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Selective ablation of antennular sensilla on the Caribbean spiny lobster *Panulirus argus* suggests that dual antennular chemosensory pathways mediate odorant activation of searching and localization of food

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Summary

In spiny lobsters and other decapod crustaceans, odorant-mediated searching behavior patterns are driven primarily by chemosensory neurons in the antennules. Two groups of antennular chemosensory neurons can be distinguished on the basis of the sensilla that they innervate and their central projections: those that innervate the aesthetasc sensilla on the lateral flagella and project into the glomerularly organized olfactory lobes, and those that innervate other (i.e. non-aesthetasc) sensilla on both lateral and medial flagella and project into the stratified and non-glomerularly organized lateral antennular neuropils. By ablating different groups of antennular sensory neurons or sensilla, we examined the role of aesthetasc and non-aesthetasc chemosensory neurons in regulating local searching behavior of Caribbean spiny lobsters, *Panulirus argus*, for food (squid) in a low-flow environment. The results show that odorant-mediated activation of searching and localization of food under these conditions requires only a subset of functional antennular chemosensory neurons, since neither aesthetasc chemosensory neurons nor non-aesthetasc chemosensory neurons are by themselves necessary for these types of behavior. However, ablation of aesthetasc chemosensory neurons together with subsets of non-aesthetasc chemosensory neurons from either the medial or lateral flagella impairs the ability of lobsters to locate the food. This reveals a large degree of functional redundancy but also some complementary functions between aesthetasc and non-aesthetasc chemosensory neurons, and hence between these dual antennular chemosensory pathways, in odorant-mediated searching behavior of lobsters under these conditions.

Key words: Crustacea, olfaction, chemoreception, chemical sense, food localization, aesthetasc, sensory, olfactory lobe, lateral antennular neuropil, *Panulirus argus*.

Introduction

The chemical senses are crucial in mediating important patterns of behavior for many animals. In crustaceans, a role for chemical senses has been demonstrated in feeding, in locating shelter and in sexual and social interactions (Gleeson, 1982, 1991; Atema, 1985; Carr, 1988; Ratchford and Eggleston, 1998, 2000; Zulandt Schneider and Moore, 1999).

Lobsters and other crustaceans possess a large variety of types of setae, including sensilla innervated by chemosensory neurons (Derby, 1982, 1989; Laverack, 1988a; Grünert and Ache, 1988; Hallberg et al., 1997). Chemosensilla are distributed over almost the entire body surface in lobsters, including the first antennae (antennules), second antennae, mouthparts, legs, cephalothorax, abdomen and telson (Derby, 1982, 1989; Derby and Atema, 1982a; Spencer, 1986; Tierney et al., 1988; Hallberg et al., 1997; Cate and Derby, 2000, 2001). However, it is the chemoreceptor neurons in the antennules, with their lateral and medial flagella, that primarily mediate long-range responses of lobsters and crayfish to odorant chemicals (McLeese, 1973; Reeder and Ache, 1980; Derby and Atema, 1982b; Devine and Atema, 1982; Giri and Dunham, 1999, 2000).

On the basis of our current knowledge, antennular chemosensory neurons can be classified into two major populations (Fig. 1). One population consists of chemosensory neurons that innervate aesthetasc sensilla on the lateral flagella (Grünert and Ache, 1988; Hallberg et al., 1997; Cate and Derby, 2001). Each aesthetasc is innervated by numerous chemoreceptor neurons (in spiny lobsters, approximately 300 neurons per sensillum). The second population consists of chemosensory neurons that innervate other antennular sensilla (which we collectively call ‘non-aesthetasc sensilla’). The non-aesthetasc chemosensilla include hooded sensilla and simple sensilla that differ from aesthetascs in that they are widely distributed over both medial and lateral flagella and are innervated by far fewer neurons (<20 per sensillum) including both chemoreceptor and mechanoreceptor neurons (Cate and

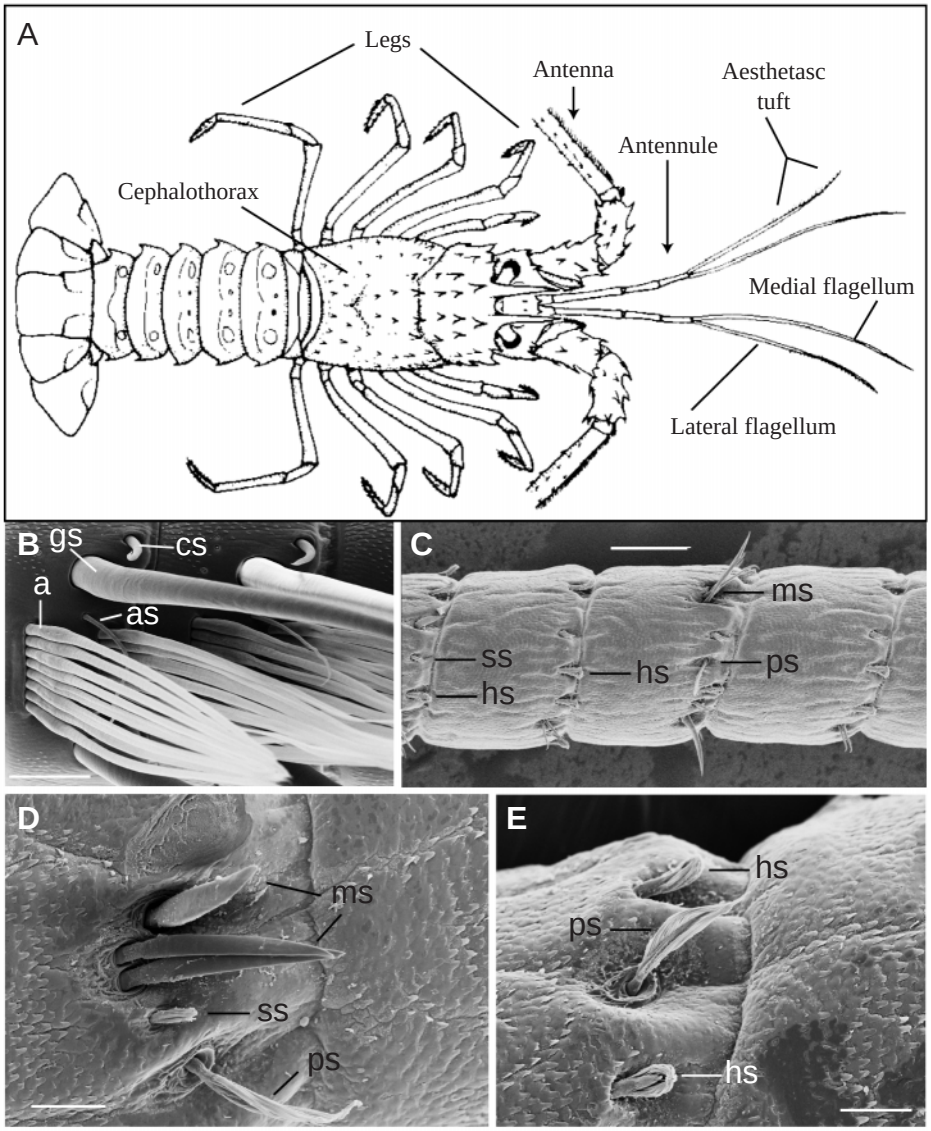

Fig. 1. An antennule and its setae of the Caribbean spiny lobster *Panulirus argus*. (A) Drawing of the spiny lobster showing the antennules with their lateral and medial flagella. The aesthetasc tuft, which contains aesthetasc sensilla and other associated setae, is located on the ventral side of the distal half of the lateral flagellum. Chemosensory neurons that innervate the setae, other than aesthetascs on the lateral and medial flagella, are collectively called 'non-aesthetasc chemosensory neurons'. (B) Scanning electron micrograph showing part of the aesthetasc tuft of the lateral antennular flagellum, with aesthetasc sensilla (a), asymmetric setae (as), guard setae (gs) and companion setae (cs). Scale bar, 100 μm. (C) Scanning electron micrograph showing the non-tuft region of the lateral flagellum. Like the medial flagellum, this region has numerous non-aesthetasc setae, including plumose (ps) and setuled setae (ss) as well as chemosensilla such as hooded sensilla (hs) and simple sensilla (ms) of three lengths (short, medium and long) (Cate and Derby, 2000, 2001). Scale bar, 200 μm. (D) High-magnification scanning electron micrograph of three medium-length simple sensilla (ms), one short setuled seta (ss) and one plumose seta (ps). Scale bar, 50 μm. (E) High-magnification scanning electron micrograph of two hooded sensilla (hs) and one plumose seta (ps). Scale bar, 50 μm.

Derby, 2000, 2001). There are many other candidate non-aesthetasc antennular chemosensilla, such as guard, companion, asymmetric and setuled setae, but the innervation of these has not yet been studied, so it is not known whether they are chemoreceptive.

Neurons that innervate aesthetasc and non-aesthetasc chemosensilla project to distinct neuropils in the brain. Aesthetasc chemoreceptors project to the olfactory lobes (OLs), which are anatomically analogous to vertebrate olfactory bulbs and insect antennal lobes (Sandeman and Denburg, 1976; Mellon and Munger, 1990; Sandeman et al., 1992; Schmidt and Ache, 1992, 1996a,b). Neurons from non-aesthetasc sensilla on the lateral and medial antennular flagella project primarily to the lateral antennular neuropils (LANs) (Schmidt et al., 1992; Schmidt and Ache, 1996a). Sensory neurons innervating non-aesthetasc sensilla on the basal segments of the antennules, as well as statocyst neurons, project to yet another neuropil – the median antennular neuropil. The organization and structure of these neuropils are clearly different. The most obvious difference is that OLs are glomerular neuropils, whereas LANs have a stratified organization.

Sensory projections to these processing centers suggest a functional distinction between the aesthetasc/OL pathway and the non-aesthetasc/LAN pathway, although their functions are poorly understood. The non-aesthetasc/LAN pathway is thought to be involved primarily in sensory-motor reflexes and movements of the antennules (Maynard, 1966; Schmidt and Ache, 1993) and may provide spatial chemical information (Devine and Atema, 1982), although there are no behavioral tests of these ideas. The aesthetasc/OL pathway is necessary for the detection of sex pheromones by male blue crabs (*Callinectes sapidus*) (Gleeson, 1982, 1991), but it is also important in processing food odorants (Schmidt and Ache, 1996b). Most other studies have focused on the roles of lateral *versus* medial flagella without exploring the roles of the aesthetasc/OL *versus* non-aesthetasc/LAN pathways in, for example, spiny lobsters *Panulirus argus* (Reeder and Ache,

1980), clawed lobsters *Homarus americanus* (McLeese, 1973; Devine and Atema, 1982) and crayfish *Procambarus clarkii* (Giri and Dunham, 1999, 2000). Selective removal of aesthetasc tuft setae (i.e. aesthetascs and their associated setae – guard, companion and asymmetric setae) (Fig. 1) significantly impaired food-odorant-mediated orientation in *H. americanus* (Devine and Atema, 1982). Aesthetasc chemosensory neurons are the most numerous of the aesthetasc tuft neurons, suggesting that aesthetascs are the main, but not necessarily the only, sensory input that mediate this type of behavior.

To examine the role of different antennular sensilla in chemosensory behavior patterns, we have conducted a series of behavioral studies using the Caribbean spiny lobster *Panulirus argus*. Various combinations of antennular sensilla were selectively removed, and the effects on food-odorant-mediated activation and short-range orientation were analyzed for lobsters in low-flow conditions.

Materials and methods

Animal maintenance and acclimation

Caribbean spiny lobsters *Panulirus argus* (55–75 mm carapace length) were collected in the Florida Keys, shipped to Georgia State University, kept under a 12 h:12 h light:dark cycle in 800 l aquaria containing aerated, recirculated, filtered artificial sea water (Instant Ocean, Aquarium Systems: Mentor, Ohio, USA) and fed squid or shrimp. Intermolt lobsters, determined by the method of Lyle and MacDonald (1983), were selected for behavioral assays on the basis of their responsiveness to a piece of squid or shrimp dropped into the aquarium. Selected lobsters were transferred to individual 80 l aquaria (60 cm long, 30 cm wide and 45 cm high) containing artificial sea water aerated with a recirculating pump, where behavioral testing was performed. A layer of crushed-coral gravel covered the bottom of the aquaria. Before testing, selected lobsters were acclimated to their new environment under a 12 h:12 h light:dark cycle for several days. During the acclimation period, lobsters were fed a few grams of squid every other day.

Ablations

Nine groups of lobsters, each with different ablations, were used in this study. These groups are listed in Table 1, together with a description of their ablation. To ablate sensilla, lobsters were immobilized by clamping them upside-down in a purpose-built seawater-filled container and subjected to the following techniques.

Distilled water ablation

Exposure to distilled water functionally ablates chemosensory neurons in lobsters and other marine crustaceans by osmotically disrupting the outer dendritic segments of the chemosensory neurons located in the permeable chemosensilla, while retaining the function of at least some of the mechanosensory neurons (Derby and Atema, 1982b; Gleeson et al., 1996) (P. Steullet, data not shown). Distilled water ablation was performed by placing the flagella of interest in a 15 ml centrifuge tube filled with distilled water for 15–30 min.

Shaving of setae

Physical removal of setae eliminates the activity of chemoreceptor neurons in them since the dendritic receptors of

Table 1. *Groups of lobsters with different ablations and a summary of their behavioral responsiveness*

			Summary of behavioral effects of ablation			
Group	Ablation type	Method of ablation	1	2	3	4
C	Sham control	No ablation; animal restrained as for other groups; sea water on medial and lateral flagella				
1	Removal of chemoreceptors on the medial and lateral flagella	Distilled water on the medial and lateral flagella	↓*	↑*	↑	↓
2	Removal of chemoreceptors on the medial flagella	Distilled water on the medial flagella				
3	Removal of chemoreceptors on the lateral flagella	Distilled water on the lateral flagella	↓		↑ (*)	↓
4	Removal of aesthetascs and asymmetric setae	Shaving of aesthetascs and asymmetric setae	↓			
5	Removal of aesthetasc tuft setae (aesthetascs, asymmetric, companion and guard setae)	Shaving of aesthetascs, asymmetric, companion and guard setae	↓	↑	↑*	↓
6	Removal of companion and guard setae	Shaving of companion and guard setae				
7	Removal of aesthetascs and asymmetric setae and chemoreceptors on the medial flagella	Shaving of aesthetascs and asymmetric setae; distilled water on the medial flagella	↓		↑*	↓
8	Removal of tuft-region setae and chemoreceptors on the medial flagella	Shaving of aesthetascs, asymmetric, companion and guard setae; distilled water on the medial flagella	↓*	↑*	↑ (*)	↓

Key for types of behavior: (1) success in finding food; (2) activation time; (3) search duration; (4) search mode.

↓*, statistically significant decrease (P<0.05); ↑*, statistically significant increase (P<0.05); ↓, strong trend for decrease; ↑, strong trend for increase.

(*) indicates that the effect was close to being significant (0.1>P>0.05).

all known chemoreceptor neurons are located in the setal shaft. Shaving was performed under a compound microscope using a hand-crafted tool whose tip was a piece of carbon steel blade 0.2 mm wide.

After completion of the behavioral assays, the ablated antennules were observed under a compound microscope to assess the quality of the ablation treatments. The results of this analysis show that ablations were highly effective in removing more than 96 % of the setae of interest, while keeping intact more than 94 % of the neighboring setae (Table 2). An example of an aesthetasc-shaved antennule (group 4) is shown in Fig. 2.

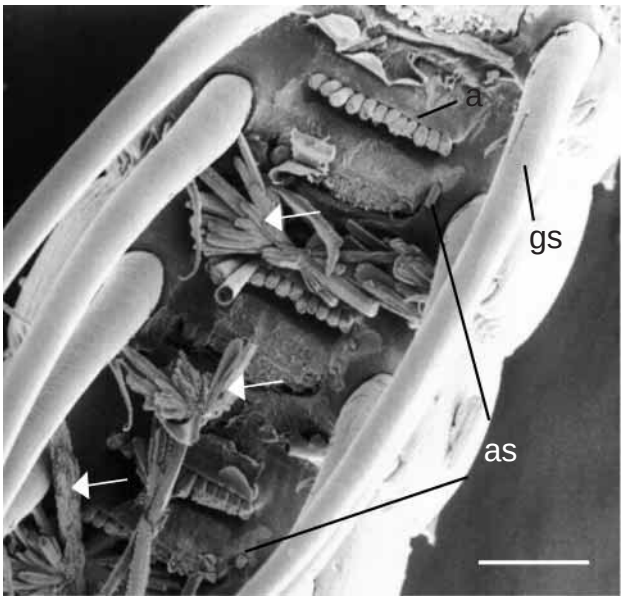

Fig. 2. Scanning electron micrograph showing part of the aesthetasc tuft on the lateral antennular flagellum of group 4 animals; i.e. after only the aesthetascs (a) and asymmetric setae (as) had been ablated by shaving, without affecting the guard setae (gs) or any other setae. Pieces of the shafts of cut aesthetascs and other debris are indicated by arrows. Scale bar, 150 μm.

Behavioral experiments

Odorant-mediated activation and search behavior

The aim of these experiments was to identify the effects of various types of ablation of antennular chemosensory neurons on the ability of resting lobsters to be aroused by chemicals released from a piece of squid and to locate the squid. Behavioral assays were performed during the dark phase of a 12 h:12 h light:dark cycle under dim red light. The recirculating pump in the aquarium was stopped 1 h prior to the trials. The assay consisted of (i) a control (pre-ablation) phase to test the responsiveness of the lobsters and to select only responsive lobsters for a subsequent ablation, and (ii) a test (post-ablation) phase to examine the effect of specific ablations on behavior. Each lobster was videotaped during each phase, and quantitative analyses were made from the recordings.

During the control phase, a trial was begun after a lobster had been inactive for at least 15 min. A piece of squid (2 cm^2) was then attached to a string and introduced into the aquarium at the opposite end from the lobster. Lobsters were then allowed 10 min to find the squid. The squid was rapidly pulled out of the aquarium if the lobster located and grabbed it. Only animals that located and grabbed the squid within 10 min for two consecutive days in the control phase were selected for the ablation treatments and subsequently tested. For each trial, three variables were quantified from the videotapes: (i) activation time, which is the time between the introduction of the squid and the initiation of searching behavior by the lobster and measures the responsiveness of animals to odorants; (ii) search duration, which is the time that an activated lobster needed to locate the squid and measures search efficiency; and (iii) search mode, which is the proportion of time that active lobsters spend walking after initiating the search and describes the dynamics of the search (constant walking *versus* walking interrupted by frequent stops).

Following ablation in the test phase, selected lobsters were tested in two trials within 36 h. During each trial, a piece of squid was introduced as described above. Each lobster was allowed 10 min to become active and to initiate a search and another 10 min after search initiation to locate and grab the squid. At the end of the trial, the squid was brought in contact with the legs of any lobsters that did not become activated or did not find the squid. Lobsters that did not subsequently grab the squid were considered to be unmotivated and eliminated from the study.

Behavioral sequences were analyzed ‘blind’ by an observer unaware of the ablation treatment. The frequency of success in finding food was compared among all groups of lobsters using

Table 2. *Evaluation of the efficacy of setal removal by shaving*

Ablation type	Percentage of aesthetascs remaining after shaving	Percentage of companion setae remaining after shaving	Percentage of guard setae remaining after shaving
Group 4: removal of aesthetascs (*N*=30)	0.3±0.05	98.9±0.46	94.3±1.66
Group 5: removal of aesthetasc tuft setae (*N*=20)	0.1±0.02	2.3±0.49	0.2±0.07
Group 6: removal of companion and guard setae (*N*=14)	97.7±0.88	3.5±0.67	0.9±0.19

N, number of antennules.
Values are means ± S.E.M.

a *G*-test. If success in finding food differed statistically among all groups, eight planned comparisons between the control sham lobsters (group C) and each of the eight groups of ablated lobsters (groups 1–8) were performed using the *G*-test at a 5 % experiment-wise error rate, as determined by a sequential test using the Dunn–Šidák method (Sokal and Rohlf, 1997). For each of the three types of behavior (activation time, search duration, search mode), the effect of each ablation was statistically evaluated using a median test including all test responses of all ablation treatments (Statistica, StatSoft Inc., Tulsa, OK, USA). If an overall significant effect of ablation treatment was found, eight planned pair-wise comparisons between the group of control sham lobsters (group C) and each group of ablated lobsters (groups 1–8) were performed for each day of the test phase using a two-sample randomization test for the median difference as a statistic (Manly, 1991; Sokal and Rohlf, 1997). For each pair-wise comparison, the *P* values obtained for the two days of the test phase were combined to determine an overall *P* value, as described by Sokal and Rohlf (1997). Two groups were considered statistically different from each other if the overall *P* value obtained was equal to or lower than the critical values for a 5 % experiment-wise error rate, as determined by a sequential test using the Dunn–Šidák method based on eight planned comparisons (Sokal and Rohlf, 1997).

Concentration-dependent activation of searching

Since the effects of ablations may be apparent only at lower odorant concentrations, we examined odorant activation using a series of concentrations of artificial oyster odor for animals before and after ablation of aesthetascs and asymmetric setae. Lobsters were isolated and acclimated for a few days in aquaria similar to those described above. Behavioral assays were performed during the dark phase of a 12 h:12 h light:dark cycle under dim red light. Different concentrations of an artificial oyster odor [for composition, see Carr and Derby (1986)] and sea water were presented to the antennules of a resting lobster. Stimulation consisted of delivering 5 ml of odorant or sea water within 2–3 s using a hand-held glass pipette positioned approximately 5 cm away from the antennules. The duration of search behavior over the 3 min following stimulation was quantified. Search responses consisted of lateral or forward movement of the lobster. Each stimulus was presented daily in random order for three consecutive days. The aesthetascs and asymmetric setae of responding lobsters were then shaved (as described previously for group 4). After two days of post-surgical recovery, lobsters were retested daily with the same set of stimuli for three consecutive days. For each day, the response to sea water was subtracted from the responses to each odorant concentration. For each lobster, a mean corrected response to each odorant concentration before (three trials) and after (three trials) ablation was calculated. These corrected responses to each odorant concentration before and after ablation were compared using one-way within-subjects analysis of variance (ANOVA) with multiple dependent measures (MANOVA; Statistica, StatSoft).

Results

'Normal' behavior (control lobsters)

Normal animals (i.e. all animals in the control phase) that initiated searches had activation times (i.e. the time between the introduction of food and initiation of searching) of 187±8 s (mean ± S.E.M., N=177). Just prior to searching, lobsters typically increased the frequency of antennular flicking and then swept their antennules vertically. The relatively long activation times were due to the low-flow conditions in our study. This resulted in a long delay between the introduction of food and the food odorant reaching the animals, as revealed by dye studies (data not shown). This delay occurred despite currents generated by the lobsters, which moved the odorants towards the animal (data not shown) (see also Atema, 1985; Breithaupt and Ayers, 1998; Breithaupt, 2001).

Activated lobsters had search durations (i.e. the time between search initiation and grabbing the food) of 65±6 s (mean ± S.E.M., N=177). Many activated lobsters moved directly to the squid, but others followed the aquarium walls before reaching the food. Because of the relatively small size of the aquarium, searching lobsters had a high probability of eventually encountering the squid even if their orientation path was not direct.

Activated lobsters had search modes in which they spent 82±2 % of their search time walking (mean ± S.E.M., N=177), occasionally and momentarily interrupting their search with short stops. During searching, the lobsters usually kept their antennules just above the gravel substratum in a relatively horizontal position and flicked their antennules only occasionally, particularly during stops.

The values for activation time and search duration in the control phase did not differ significantly among the various groups of lobsters (median test, P>0.05). Furthermore, the responses of lobsters on the first and second days of pre-ablation testing were not significantly different (Wilcoxon matched-pairs test, P>0.05).

Effects of ablations on odorant activation and searching for food

Success in finding the squid

Success in finding squid was affected by several ablation treatments. In the test phase, intact lobsters (sham control, group C) located the food in 77 % of all trials. Overall, ablated animals (groups 1–8) were less successful (*G*-test, P<0.0001), with the trend that greater degrees of ablation resulted in lower success (Fig. 3). Two groups of lobsters with extensive ablations, group 1 (removal of chemoreceptors on the medial and lateral flagella) and group 8 (removal of aesthetasc tuft setae and chemoreceptors on the medial flagella), had significantly lower success in finding food than did sham control lobsters (Fig. 3). In contrast, the two groups with the least extensive ablations in terms of the number of affected chemoreceptor neurons, group 2 (removal of chemoreceptors on the medial flagella) and group 6 (removal of companion and guard setae), were unaffected. Other groups, including group

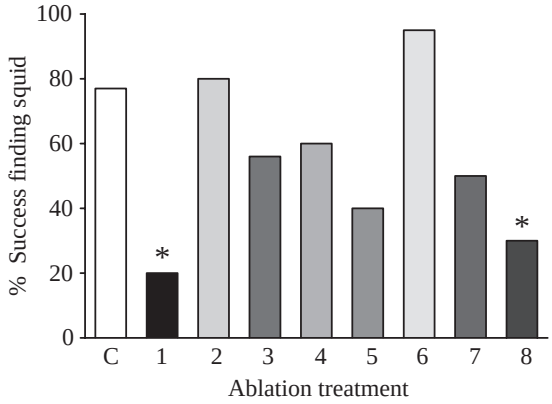

Fig. 3. Success in finding food (the percentage of lobsters that successfully found a piece of squid) for sham control animals (group C) and eight groups of animals (groups 1–8) with specific sensory ablations of the antennules (see Table 1 for a description of the groups). An asterisk indicates that the success of a group of ablated lobsters is significantly different from that of the group of sham control lobsters (*G*-test; *P* lower than the critical value for a 5% experiment-wise error rate as determined by a sequential test using the Dunn–Šidák method for eight planned comparisons) (Sokal and Rohlf, 1997). The number of trials was 20 (two trials for each of 10 lobsters) for all groups except group C (22 trials, 11 lobsters) and group 3 (18 trials, 10 lobsters).

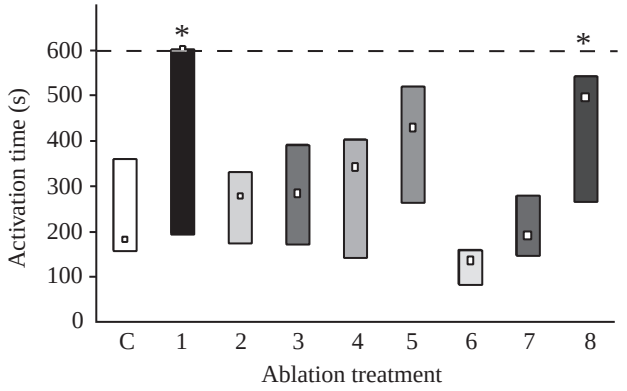

Fig. 4. Activation times (the latency for initiating searching) for sham control animals (group C) and eight groups of animals with specific sensory ablations of the antennules (groups 1–8). The groups are defined in Table 1 and are the same as in Fig. 3. An asterisk indicates that the activation time for a group of ablated lobsters is significantly different from that for the sham control lobsters (two-sample randomization test for median difference as the statistic; *P* lower than the critical value for a 5% experiment-wise error rate as determined by a sequential test using the Dunn–Šidák method for eight planned comparisons) (Sokal and Rohlf, 1997). The horizontal dashed line indicates the maximum time (10 min) allowed for each lobster to become activated. For each group of lobsters, the median (□) and upper and lower quartiles are shown. Each group of ablated lobsters represents 10 animals, and the group of sham control lobsters contains 11 animals. This graph was prepared using the mean activation time from the two trials for each animal.

3 (removal of chemoreceptors on the lateral flagella), group 4 (removal of aesthetascs and asymmetric setae), group 5 (removal of aesthetasc tuft setae) and group 7 (removal of aesthetascs and asymmetric setae and chemoreceptors on the medial flagella), had lower success values than the sham control group, but these effects were not statistically significant (Fig. 3). Overall, this indicates that the ability of lobsters to find and grab the squid does not depend solely on the sensory neurons from either the medial flagella or the lateral flagella, but rather involves the aesthetasc and non-aesthetasc chemosensory neurons of both flagella.

Success in finding the squid was the result of two temporally distinct types of behavior: odorant-mediated activation leading to the initiation of searching, and locating the squid during the search. As presented in the next two sections, the various ablations of the sensory system of the antennules differentially affected these two types of behavior.

Activation time

Activation time (i.e. the latency for initiation of searches) was significantly affected by ablations (median test, P=0.0063) (Fig. 4). There was a trend for longer activation times as progressively more antennular setae were ablated. For each group of lobsters, the activation time did not differ between the first and second days of testing (sign test, P>0.05). Planned comparisons showed that the two groups with the most extensive ablations, group 1 (removal of chemoreceptors on the medial and lateral flagella) and group 8 (removal of aesthetasc tuft setae and chemoreceptors on the medial flagella), had significantly longer activation times than group C (sham control) (Fig. 4). In fact, animals in group 1 did not even become activated in 15 out of 20 trials. Group 2 (removal of chemoreceptors on the medial flagella) and group 3 (removal of chemoreceptors on the lateral flagella) had median activation times that were 1.5 times higher than those of sham control animals, but these effects were not statistically significant (Fig. 4). Group 4 (removal of aesthetascs and asymmetric setae) and group 5 (removal of aesthetasc tuft setae) had values 2–2.5 times higher than those of sham controls, but these values were not statistically significant. Finally, the activation times of group 6 (removal of companion and guard setae) and group 7 (removal of aesthetascs and asymmetric setae and chemoreceptors on the medial flagella) were similar to those of the sham controls.

Search duration

The overall effect of ablation on search duration (i.e. the time for activated lobsters to locate the squid) was significant (median test, P=0.0023) (Fig. 5). For each group of lobsters, the search duration did not differ between the first and second days of testing (sign test, P>0.05). Median search durations for lobsters with ablation of aesthetascs and different subsets of non-aesthetasc setae on either the medial or the lateral flagella (groups 3, 5, 7, 8) were 6–8 times longer than the search duration in the sham control lobsters (group C) (Fig. 5). However, because of the high variability in search durations among treated lobsters and the small number of active lobsters

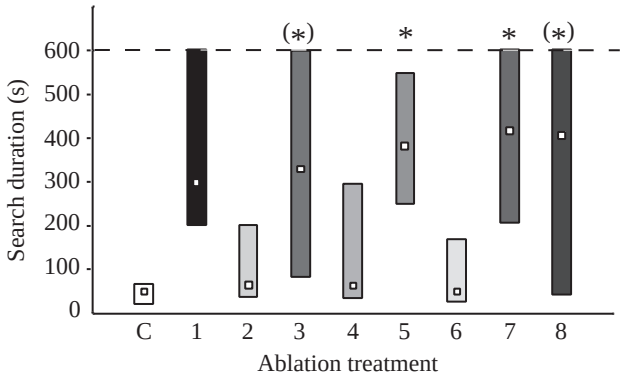

Fig. 5. Search duration (the time to find squid following activation) for sham control animals (group C) and eight groups of animals with specific sensory ablations of the antennules (groups 1–8). The groups are defined in Table 1 and are the same as in Fig. 3. An asterisk indicates that the search duration of the group of ablated lobsters is significantly different from that of the group of sham control lobsters (two-sample randomization test for median difference as the statistic; *P* lower than the critical value for a 5% experiment-wise error rate as determined by a sequential test using the Dunn–Šidák method for eight planned comparisons) (Sokal and Rohlf, 1997). An asterisk in parentheses indicates that the search duration of the group of ablated lobsters is close to being significantly different from that of the group of sham control lobsters (two-sample randomization test for median difference as the statistic; *P* values for group 8 and group 3 correspond respectively to 5.5% and 7.6% experiment-wise error rate as determined by a sequential test using the Dunn–Šidák method for eight planned comparisons) (Sokal and Rohlf, 1997). The horizontal dashed line indicates the maximum time (10 min) given to each activated lobster to find squid. For each group of lobsters, the median (□) and upper and lower quartiles are shown. The number of activated lobsters in groups C and 1–8 is 10, 3, 9, 8, 10, 7, 10, 10 and 9, respectively. The mean search durations were calculated only from those trials for which animals were activated to search.

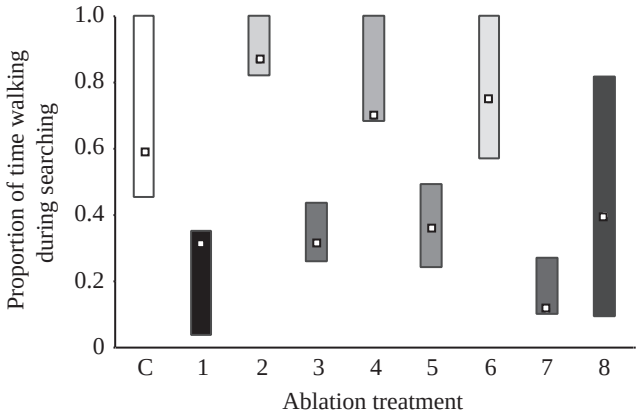

Fig. 6. Search mode (the proportion of time spent walking during searching) for sham control animals (group C) and eight groups of animals with specific sensory ablations of the antennules (groups 1–8). The groups are defined in Table 1 and are the same as in Fig. 3. For each group of lobsters, the median (□) and upper and lower quartiles are shown. The numbers of activated lobsters in groups C and 1–8 are the same as in Fig. 5. The values were calculated only from those trials for which animals were activated to search.

in most groups, only group 5 (removal of aesthetasc tuft setae) and group 7 (removal of aesthetascs and asymmetric setae and chemoreceptors on the medial flagella) took significantly longer to find squid than did sham control lobsters (Fig. 5). The search durations for group 3 (removal of chemoreceptors on the lateral flagella) and group 8 (removal of aesthetasc tuft setae and chemoreceptors on the medial flagella) were very close to being significantly different from that for sham control lobsters (Fig. 5). Median search duration for group 1 (removal of chemoreceptors on the medial and lateral flagella) was six times longer than that for sham control lobsters (Fig. 5), but this difference was not significant because of the small number of activated lobsters in group 1. In contrast, lobsters in group 4 (removal of aesthetascs and asymmetric setae), group 6 (removal of companion and guard setae) and group 2 (removal of chemoreceptors on the medial flagella) found squid within a similar time to that of sham controls (Fig. 5).

Search mode

The proportion of time that active lobsters spent walking after initiating a search represents the dynamics of searching behavior. For example, some animals walked steadily during the search, and others repeatedly started and stopped. The proportion of time spent walking during the search was significantly affected by the ablation treatments (median test, P=0.0025) (Fig. 6). Lobsters in groups 3, 5, 7 and 8 (removal of aesthetascs and different subsets of non-aesthetasc setae) tended to search with hesitation and stopped more often and for longer (1.5–5 times) than the sham control lobsters (group C) (Fig. 6). Many of the lobsters in these groups even stopped a few centimeters away from the squid and failed to grab it. Moreover, the few lobsters in group 1 (removal of chemoreceptors on the medial and lateral flagella) that were activated also tended to stop twice as often as control sham lobsters (Fig. 6). However, despite the strong and significant effect of ablation treatments on searching behavior, no treated groups were significantly different from the sham controls (two-sample randomization test, P>0.05) because of the rather high variability in searching behavior and the small number of active lobsters in most of the groups. Although the results suggest a trend that groups 3, 5, 7 and 8 hesitated more during searching than sham control lobsters, the results also show that group 2 lobsters (without chemoreceptors on the medial flagella), group 4 lobsters (without aesthetascs and asymmetric setae) and group 6 lobsters (without companion setae and guard setae) did not search with more hesitation and stops than sham control lobsters (Fig. 6).

Concentration-dependent effects of aesthetasc and asymmetric seta ablation

To determine whether the effects of aesthetasc ablations were dependent on odorant concentration, we examined the search response (i.e. the time spent walking) evoked by a series of concentrations of artificial oyster odor prior to and after

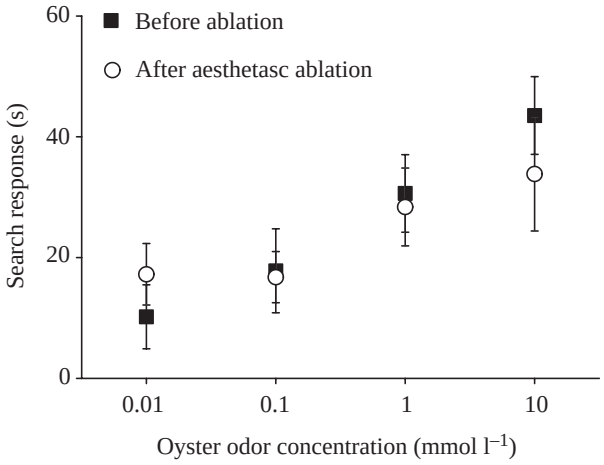

Fig. 7. Effects of the removal of aesthetascs and asymmetric setae on the search responses (the time spent walking) over 3 min following stimulation of the antennules with a series of concentrations of artificial oyster odor. The responses before (filled squares) and after (open circles) shaving the aesthetascs and asymmetric setae are not significantly different (one-way MANOVA, $P>0.05$). Values are means ± S.E.M. from seven animals (three trials per animal).

removal of the aesthetascs and asymmetric setae (group 4). Animals had concentration-dependent responses before and after ablation, and the ablation did not significantly change the search response to any of the oyster odor concentrations (one-way MANOVA, $P>0.05$) (Fig. 7). This suggests that the threshold and concentration-dependence of this behavioral response to odorants were not significantly affected by the absence of aesthetasc chemosensory neurons.

Discussion

Our results show that ablation of selected sets of antennular sensory neurons affects the behavioral responses of lobsters to food odorants in low-flow conditions. Non-aesthetasc sensory neurons in both the lateral and medial antennular flagella and aesthetasc chemosensory neurons in the lateral flagella contribute to odor-mediated activation of searching behavior and short-range localization of food. A high degree of functional redundancy between these two sets of chemoreceptors exists, although some complementary functions are also apparent.

Either aesthetasc or non-aesthetasc chemosensory neurons can mediate odorant activation of searching behavior

Our experiments show that activation of searching behavior by food odorants occurred in animals with only a subset of antennular chemoreceptor neurons (Fig. 4). As greater proportions of sensilla or chemosensory neurons on the antennular flagella were ablated, activation times (the time between food presentation and initiation of searching) tended to increase progressively (Fig. 4). While both aesthetasc and non-aesthetasc chemosensory neurons contribute to odorant-mediated activation, neither was necessary. Removal of the aesthetascs and asymmetric setae (group 4) did not significantly affect either the activation time (although it did double the time compared with controls) (Fig. 4) or the intensity of the search response at any odor concentration tested (Fig. 7). These results are noteworthy because ablation of aesthetascs removes over 800 000 chemoreceptor neurons, which is by far the greatest number of neurons innervating any single type of sensillum in the antennules (Cate and Derby, 2001). In addition, other experiments have shown that lobsters with ablation of all antennular flagellar setae except for aesthetascs and asymmetric setae were still able to learn associative olfactory tasks and to discriminate between highly related odorant mixtures (Steullet et al., 2000).

While both aesthetasc and non-aesthetasc chemosensory neurons on the antennules clearly play a primary role in odorant-mediated activation of searching, receptors on other body regions may also play a minor role. Although most animals with ablation of chemoreceptors of both antennular flagella (group 1) did not become activated, some animals did become active (Fig. 4) and even succeeded in finding the food (Fig. 3). One possible explanation is that chemo- and mechanosensilla on the three basal segments of the antennules and/or other body regions such as antennae II, legs, cephalothorax, abdomen and telson (Cate and Derby, 2000, 2001) mediate these responses. This idea is consistent with observations on food-odorant-mediated activation in *Homarus americanus*, which occurs after ablation of all antennular flagella, albeit following a delay (McLeese, 1973).

We speculate that odorant-mediated activation occurs when the sum of the inputs from aesthetasc and non-aesthetasc antennular chemosensors, and possibly chemosensors from other body parts, reaches a threshold in the central nervous system sufficient to produce a behavioral response. Support for this idea in other animals comes from studies of *Drosophila melanogaster*, in which the intensity of odorant-mediated responses depends on the sum of olfactory information reaching the brain through either the antennae or palps (Charro and Alcorta, 1994). Because lobsters have millions of chemosensory neurons in their cephalic and thoracic appendages (Derby and Atema, 1988; Laverack, 1988a,b; Derby, 1989; Derby and Steullet, 2001), even a small fraction of intact chemosensory neurons may still constitute a considerable number of sensors, sufficient to initiate search activation. Our results suggest that chemosensory information to the olfactory lobes (which receive aesthetasc input), the lateral antennular neuropils (which receive non-aesthetasc input) (Schmidt and Ache, 1992, 1996a,b; Schmidt et al., 1992) and possibly other neuropils, such as the antenna II neuropils and the tegumentary neuropils (which receive sensory inputs from the antennae II and the carapace, respectively) (Schmidt and Ache 1996a), might eventually converge onto the same integrative units that control odorant-mediated activation. This might occur in the terminal medullae (Derby and Blaustein, 1988; Mellon and Alones, 1997; Mellon, 2000) or in other neuropils in the brain proper (Sandeman et al., 1992) or might even involve direct cross-talk between the olfactory lobes and

the lateral antennular neuropils (Arbas et al., 1988; Mellon and Alones, 1994).

Both aesthetasc and non-aesthetasc chemosensory neurons contribute to the ability to locate an odorant source

Ablation of antennular chemosensory neurons affects the ability of activated lobsters to find an odorant source, but no single antennular chemosensory pathway is solely responsible for mediating this type of behavior. The present study confirms results from previous work showing that sensory neurons in the antennular lateral flagella of lobsters play a central role in locating an odorant source, while sensory neurons in the medial antennular flagella are not necessary and play only a minor role (McLeese, 1973; Reeder and Ache, 1980; Devine and Atema, 1982). Impairment of the ability of *H. americanus* to locate an odorant source when aesthetasc tuft setae were shaved suggested to Devine and Atema (1982) that aesthetascs are the main, but not the only sensory input, used in orientation. Our study on *P. argus* also shows that ablation of all chemosensory neurons on the lateral flagella, or ablation of the aesthetasc tuft setae alone, impairs the ability of lobsters to locate an odorant source.

Our results further show, however, that aesthetascs alone are not necessary and that non-aesthetasc chemosensory neurons are sufficient for lobsters to locate food, at least under the conditions used in our experiments, because spiny lobsters were able to locate food following aesthetasc ablation (Figs 3, 5). The removal of aesthetascs affects the ability of activated lobsters to find food only when their removal is combined with removal of a subset of non-aesthetasc chemosensory neurons. Conversely, removal of a subset of non-aesthetasc sensory neurons only impairs the ability of activated lobsters to find food when combined with removal of aesthetascs. Lobsters without aesthetascs and at least some non-aesthetasc sensors often hesitated during their search, sometimes stopping very close to the squid without finding it. Such occasional failure to find the squid did not depend on the motivational state of the animal but rather on a lack of appropriate sensory information, since animals that did not find the food grabbed and ate it when it was brought into contact with their legs.

These results demonstrate the involvement of non-aesthetasc chemosensory neurons in locating a food source under the conditions used in our experiments. Bimodal (i.e. chemo- and mechano-) sensors such as hooded sensilla and simple sensilla (Cate and Derby, 2000, 2001) may provide much of the non-aesthetasc input for locating a food source. In addition, guard and companion setae may contribute, as suggested by the longer search durations (Fig. 5) and a change in search mode (Fig. 6) in animals lacking aesthetasc tuft setae (aesthetascs and asymmetric, guard and companion setae; group 5) compared with animals lacking only aesthetascs and asymmetric setae (group 4). The innervation of guard and companion setae has not been studied either anatomically or physiologically, but our behavioral data suggest that their input and the input from other non-aesthetasc sensors may provide information about either the odorants themselves and/or about the fluid dynamics of the odorant environment. This information might be used by lobsters and other crustaceans to orient within an odorant plume (Atema, 1996; Weissburg, 1997, 2000; Moore and Grills, 1999; Horner et al., 2000; Derby et al., 2001).

These results, together with preliminary studies under faster flow conditions ($5\,cm\,s^{-1}$) in a large flume with the odorant released 2 m upstream of the lobster (Horner et al., 2000; Derby et al., 2001), support the notion that aesthetasc and non-aesthetasc chemosensory inputs, and hence the two antennular chemosensory pathways (aesthetasc/olfactory lobe pathway and non-aesthetasc/lateral antennular neuropil pathway), provide overlapping but also complementary information important for the efficient localization of an odorant source. Our results further suggest that a glomerularly organized chemosensory neuropil (the olfactory lobe) is not essential for processing chemical signals that lead to activation of searching and localization of an odorant source. Similarly, a normal glomerular organization of the antennal lobes in the moth *Manduca sexta* is not necessary to initiate or sustain odorant-modulated flight (Willis et al., 1995).

Why have multiple antennular chemosensory systems?

Our study indicates that aesthetasc and non-aesthetasc sensilla overlap in function, so why have this diversity of sensillar types and in particular two distinct antennular chemosensory pathways? One possible explanation is that a diversity of detectors and parallel sensory pathways may help lobsters efficiently detect, discriminate and locate a variety of odor types, including those from food, conspecifics and mates. For example, detection of pheromones by spiny lobsters (Ratchford and Eggleston, 1998, 2000) might be mediated uniquely by the aesthetasc system, as proposed for blue crabs (Gleeson, 1982, 1991). Second, a diversity of receptors may provide information that enables lobsters to locate odor sources in a variety of environments with different flow conditions. For example, spiny lobsters are able to locate odorant sources in both turbulent (Reeder and Ache, 1980; Horner et al., 2000; Derby et al., 2001) and low-flow (present study) conditions, which represent the diversity of flow conditions in their natural environment, from grass beds, to crevices in rock and coral, and open sandy areas (Cobb and Phillips, 1980; Denny, 1988; Finelli et al., 1999). Since odorant access to chemosensory neurons depends on the morphology and organization of setae on the appendages (Moore et al., 1991; Mead et al., 1999) and on the fluid dynamics, lobsters may rely on different subsets of setae and on their mechano- and chemosensors to provide the information about flow conditions and odor distribution that is used in orientation. Third, non-aesthetasc sensilla on the antennular flagella, which are innervated by both chemo- and mechanosensory neurons (Cate and Derby, 2000, 2001), might provide a coupling between mechanical and chemical signals that is represented in the lateral antennular neuropils as an overlapping chemo-mechanical spatial map. However, information processed by the olfactory lobes, which receive input from the aesthetasc chemosensory neurons, is not *a priori*

designed to provide such tight coupling between mechanical and chemical signals because aesthetascs do not also contain mechanoreceptors.

In summary, the present study provides evidence that both aesthetasc and non-aesthetasc chemosensory neurons function in odorant-mediated activation of searching behavior and in localization of a food odorant source and that information from both antennular chemosensory pathways is integrated and used to respond appropriately to odorants. Further studies are needed to assess the degree of redundancy and functional overlap of aesthetasc and non-aesthetasc chemosensory neurons, and hence, between the two antennular chemosensory pathways, in other behavioral contexts, such as the detection of pheromones and odor discrimination.

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