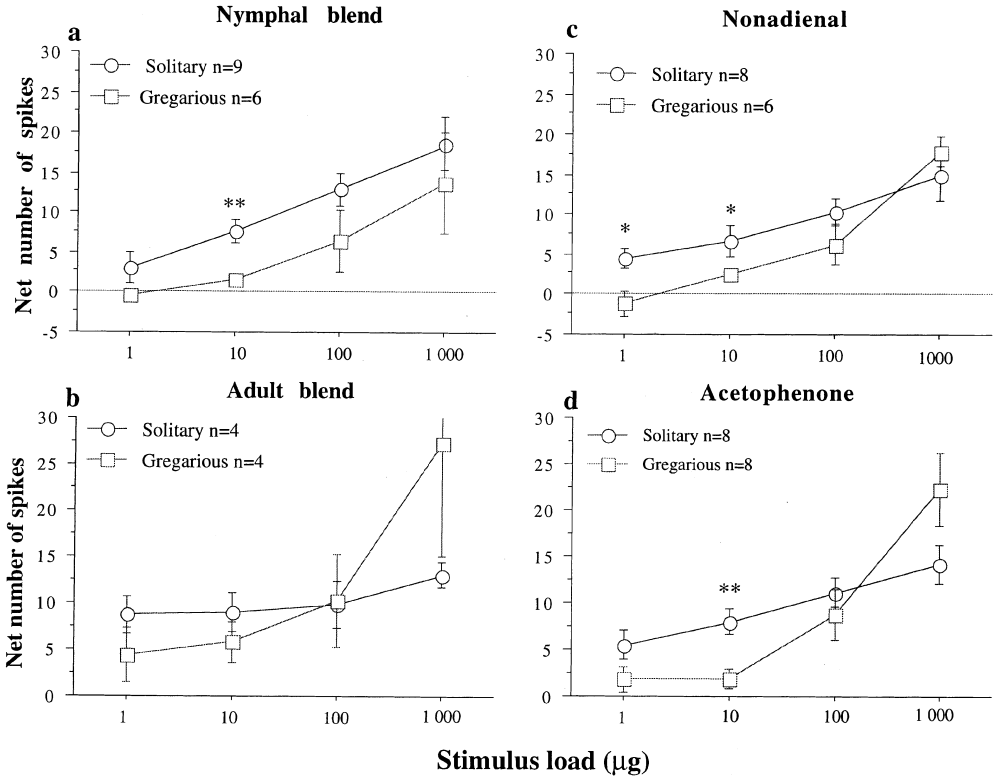


Fig. 7a–d Dose-dependent response of antennal lobe projection neurons in solitary and gregarious fifth-instar locusts to **a** the nymphal aggregation pheromone blend, **b** adult aggregation pheromone blend, **c** the putative sex pheromone (*E*,*Z*)-2,6-nonadienal and **d** the egg-laying attractant acetophenone. Neurons of solitary locusts generally showed a higher sensitivity to low concentrations of the tested components. The net number of spikes with *asterisks* at different stimulus loads were significantly different (*$P < 0.05$, **$P < 0.01$) between phases (mean ± SE)

Table 3 Percentage of antennal lobe neurons of fifth-instar ($n = 130$) and adult ($n = 168$) (Anton and Hansson 1996; unpublished data) gregarious desert locusts, *Schistocerca gregaria* responding to aggregation pheromone components and blends. Antennal lobe neurons of adult locusts responded significantly more frequently to PAN than neurons of fifth-instar locusts (*$P < 0.05$) and a similar but statistically not significant trend was observed for benzaldehyde

	Adult blend	Nymphal blend	PAN	Guaiacol	Phenol	Benzaldehyde
Adult locusts	48	45	40 (*)	41	25	26
Fifth instar	46	49	22	40	29	17

neurons of fifth-instar gregarious nymphs, indicating a converging innervation of specific RNs onto pheromone-generalist neurons. The significantly higher number of sensilla, enclosing RNs specific to aggregation pheromone components, in solitary compared to gregarious locusts (Ochieng' et al. 1998; Ochieng' 1997) may in accordance with the arguments above explain the observed phase-specific differences in the percentage of PNs responding to a sub-set of aggregation pheromone components.

Differential innervation of RNs has also been studied in the hemimetabolous insect *Periplaneta americana*, where the number of RNs from male specific sensilla innervating the AL shows a significant increase during the final moult leading to sexually dimorphic behaviour (Prillinger 1981). The increasing number of RNs innervate a constant number of deutocerebral neurons (Prillinger 1981) implying the importance of sensory input in regulating sex- and stage-specific behaviour in *P. americana*. A constant number of central nervous neurons throughout postembryonic development also seems to be the general case in other hemimetabolous insects (*Blaberus craniifer*: Chambille and Rospars 1985; *Acheta domesticus*: Gymer and Edwards 1967; *S. gregaria*: Sbrenna 1971). With respect to these developmental data, our results indicate a stage-dependent integration pattern of aggregation pheromone components in the AL, an integration highly correlated with the preferential behavioural attraction to nymphal or adult aggregation pheromones in the two developmental stages (Obeng-Ofori 1993, 1994a; Torto et al. 1994, 1996). In addition, similar response spectra of pheromone-specific neurons of the two phases may explain the phase-independent behavioural responses to stage-specific pheromones observed in *S. gregaria* (Njagi et al. 1996). In contrast, phase-specific behaviours (Roessingh et al. 1993) may be the result of the phase-dependent olfactory integration.

In addition to aggregation pheromone components, pheromone-generalist neurons responded frequently to oviposition-aggregating pheromone components and the putative sex pheromone and also, but to a lesser extent, to other tested behaviourally active and putative pheromone components. The response spectra of pheromone-generalist neurons thus suggest that these neurons play an important role for locusts in detecting and integrating species-specific attractants in the environment.

Dose-dependent responses of PNs to the tested stimuli were found in both locust phases with PNs of

solitary locusts generally showing higher sensitivity to the tested compounds. Chapman (1982), Greenwood and Chapman (1984) and Ochieng' et al. (1998) suggested that social facilitation, resulting from a gregarious habit, permit a decrease in individual sensitivity with an associated reduction in the number of olfactory sensilla. The higher number and higher sensitivity of RNs in the solitary as compared to the gregarious phase (Ochieng' 1997) may thus indicate an increased convergence of RNs onto AL neurons and thus a subsequent increase in the sensitivity of AL neurons.

Absolute sensitivity of PNs of solitary and gregarious locusts match with what has been found at the receptor level (Ochieng' 1997). This fact implicates a low degree of convergence of RNs onto PNs of locusts compared to a high degree of convergence, enhancing the olfactory signal in sex-pheromone communication systems as described in, for example, *Agrotis segetum* and in *M. sexta* (Christensen and Hildebrand 1987; Hansson et al. 1994; Hartlieb et al. 1997).

Projection neurons generally showed excitatory or inhibitory responses to the tested stimuli, response patterns which have been found earlier in adult locusts and in other insects (Anton and Hansson 1996; Hansson 1995). In addition, delayed inhibitory and excitatory responses were found in both fifth-instar solitary and gregarious nymphs. Christensen et al. (1993) suggested that local-circuit interaction of local interneurons in the AL of *M. sexta* leads to delayed excitatory and inhibitory responses. Delayed responses at the receptor level (SA Ochieng', personal communication) may also contribute to the observed delayed responses of AL neurons. Receptor neurons present in sensilla basiconica and sensilla coeloconica show excitatory and inhibitory responses, respectively, in response to aggregation pheromone components (Hansson et al. 1996; Ochieng' 1997) which implicate the possibility of a two-channel system for the integration of these stimuli, Differences in the temporal representation of the two channels in the AL, either through direct synaptic connection between RNs and PNs or via local interneurons, may explain the physiological characteristics of the inhibitory-excitatory-inhibitory responses observed in a PN. The dual-response characteristics of this neuron further suggests that locust PNs are able to discriminate among similar pheromone blends.

Our study reveals important aspects regarding the effects of differential innervation of PNs by RNs in the AL of the desert locust, *S. gregaria*. We suggest that developmental and environmental effects on the number of olfactory sensilla alter the response spectra of AL neurons. Stage-specific differences in the response spectra of AL neurons may explain how different developmental stages discriminate among similar aggregation pheromones. The observed phase-specific integration further supports the hypotheses of phase-specific olfactory input.

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