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JUVENILE-HORMONE-MEDIATED PLASTICITY OF AGGREGATION BEHAVIOUR AND OLFACTORY PROCESSING IN ADULT DESERT LOCUSTS

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Summary

In desert locusts *Schistocerca gregaria*, aggregation behaviour is elicited by aggregation pheromones. In this study, we show that the behavioural response to the major and most potent adult aggregation pheromone component, phenylacetonitrile, is age- and juvenile-hormone-dependent. Furthermore, we show that juvenile hormone influences the responsiveness of olfactory interneurons in the antennal lobe to aggregation pheromone, whereas the responsiveness of antennal receptor neurons is not changed. Old locusts and locusts injected with juvenile hormone, in contrast to young locusts and locusts deprived of juvenile hormone through allatectomy, i.e. after surgical removal of the gland producing this hormone, do not display any aggregation behaviour, as indicated by long-term behavioural observations. The lack of positive olfactory-guided behaviour coincides with an impairment of the central olfactory system, which displays a lower number of neurons responding to aggregation pheromone. Indirect and direct actions of juvenile hormone at different levels of the central nervous system may thus contribute to the regulation and modulation of behavioural responsiveness in the locust.

Key words: *Schistocerca gregaria*, juvenile hormone, behaviour, electrophysiology, antennal lobe, olfactory processing.

Introduction

In insects, juvenile hormone (JH) plays a decisive role in regulating a multitude of physiological processes (for a review, see Kumaran, 1990). In *Schistocerca gregaria* and other locusts, which by definition are able to occur in two distinct phases, research has been focused on phase-related differences in JH titre (for reviews, see Applebaum et al., 1997; Pener, 1991; Pener and Yerushalmi, 1998). Juvenile hormone affects a number of aspects of behaviour in desert locusts, including phase behaviour (Wiesel et al., 1996), sexual behaviour (Amerashinge, 1978) and spontaneous locomotor activity (Odhiambo, 1966). Although the aggregation behaviour of gregarious locusts has attracted attention over recent years, with emphasis on its behavioural significance (Obeng-Ofori et al., 1993; Obeng-Ofori et al., 1994; Torto et al., 1994) and the central nervous integration of aggregation pheromones (Anton and Hansson, 1996; Hansson et al., 1996; Ignell et al., 1998; Ignell et al., 1999; Ochieng' and Hansson 1999), nothing is known about the endocrine regulation of this behaviour (Pener, 1991). The aggregation of locusts, long known to be a crucial stage in the gregarisation process, i.e. the transition between the solitary and gregarious phases (Hassanali and Torto, 1999; Pener, 1991; Roffey and Popov, 1968), is fundamental to our understanding of the behavioural ecology and physiology of these species.

In the present study, we evaluated age-dependent changes in the behavioural response of adult gregarious desert locusts to the major aggregation pheromone component phenylacetonitrile, which has been reported to be intrinsically the most potent component in eliciting the aggregation response in adult locusts (Obeng-Ofori et al., 1994; Torto et al., 1994). Previous data reporting age-dependent changes in the rate of JH biosynthesis by the corpora allata (CA), the retrocerebral gland producing JH (Avruch and Tobe, 1978; Dale and Tobe, 1986; Tawfik, 1995; Tobe and Pratt, 1975), enable us to postulate a link between JH and behavioural changes. The rate of JH biosynthesis in both male and female adult locusts is low during the first 2 days following adult emergence but increases gradually during the first week (Avruch and Tobe, 1978; Dale and Tobe, 1986; Tobe and Pratt, 1975). During the second and third weeks, the daily rate of JH biosynthesis displays significant fluctuations but remains high in both males and females. Reflecting changes in the rate of JH biosynthesis, haemolymph JH titre also increases during the first 2 weeks following emergence (Couillaud et al., 1985). Although daily JH titre measurements are lacking, it seems that the JH titre remains high until at least 4 weeks of age because a high JH titre has been reported in 30-day-old locusts (Tawfik, 1995).

To measure possible behavioural changes induced by the naturally fluctuating JH titre, we employed a simple

classification of locust behaviour suggested by previous studies (Obeng-Ofori et al., 1993; Roessingh et al., 1993). Behavioural tests were also performed on allatectomized locusts (i.e. locusts deprived of the corpora allata) and JH-injected locusts to establish the direct and indirect effects of JH on their aggregation behaviour.

The aggregation behaviour of adult locusts is elicited by aggregation pheromones emitted by sexually mature males. These pheromone components are detected by antennal receptor neurons (RNs) (Hansson et al., 1996; Ochieng' and Hansson, 1999) and integrated by interneurons in the antennal lobe (Anton and Hansson, 1996; Ignell et al., 1998; Ignell et al., 1999). Electrophysiological studies in other species have emphasised the age- and JH-dependent plasticity of antennal RNs (Diptera, Bowen, 1991; Crnjar et al., 1990; Hymenoptera, Masson and Arnold, 1984; Sigg et al., 1997; Vetter and Vissher, 1997; Lepidoptera, Seabrook et al., 1979) or of antennal lobe interneurons (Lepidoptera, Anton and Gadenne, 1999), thereby regulating the behavioural responsiveness of the insect.

To evaluate the influence of JH on the central nervous integration of aggregation pheromones, we examined both the antennal response and the response characteristics of antennal lobe interneurons in adult gregarious locusts of different ages and of different hormonal status.

Materials and methods

Animals

Fifth-instar gregarious desert locusts *Schistocerca gregaria* (Forskål) originating from Blades Biological, Cowden, Edenbridge, UK, were reared as described previously (Ignell et al., 1998). After adult emergence, locusts were marked with number tags enabling the identification of individuals throughout the experiments. For behavioural experiments, adult male and female locusts were divided into four experimental groups: (i) control; (ii) allatectomized; (iii) sham-operated; and (iv) JH-injected. Surgical removal of the corpora allata (Strong, 1965) and sham operations were performed 2 days after adult emergence. Juvenile hormone III, the JH type identified in *S. gregaria* (JHIII; racemic mixture, Sigma; 50 μg in 1 μl of olive oil), was injected into the abdomen of the locusts on days 1, 2 and 3 after adult emergence.

Behavioural assay

Bioassays were performed in a single-chamber two-choice olfactometer (Obeng-Ofori et al., 1993). Compressed air, after purification through a charcoal filter, was split into two streams, with each stream passing through a 2 l round-bottomed flask before being directed into either the control or the treatment area of the olfactometer (60 cm×30 cm×30 cm) through perforations in its floor. The flow rate was 120 ml min^{-1} for each stream. The test stimulus, 1 mg of phenylacetonitrile (Sigma), was dissolved in 2 ml of paraffin oil (Merck) in a 3.7 ml vial (Torto et al., 1994) and placed in one of the flasks of the olfactometer. Paraffin oil, acting as the control, was placed in a similar vial in the other flask. The position of the two flasks was changed randomly between trials, and connector tubings and flow meters were flushed with clean air and changed regularly to minimise contamination. In addition, the olfactometer was cleaned with acetone, and the metal top and bottom were heated at 300 °C for 2 h to prevent contamination effects.

Shortly (1–2 h) prior to the experiments, locusts were placed individually in 250 ml plastic jars and transferred to the room in which the experiments were performed, which was kept under the same conditions as the rearing room. Single locusts were introduced into the olfactometer by tilting the jar a few centimetres above the non-perforated central part of the olfactometer. This procedure did not seem to affect the behaviour of the locusts.

Aggregation behaviour was assayed 1, 8, 15, 22 and 29 days after adult emergence for control locusts, with each day representing a trial period, and at 8, 15, 22 and 29 days after adult emergence for allatectomized locusts. To disregard any effects of the operation *per se*, sham-operated locusts were also assayed at the same times as allatectomized locusts. JH-injected locusts were assayed only at day 8 after adult emergence. The experiments were performed on 30 individuals per trial period for both male and female locusts. To assess behavioural changes in individuals over a longer period, we assayed half the locusts in each experimental group throughout the treatment period. The remaining locusts in each experimental group were assayed only once within one trial period and then discarded. No significant behavioural differences, tested using a two-way analysis of variance (ANOVA) without cell replication, were found between these groups, and we therefore considered each behavioural observation as independent.

Aggregation behaviour was assessed by an observer positioned approximately 1.2 m from the olfactometer. The experiment was divided into two 15 min periods. During the first 15 min period, individual behavioural variables, chosen after preliminary observations, were registered every 10 s for both the control and the treatment area. Behavioural variables included (a) the number of times the insect was inactive, (b) the number of walking bouts (a walk was defined as an unbroken period of locomotion of more than half a body length), (c) the number of times leg movements occurred, (d) the number of times antennal movements occurred, (e) the number of times grooming occurred, (f) the number of times wing-fanning occurred, (g) the number of times jumping occurred and (h) the time spent at the sides of the olfactometer (see Roessingh et al., 1993). Of these variables, the first four were found to display age-dependent changes. Frequency was defined as the number of observed behavioural variables per 10 s interval divided by assay duration. For example, if the locust was walking for 25 of the (10 s) intervals monitored, the final frequency of walking bouts was calculated as 25/90=0.27. After the end of the initial time period, the observer left the room; the observer returned after 15 min to register the final

location of the insect, which was used to calculate an aggregation index.

Analysis of behavioural data

The aggregation index (AI) (Obeng-Ofori et al., 1993; Obeng-Ofori et al., 1994; Torto et al., 1994) was calculated as $100(T-C)/N$, where T is the number of locusts found in the treatment area, C is the number of locusts found in the control area and N is the total number of locusts tested. Differences between experimental groups were analysed using the χ^2-test on raw data, applying the Bonferroni correction to limit the overall experiment-wise error rate (Obeng-Ofori et al., 1993).

The net behavioural response to phenylacetonitrile was calculated by subtracting the frequency of each behavioural variable exhibited in the control area from the frequency of each behavioural variable exhibited in the treatment area. The behaviour displayed in the control area was generally found to be constant throughout the trial period. Differences in behavioural variables between experimental groups and between sexes were analysed using the χ^2-test on raw data, applying the Bonferroni correction to limit the overall experiment-wise error rate.

Electroantennograms

Electroantennograms (EAGs) were recorded from excised antennae of (i) 8- and 29-day-old control and allatectomized locusts and (ii) 8-day-old JH-injected locusts using standard procedures (e.g. Hansson et al., 1991). Stimulation was performed as described previously (Ignell et al., 1998): a random application of stimuli with a minimum interstimulation period of 1 min. Representative behaviourally significant compounds were used as stimuli: (i) a blend resembling the adult aggregation pheromone (phenylacetonitrile:guaiacol:phenol:benzaldehyde:veratrole in the proportion 80:3:3:5:4); (ii) a possible *S. gregaria* sex pheromone (*E*,*Z*)-2,6-nonadienal, which is also a minor component of a preferred host plant *Tribulus terrestris* (Zygophyllaceae) and (iii) an oviposition aggregating pheromone component, acetophenone. Each compound was prepared as a serial dilution in diethyl ether in increasing concentrations from $1\,ng\,\mu l^{-1}$ to $100\,\mu g\,\mu l^{-1}$. For each stimulus dosage, a 10 µl sample was applied to a filter paper. A green leaf volatile, *E*-2-hexenal (100 µg), was used to check the responsiveness of the antennae. Differences in EAG amplitude between experimental groups and between sexes were tested using repeated-measures ANOVAs (Ochieng', 1997).

Intracellular electrophysiology

Intracellular recordings from antennal lobe neurons were performed on 8- and 29-day-old controls, on 29-day-old allatectomized and on 8-day-old JH-injected male and female locusts. Responses of antennal lobe neurons were recorded in at least 20 locusts for each experimental group and trial period, with the total number of recorded neurons ranging from 93 to 130 in each case. Standard intracellular recording methods were used (Christensen and Hildebrand, 1987). The preparation and stimulation procedure have been described in detail elsewhere (Anton and Hansson, 1996; Ignell et al., 1998). As stimuli, we used behaviourally active blends resembling adult- and nymph- (guaiacol:phenol 60:40) produced aggregation pheromones and their single components, as well as acetophenone, (*E*,*Z*)-2,6-nonadienal and plant volatiles (hexanoic acid, *E*-2-hexenal). The responses of antennal lobe neurons to this range of compounds were tested using 1 mg of each component on the filter paper in the stimulating pipette. Up to seven antennal lobe neurons were tested in each individual locust.

A neuron was defined as non-responding if its spike frequency over the 600 ms after the onset of stimulation did not exceed its spontaneous spike frequency over the 600 ms before stimulation by more than 10 % for any of the stimuli tested. Only neurons for which at least all blends and all single components not present in the blends had been tested were taken into account. A locust was defined as non-responding if at least three neurons had been tested and none of them responded to any stimulus. Differences in the number of non-responding neurons and locusts between experimental groups were analysed using the χ^2-test on raw data, applying the Bonferroni correction to limit the overall experiment-wise error rate.

Results

Behavioural responses of control and sham-operated locusts

Control and sham-operated locusts displayed no significant differences in their behavioural response to phenylacetonitrile, and the results were therefore combined. Male and female locusts displayed different age-dependent changes, as shown by their aggregation indices (Fig. 1), and in four of the observed behavioural variables: the frequency of inactivity, of walking, of leg movement and of antennal movement (Fig. 2).

Aggregation indices and behavioural variables of control and sham-operated male locusts displayed age-dependent changes. The AI increased between day 1 and day 8 and then decreased stepwise to reach values close to zero at the end of the observation period, i.e. male locusts were initially attracted by the aggregation pheromone and later did not show any preference for either of the experimental areas (Fig. 1). The frequency of walking and of leg and antennal movements in the treatment area increased up to day 15 and decreased after day 22 (Fig. 2).

The behavioural responses of control and sham-operated female locusts were similar to those of male locusts. We observed some sex-dependent differences, but they were not significant. In female locusts, the decrease in the AI was not evident until day 22 (Fig. 1), and females had a generally lower behavioural activity than males, with a peak in their behavioural activity between days 8 and 15.

Behavioural responses of allatectomized locusts

Allatectomized (–CA) locusts generally displayed high AIs and did not show clear differences in AI with age (Fig. 1).

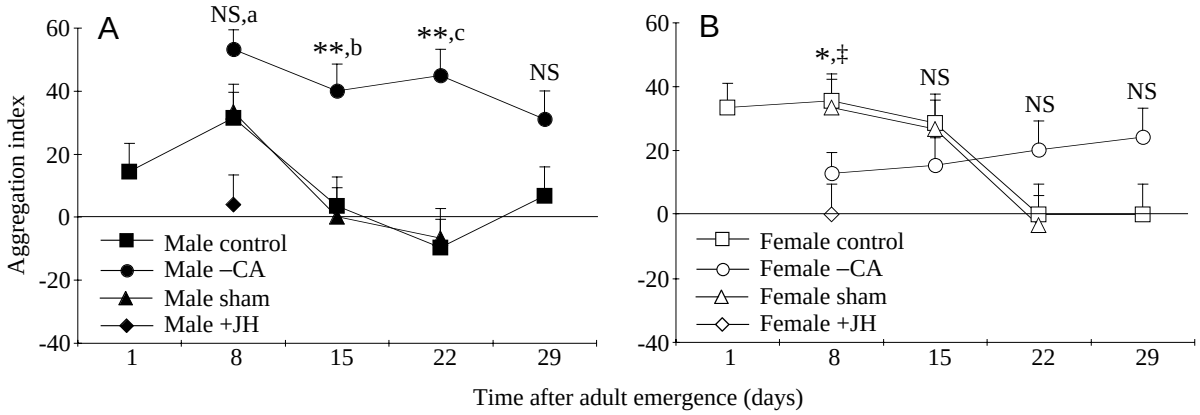

Fig. 1. Aggregation indices (AIs; see Materials and methods) of male (A) and female (B) control, sham-operated (sham), allatectomized (–CA) and juvenile hormone (JH)-injected (+JH) locusts in response to phenylacetonitrile, assayed weekly after adult emergence, in a two-choice olfactometer. Aggregation indices were calculated after 30 min. Significant differences ($^{a}P<0.001$, $^{b}P<0.01$ and $^{c}P<0.01$) are indicated between male and female allatectomized locusts. ‡ indicates a significant difference ($P<0.01$) between control and JH-injected females. Significant differences between experimental groups are indicated: NS, not significant; *$P<0.05$; **$P<0.01$ (χ^2-test). Values are means ± S.E.M. ($N=30$).

Their behavioural activity did, however, show clear age-dependent changes (Fig. 2).

Allatectomized males displayed AIs (Fig. 1) and behavioural activities (Fig. 2A–D) at day 8 similar to those of control males during the same period. The aggregation indices and behavioural activity of allatectomized males in the treatment area were generally significantly higher than the AIs and behavioural activity of control animals at corresponding times from day 15 onwards (Figs 1, 2). In allatectomized males, behavioural activity reached a peak at day 15 (Fig. 2A–D), and walking and leg movement frequencies decreased over the subsequent trial periods (Fig. 2B,C), with corresponding changes in the frequency of inactivity (Fig. 2A). However, no changes were found in the frequency of antennal movement after day 15.

Allatectomized females displayed lower AIs than allatectomized males up to day 22 and lower AIs than control females at day 8 (Fig. 1), whereas the AIs of day 22 and day 29 females resembled those of day 1 females. The behavioural activity (Fig. 2E–H) of allatectomized female locusts up to day 15 resembled the behavioural activity of control females during the same period, whereas older allatectomized females were significantly more active (Fig. 2E–H).

The behavioural observations showed that allatectomized male and female locusts displayed sex-dependent differences in their response to the tested stimulus at day 15 (Figs 1, 2). These differences decreased as a result of a decrease in behavioural activity in males and an increase in behavioural activity in females, so that both sexes had similar levels of activity by the end of the experimental period (Fig. 2).

Behavioural responses of JH-injected locusts

Day 8 male and female JH-injected locusts did not display any preference for either the treatment or the control area, i.e. they had AI values close to zero, resembling the AI of control locusts at 22 and 29 days of age (Fig. 1).

The behavioural activity of JH-injected locusts at day 8 was similar to that of control locusts at day 29 (Fig. 2). A difference in the AI of JH-injected locusts compared with control locusts at day 8 was present in both males and females, although the difference was only significant for females (Fig. 1). No significant differences were found in the measured behavioural activity between JH-injected and control locusts at day 8 (Fig. 2).

Electroantennograms

A clear dose–response relationship was found in EAGs for all stimuli tested in males and females. No significant differences were observed in EAG amplitude, either between sexes or among the different experimental groups (Fig. 3).

Central nervous processing

Intracellular recordings were made from 898 antennal lobe neurons in 161 adult locusts. The proportion of responding neurons and of responding control locusts decreased significantly with age. At day 8, more than 90 % of the locusts had responding neurons (Fig. 4A,B), of which more than 70 % of those tested responded to aggregation pheromones (Fig. 4C,D). In 29-day-old locusts, fewer than 60 % of individuals displayed responding neurons (Fig. 4A,B) and

Fig. 2. Net behavioural responses of male (A–D) and female (E–H) control, sham-operated (sham), allatectomized (–CA) and juvenile hormone (JH)-injected (+JH) locusts to phenylacetonitrile, assayed weekly after adult emergence. High frequencies of observed behavioural variables indicate a high behavioural activity in the treatment area compared with the control area and *vice versa*. Significant differences between male and female allatectomized locusts are indicated ($^{a}P<0.01$, $^{b}P<0.5$). Differences between allatectomized locusts and other experimental groups are indicated: NS, not significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$ (χ^2-test). Values are means ± S.E.M. ($N=30$).

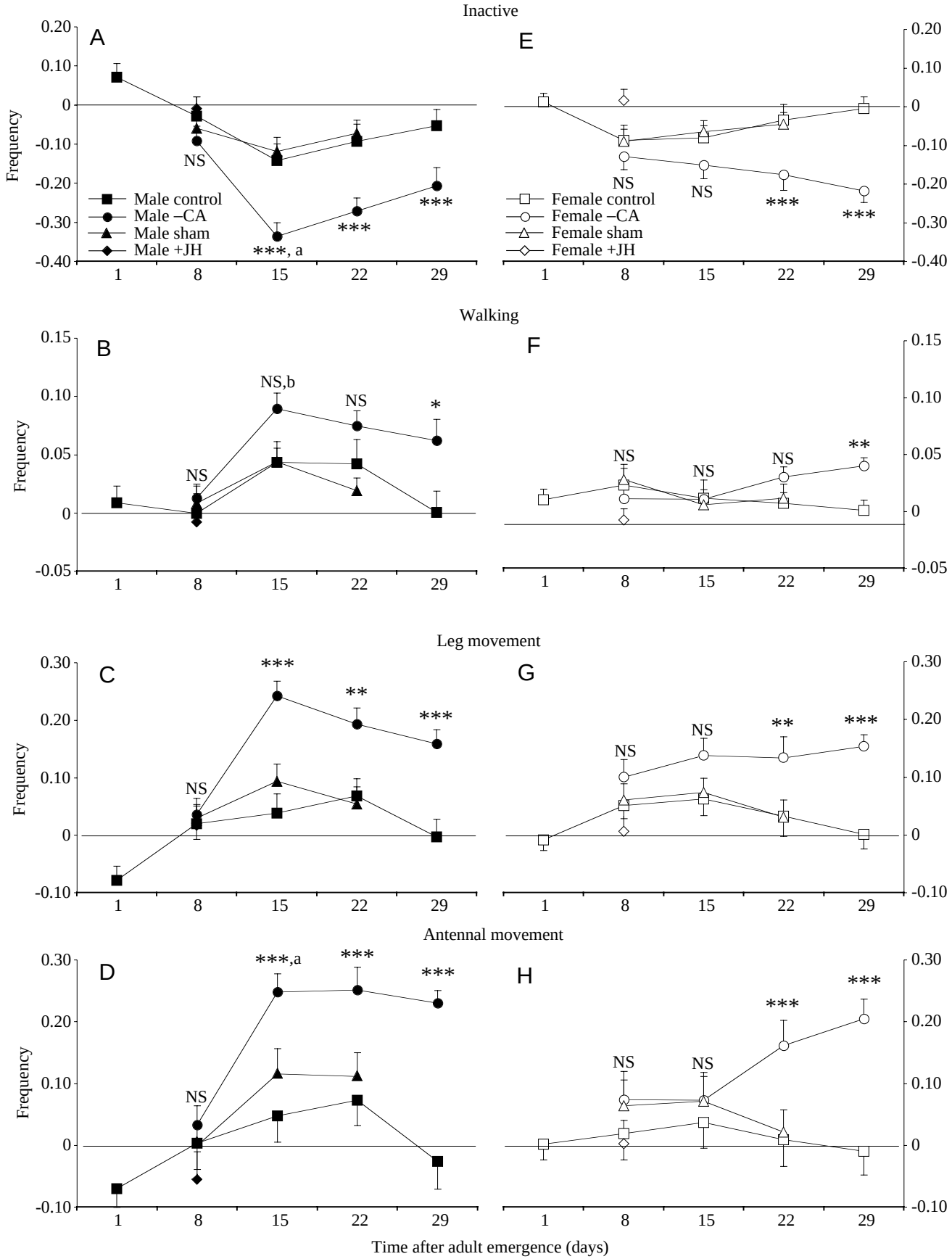
Inactive
A
E
Walking
B
F
Leg movement
C
G
Antennal movement
D
H
Frequency
Time after adult emergence (days)
Male control
Male –CA
Male sham
Male +JH
Female control
Female –CA
Female sham
Female +JH
NS
NS,b
***, a
***,a
*
**
***

Fig. 2

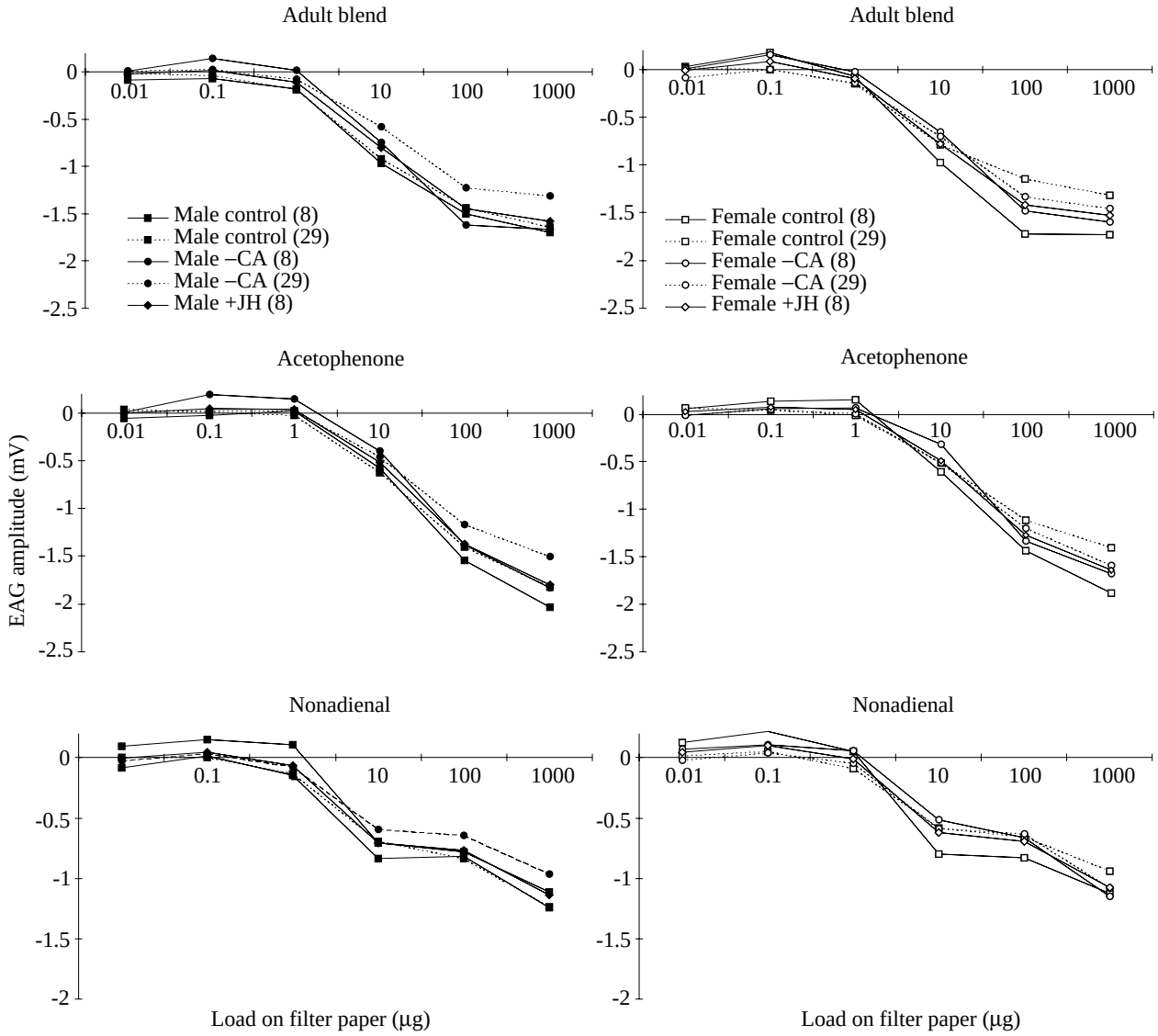

Fig. 3. Mean electroantennogram (EAG) amplitudes (*N*=5) in response to the adult aggregation pheromone (adult blend), acetophenone and (*E*,*Z*)-2,6-nonadienal in control, allatectomized (–CA) and juvenile hormone (JH)-injected (+JH) locusts. Numbers within parentheses indicate the age (in days after adult emergence) of the animal tested. Standard errors are not indicated to enhance the clarity of the figure.

only approximately 30 % of the tested neurons responded to aggregation pheromones (Fig. 4C,D). The proportion of neurons responding to aggregation pheromone and the proportion of locusts with responding neurons in 29-day-old allatectomized locusts did not differ from those in 8-day-old control locusts (Fig. 4). The proportion of responding neurons and of locusts with responding neurons in 8-day-old JH-injected individuals was not significantly different from those in 29-day-old control locusts (Fig. 4). Differences in the proportion of non-responding locusts and non-responding neurons between locusts with different JH levels were more pronounced in females (Fig. 4B,D) than in males (Fig. 4A,C).

The responses of antennal lobe neurons to the range of compounds tested varied among experimental groups for some stimuli only. Note, however, that the number of responding neurons was very low in 29-day-old control and in 8-day-old JH-injected locusts (Fig. 4C,D). For JH-injected locusts, there was a tendency for fewer neurons to be responsive to aggregation pheromones than in 8-day-old control and in allatectomized individuals (Fig. 5). This effect was found to be less pronounced in 29-day-old locusts, probably because of the extremely low number of responding neurons (Fig. 5). For the plant volatile nonadienal, no difference in the proportion of responding neurons was found among treatments (Fig. 5).

Discussion

In the present study, we present data that demonstrate age-dependent plasticity of olfactory-guided aggregation behaviour and of the central nervous processing of olfactory cues. In addition, we show that changes in the central olfactory processing and subsequent changes in behavioural

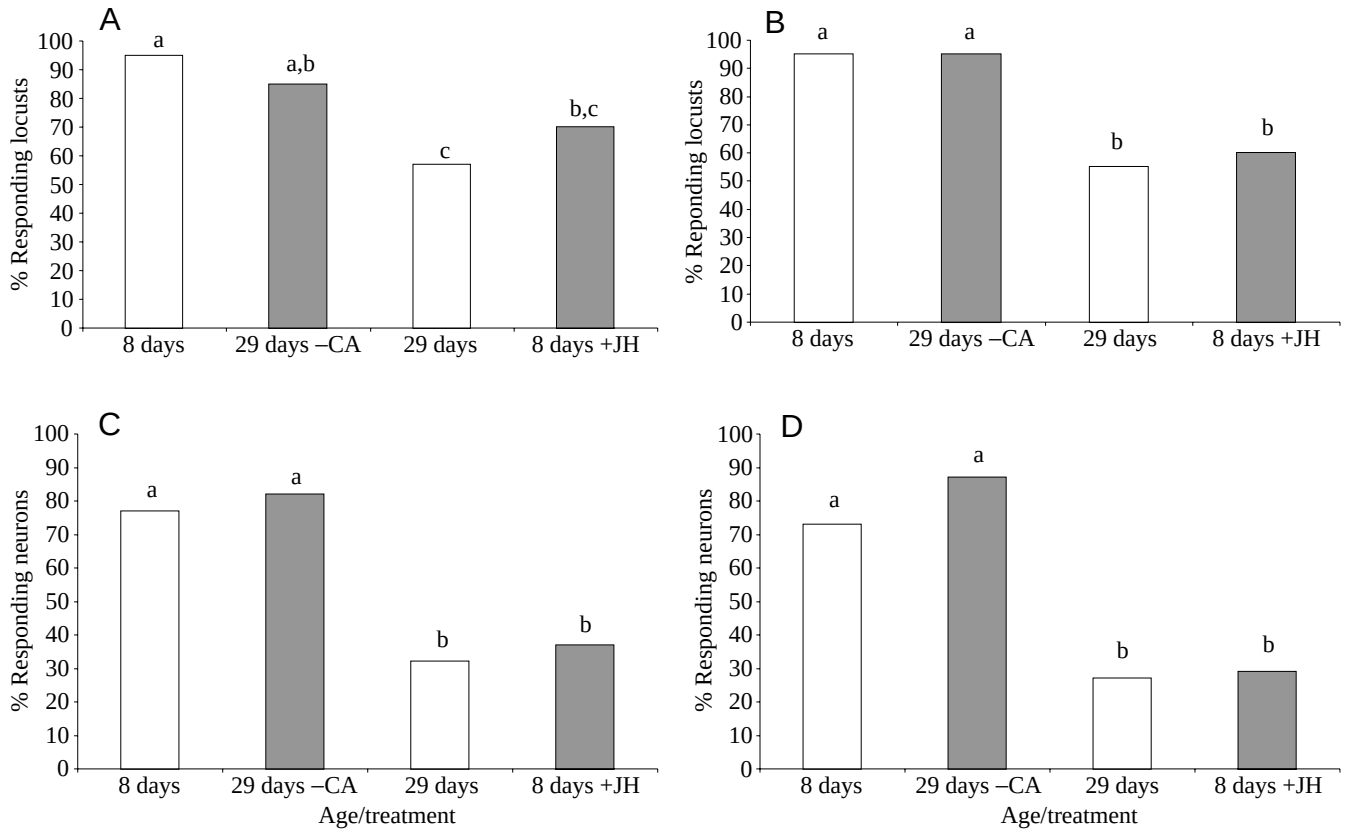

Fig. 4. The percentage of locusts with responding antennal lobe neurons (A,B) and the total percentage of antennal lobe neurons responding to aggregation pheromones (C,D) in 8- and 29-day-old control locusts (open columns), in 29-day-old allatectomized locusts (–CA) and in 8-day-old juvenile hormone (JH)-injected (+JH) male (A,C) and female (B,D) locusts. Different letters above columns indicate significant differences at $P<0.05$ (A,B) and at $P<0.001$ (C,D) (χ^2-test).

responsiveness and activity are JH-mediated. Our data suggest that an experimentally increased JH titre in young locusts ages them artificially and that depriving locusts of JH keeps old locusts 'young' with respect to their olfactory-guided behaviour and central olfactory processing.

Juvenile hormone has previously been reported to stimulate sexual behaviour (Amerashinge, 1978; Loher, 1960; Pener, 1965; Pener, 1967), spontaneous locomotor activity (Odhiambo, 1965; Odhiambo, 1966) and the excitability (Cassier, 1963) of adult locusts. An increased JH titre induced a 'solitarization' effect, i.e. a significant reduction in social aggregation, and stimulated the marching behaviour of *S. gregaria*, both important characteristics of the phase behaviour (Wiesel et al., 1996). The observed increase in behavioural activity and decrease in AIs of untreated locusts, up to day 22, coinciding with an age-dependent increase in JH biosynthesis during this period (Avruch and Tobe, 1978; Couillaud, 1986; Dale and Tobe, 1986; Injeyan and Tobe, 1981; Tawfik, 1995; Tobe and Pratt, 1975) conforms with previous observations of a JH-mediated change in behavioural activity and in social aggregation. Behavioural observations of untreated locusts at day 29, previously reported to have a high haemolymph JH titre (Tawfik, 1995), of JH-injected locusts and of allatectomized locusts, however, indicate that JH may modulate the behavioural activity not only directly but also indirectly in combination with other hormones (see below).

The observed behavioural activity of locusts with high levels of JH (JH-injected and 29-day-old locusts) may be explained by the impairment of the olfactory system discussed below. However, the changes in antennal lobe neuron responses cannot explain the observed sex-dependent differences in behavioural activity of allatectomized locusts. Furthermore, they cannot explain the observed differences in behavioural activity of untreated and allatectomized locusts. We postulate, therefore, that aggregation behaviour may be regulated indirectly by JH *via* other hormones or neuroactive substances expressed differentially in the two sexes.

One possibility for such modulators are the ecdysteroids, which have been shown previously to induce a reduction in the flight activity of *S. gregaria* (Michel, 1972) and in the marching behaviour of the migratory locust *Locusta migratoria* (Carlisle and Ellis, 1963). Biosynthesis of ecdysteroids is JH-dependent to different extents in the two sexes of different locust species (females, Lagueux et al., 1977; Glass et al., 1978; Goltzene et al., 1978; Kappler et al., 1986; Koeppe et al., 1985; Romana et al., 1995; males, Loher, 1960; Pener, 1965; Cantacuzène, 1967). The differential effects of JH on ecdysiogenic tissues may therefore explain the observed

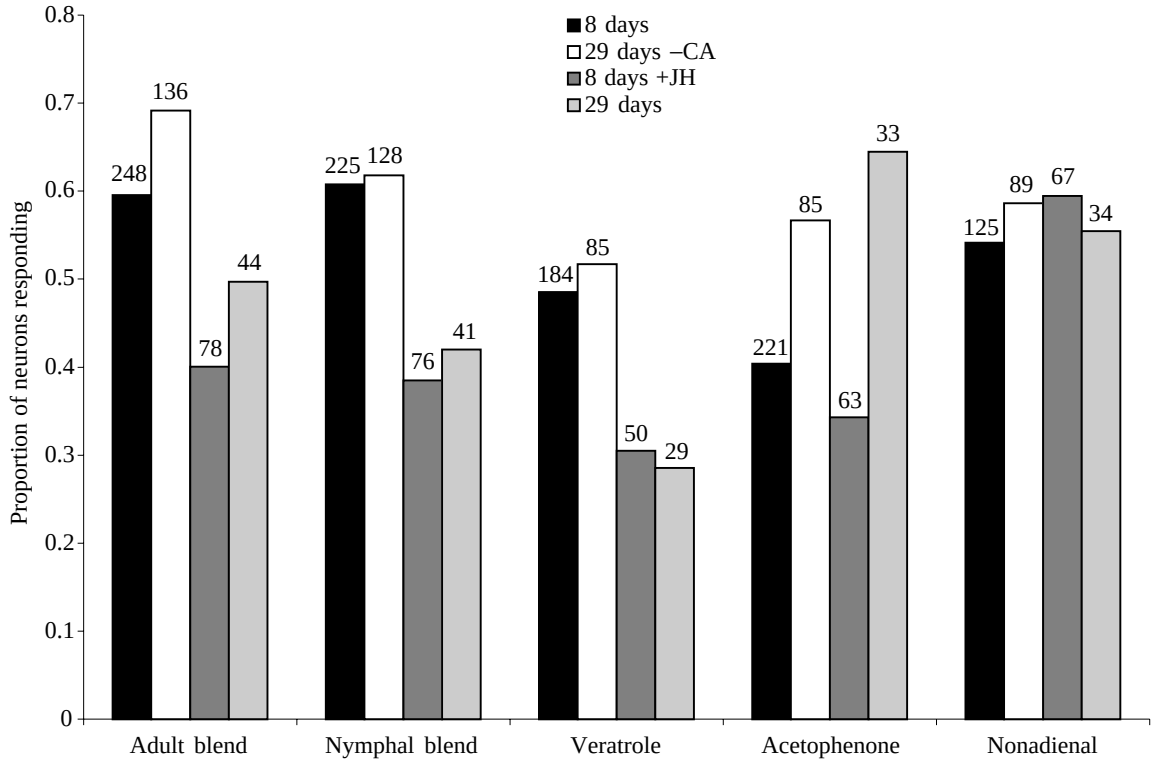

Fig. 5. Changes in response profiles of antennal lobe neurons of male and female locusts in the different experimental groups. The proportion of neurons responding to each of the components tested (see Materials and methods) is shown. Note that the proportion of neurons responding to the plant odour nonadienal does not change with treatment, while the proportion of neurons responding to aggregation pheromones (adult blend, nymph blend and veratrole) and to the egg-laying aggregation pheromone component acetophenone is generally much higher in locusts low in juvenile hormone (JH) (8-day-old control and 29-day-old allatectomized locusts) than in 8-day-old JH-injected locusts and in 29-day-old locusts. Numbers indicate the number of responding neurons tested for each experimental group.

differences and changes in behavioural activity of treated compared with untreated locusts. Furthermore, the differential ecdysteroid titre of male and female locusts may explain the observed sex-dependent differences in behavioural activity.

Age- and JH-dependent plasticity of RNs has been shown previously to regulate the behavioural responsiveness of insects (Bowen, 1991; Crnjar et al., 1990; Masson and Arnold, 1984; Seabrook et al., 1979; Sigg et al., 1997; Vetter and Vissher, 1997). Electroantennograms from the antennae of untreated, allatectomized and JH-injected locusts, however, were unable to differentiate any age- or JH-dependent plasticity of locust RNs. Although we are unable to exclude a JH-dependent effect at this level using EAGs alone, single-cell recordings from sensory neurons of young and old locusts (S. A. Ochieng', personal communication) did not reveal any age-dependent effects. However, age- and JH-dependent plasticity in the proportion of responding antennal lobe neurons was observed. These observations coincided with changes in the aggregation behaviour of both male and female locusts. Old (29-day-old) locusts, known to contain a high haemolymph JH titre (Tawfik, 1995), and locusts with an artificially increased level of JH contained a high proportion of non-responding neurons.

Thus, behavioural observations and electrophysiological data emphasise that locusts containing high levels of JH may not display a positive olfactory-guided behaviour as a result of an impairment of the central olfactory system. In contrast, locusts containing low levels of JH (8-day-old or allatectomized locusts) were behaviourally responsive and had a highly functional central olfactory system. The somewhat higher number of responding neurons and locusts in the JH-injected group compared with the 29-day-old control group, in spite of a high dose of injected JH, might be due to the following causes: either the amount of active JH reaching the effectors does not attain the same level as in 29-day-old locusts because of transport problems or degradation or there is a developmental factor involved that prevents 8-day-old locusts reaching the same low response level as 29-day-old locusts, even with high doses of JH.

Age- and JH-mediated plasticity of olfactory-guided behaviour, related to changes in the responsiveness of the central olfactory system, has been reported previously in the black cutworm *Agrotis ipsilon* (Anton and Gadenne, 1999; Gadenne et al., 1993) and was suggested to control host-seeking behaviour in female mosquitoes *Culex pipiens* (Bowen, 1991). In the black cutworm, in contrast to locusts, central olfactory neurons are dependent on JH as a 'switch' to reach a high sensitivity (Anton and Gadenne, 1999), suggesting

a differential modulation of the two systems. Whether JH acts directly on central olfactory neurons or indirectly *via* other neuroactive substances is, however, unknown. Previous studies have demonstrated age-dependent plasticity in insect brain and neuronal morphology (Fahrbach and Robinson, 1996; Gronenberg et al., 1996; Strambi et al., 1999; Winnington et al., 1996; Withers et al., 1995). Juvenile hormone has been shown to induce neuronal plasticity, including neuronal reorganisation, changes in morphology and sensitivity, synaptic plasticity and neurogenesis (for a review, see Strambi et al., 1999). An age-dependent decrease in the number of neuronal side branches and varicosities (Corfas and Dudai, 1991) and in the number of receptor synapses (Fröhlich and Meinertzhagen, 1982) and changes in the dendritic and axonal morphology (Strambi et al., 1999) may explain the observed differences in central olfactory processing. In vertebrates, olfactory-guided behaviour is believed to be influenced by steroids that may regulate synaptic transmission in central olfactory areas (Kawata, 1995; Stumpf and Sar, 1982; Swann, 1997). Juvenile hormone may, therefore, like steroids (Kawata, 1995; Wood et al., 1992), act directly *via* membrane receptors (Ilenchuk and Davey, 1985; Yamamoto et al., 1988) on central olfactory neurons. Juvenile hormone may also act indirectly *via* other neuroactive substances, e.g. serotonin, which has been shown to modulate the growth and the response of olfactory neurons in the sphingid moth *Manduca sexta* (Hildebrand, 1995; Kloppenburg and Hildebrand, 1995, Mercer et al., 1995; Kloppenburg et al., 1999). A third possible mode of action of JH might be a nuclear effect: in crickets, blocking of transcription or translation factors abolishes the effect of JH on auditory interneurons (Stout et al., 1998).

Our long-term behavioural observations and examinations of the central olfactory responsiveness of adult desert locusts indicate that JH plays an important role in mediating changes and differences in the aggregation behaviour of male and female locusts. The present study suggests that JH acts indirectly or directly at different levels of the central nervous system to regulate or modulate behavioural responsiveness and behavioural activity following exposure to aggregation pheromones.

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