

# Dual antennular chemosensory pathways can mediate orientation by Caribbean spiny lobsters in naturalistic flow conditions

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## Summary

Benthic crustaceans rely on chemical stimuli to mediate a diversity of behaviors ranging from food localization and predator avoidance to den selection, conspecific interactions and grooming. To accomplish these tasks, Caribbean spiny lobsters (*Panulirus argus*) rely on a complex chemosensory system that is organized into two parallel chemosensory pathways originating in diverse populations of antennular sensilla and projecting to distinct neuropils within the brain. Chemosensory neurons associated with aesthetasc sensilla project to the glomerular olfactory lobes (the aesthetasc pathway), whereas those associated with non-aesthetasc sensilla project to the stratified lateral antennular neuropils and the unstructured median antennular neuropil (the non-aesthetasc pathway). Although the pathways differ anatomically, unique roles for each in odor-mediated behaviors have not been established. This study investigates the importance of each pathway for

orientation by determining whether aesthetasc or non-aesthetasc sensilla are necessary and sufficient for a lobster to locate the source of a 2 m-distant food odor stimulus in a 5000-liter seawater flume under controlled flow conditions. To assess the importance of each pathway for this task, we selectively ablated specific populations of sensilla on the antennular flagella and compared the searching behavior of ablated animals to that of intact controls. Our results show that either the aesthetasc or the non-aesthetasc pathway alone is sufficient to mediate the behavior and that neither pathway alone is necessary. Under the current experimental conditions, there appears to be a high degree of functional overlap between the pathways for food localization behavior.

Key words: olfaction, Crustacea, aesthetasc, odor, flume, *Panulirus argus*.

## Introduction

The ability to detect and locate the source of a distant chemical stimulus is an essential process in the lives of benthic crustaceans. Decapod crustaceans, including Achelata (spiny lobsters), Homarida (clawed lobsters), Astacida (crayfish) and Brachyura (crabs), rely on chemical signals to drive a diversity of behaviors ranging from conspecific interactions (Atema, 1995; Karavanich and Atema, 1998; Giri and Dunham, 1999, 2000; Gleeson, 1982, 1991) and predator avoidance (Berger and Butler, 2001) to den selection (Berger and Butler, 2001; Ratchford and Eggleston, 1998, 2000; Nevitt et al., 2000), grooming behaviors (Barbato and Daniel, 1997; Daniel et al., 2001) and food detection and localization (Kanciruk, 1980; Reeder and Ache, 1980; Devine and Atema, 1982; Giri and Dunham, 1999; Dunham et al., 1997; Keller et al., 2003).

Chemical stimuli are detected by a multitude of chemoreceptive structures on crustaceans. Although chemoreceptive sensilla can be found on virtually all body surfaces, they are most concentrated on the appendages, particularly the antennules, antennae, dactyls and mouthparts (Ache and Macmillan, 1980; Derby, 1982; Schmidt, 1989;

Schmidt and Gnatzy, 1984; Cate and Derby, 2001, 2002a; Garm et al., 2003). The antennules in particular have long been considered to be the primary chemoreceptive organ of the spiny lobster (Fig. 1). Each antennule is composed of four segments, the most distal of which bifurcates into a lateral flagellum and a medial flagellum. Each flagellum is composed of annuli that bear a complement of chemo- and mechanosensory sensilla that vary in morphology, distribution and pattern of innervation. Many studies have shown that the antennules are important for distance chemoreception in lobsters (Reeder and Ache, 1980; Devine and Atema, 1982) and other decapod crustaceans (Hazlett, 1971a; Kraus-Epley and Moore, 2002); however, it is not clear which populations of antennular sensilla are involved in this behavior.

Chemosensory information from the antennular sensilla is transmitted to the central nervous system in two parallel pathways: the aesthetasc/olfactory lobe pathway and the non-aesthetasc/lateral antennular neuropil pathway (Schmidt and Ache, 1992, 1996a,b; Schmidt et al., 1992). The aesthetasc/olfactory lobe pathway originates in clusters of olfactory

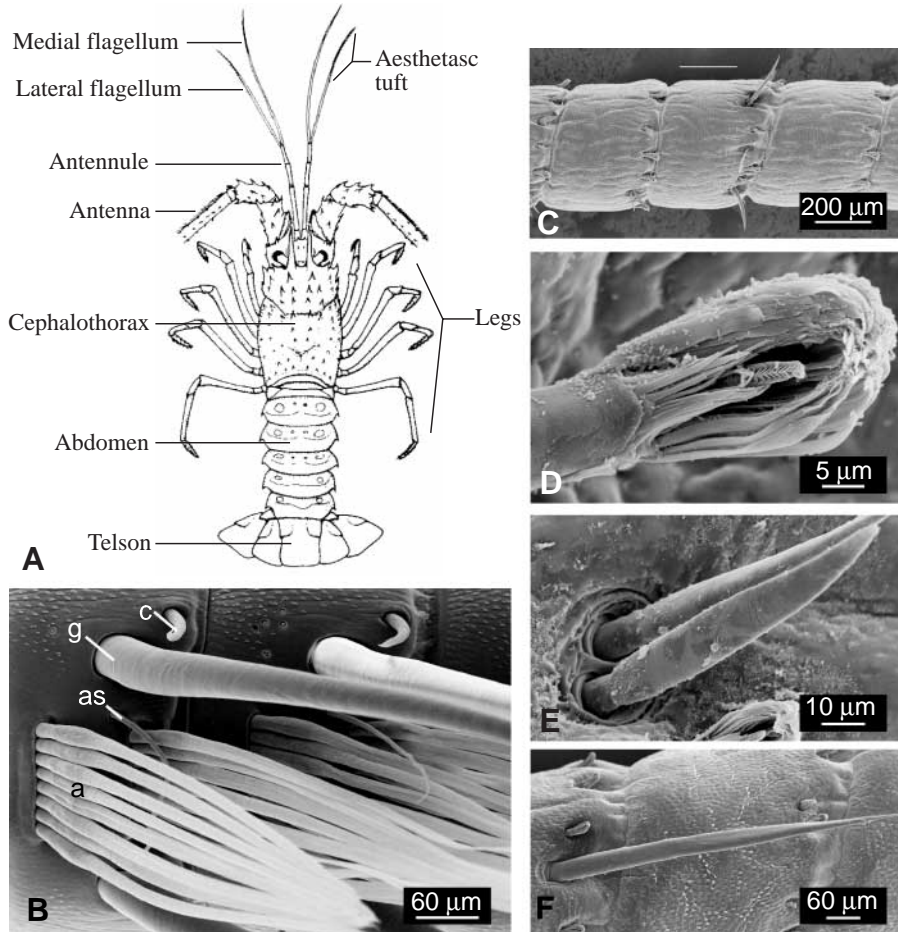


Fig. 1. Spiny lobster and antennular sensilla. (A) Diagram of the spiny lobster showing the major components of the chemosensory system. (B) High-magnification scanning electron micrograph of a portion of the aesthetasc tuft region of the lateral flagellum. Aesthetasc (a), guard (g), companion (c) and asymmetric sensilla (as) are visible. (C) Scanning electron micrograph of a portion of the medial flagellum showing various types of non-aesthetasc sensilla. (D–F) High-resolution scanning electron micrographs of three bimodal chemo-mechanosensilla: (D) hooded sensillum, (E) medium simple sensillum and (F) long simple sensillum. Modified from Cate and Derby (2001).

receptor neurons innervating the prominent aesthetasc sensilla. Aesthetascs are the most numerous sensilla on the antennules of the Caribbean spiny lobster, *Panulirus argus*, and are located exclusively in a distal tuft on the ventral face of each lateral flagellum. Aesthetascs are unique among antennular sensilla characterized thus far because they are innervated exclusively by chemosensory neurons. Each aesthetasc is innervated by the dendrites of approximately 300 olfactory receptor neurons (Grünert and Ache, 1988; Steullet et al., 2000; Derby et al., 2003), whose axons project to glomeruli within the paired olfactory lobes of the brain (Schmidt et al., 1992; Schmidt and Ache, 1996b; Sandeman and Mellon, 2002). Aesthetascs were traditionally believed to be the most important structures for detecting, discriminating and localizing odors because of their great numbers and extensive innervation by chemosensory neurons. Indeed, several studies have shown that ablation of the lateral flagellum impairs odor-mediated behaviors (Devine and Atema, 1982; Reeder and Ache, 1980; Giri and Dunham, 1999; Kraus-Epley and Moore, 2002; Wroblewska et al., 2002). These behavioral impairments were often attributed exclusively to the loss of aesthetasc sensilla because they are the most numerous sensillar type on the lateral flagellum. However, more recent work has shown that the aesthetascs are not the only structures on the antennule capable of driving

food-odor-mediated behaviors (Derby et al., 2001; Steullet et al., 2001, 2002).

Nine other types of sensilla, collectively referred to as 'non-aesthetascs', are widely distributed on the antennules of *P. argus*, and at least four of these (hooded, long simple, medium simple and asymmetric sensilla) are bimodal and innervated by distinct populations of chemoreceptive and mechanoreceptive neurons (Cate and Derby, 2001, 2002b; Schmidt et al., 2003). Backfills of the antennular nerve have revealed that presumptive chemo- and mechanosensory neurons innervating non-aesthetasc sensilla on the antennular flagella project to the stratified lateral antennular neuropils, while those on the proximal segments and statocysts project to the unstructured median antennular neuropil (Schmidt et al., 1992; Schmidt and Ache, 1993, 1996a; Cate and Roye, 1997), thus forming the non-aesthetasc chemosensory pathway.

Furthermore, these pathways remain anatomically distinct at the next synaptic pathway. Output interneurons from the olfactory lobes and from the lateral antennular neuropil project to different regions of the terminal medullae (Sullivan and Beltz, 2001). It should be noted, however, that there is some connectivity between these two neuropils; for example, local olfactory interneurons exist that connect the ipsilateral olfactory lobe and lateral antennular neuropil (Mellon and Alones, 1994; Schmidt and Ache, 1996b).

Although the two pathways have distinct anatomical arrangements, the functional significance of this dual organization remains unclear. To date, no published studies have conclusively demonstrated unique functions in food-odor-mediated behaviors for either pathway in spiny lobsters. In fact, previous work has generally found an overlap in the functions of the aesthetasc and non-aesthetasc pathways for behaviors such as odorant activation of searching behavior, odor learning and discrimination of food odors in small-scale, low-flow arenas (Steullet et al., 2001, 2002).

The importance of each pathway for behaviors over a larger spatial scale in more complex flows, such as those occurring during orientation to distant food-odor stimuli, has not been as thoroughly studied. Odor plumes emanating from sources in realistic flow conditions are spatially and temporally complex (Webster and Weissburg, 2001), and perhaps extracting orientational information from these signals requires a specialized neural pathway. Previous studies examining orientation behavior in flumes have focused more on uncovering the organism's method of orientation (e.g. tropotaxis, odor-gated rheotaxis) or on the role of entire antennular flagella than on determining the specific sensilla or chemosensory pathways involved in orientation (McLeese, 1973; Reeder and Ache, 1980; Devine and Atema, 1982; Atema, 1995; Beglane et al., 1997; Weissburg, 2000; Kozlowski et al., 2001). In several of these studies, entire flagella (including both aesthetasc and non-aesthetasc sensilla) were ablated, while in others, ablations were only performed unilaterally. Because the ablations were not specific to a single population of sensilla, the importance of each pathway for orientation remains unknown.

Therefore, the goal of this work is to determine whether the aesthetasc pathway or the non-aesthetasc pathway is necessary and sufficient for locating the source of a distant food odor stimulus. To assess the importance of each pathway for this task, we systematically ablated different populations of antennular sensilla and compared the behavior of ablated animals to that of intact controls. Under the conditions tested, both the aesthetasc and non-aesthetasc pathways were sufficient for orientation, but neither pathway alone was necessary. Overall, the results suggest that there is an overlap

in the function of the pathways and that food searching is not a unique function of either pathway alone.

## Materials and methods

### Animals

Caribbean spiny lobsters, *Panulirus argus* (Latreille 1804), ranging in carapace length from 48 to 74 mm (mean  $\pm$  S.E.M.,  $62.3 \pm 0.86$  mm,  $N=70$ ) were collected in the Florida Keys, shipped to Georgia State University and held in 800-liter aquaria containing aerated, recirculated, filtered artificial seawater (Instant Ocean<sup>®</sup>; Aquarium Systems, Mentor, OH, USA). Animals were maintained on a 12 h:12 h light:dark cycle and fed shrimp or squid three times a week. Intermolt animals (as determined by the method of Lyle and MacDonald, 1983) were selected for the behavioral assays if they approached and consumed a piece of shrimp that had been dropped into the aquarium. At least three days prior to the start of the trials, experimental animals were transported to holding aquaria (0.90 m long  $\times$  0.58 m wide  $\times$  0.67 m high) at Georgia Institute of Technology, where they remained throughout the course of the experiment.

### Ablations

To assess the importance of different populations of antennular sensilla for orientation, we performed four bilateral ablations, which are described below and summarized in Table 1. The four ablations have been used previously, and their effectiveness has been confirmed both morphologically and electrophysiologically (Steullet et al., 2001, 2002). All ablations were performed on non-anesthetized spiny lobsters immobilized on a plastic restraining device within a shallow container of artificial seawater. Ablations requiring surgical removal of sensilla were performed once, at least three days prior to the start of a series of experimental trials, using a hand-tooled narrow blade (0.2 mm wide; Steullet et al., 2001). Chemical ablations were performed with distilled water within 24 h of the start of each trial. At the conclusion of each series of experimental trials, ablated antennules were excised and the efficacy of ablation was evaluated using light microscopy to count the number of sensilla that remained intact on each

Table 1. Summary of the effects of each ablation type on antennular and non-antennular sensilla

Sensilla type	Ablation				
	Control	Aesthetascs ablated	Non-aesthetasc chemoreceptors ablated	Non-aesthetasc chemo- and mechanoreceptors ablated	All antennular chemoreceptors ablated
Aesthetascs	+	–	+	+	–
Non-aesthetasc chemoreceptors	+	+	–	–	–
Non-aesthetasc mechanoreceptors	+	+	+	–	+
Non antennular chemo- and mechanoreceptors	+	+	+	+	+

+, Intact/functional; –, ablated/non-functional; \*, some types of mechanoreceptors are more susceptible to distilled water ablations than others, and thus may have been inactivated during treatment. See text for details.

antennule. This analysis confirmed that shaving was a highly reliable method for removing sensilla. Shaving removed  $99.8 \pm 0.04\%$  (mean  $\pm$  S.E.M.,  $N=13$ ) of all aesthetascs on the antennule, which is similar to values obtained in other studies (Steullet et al., 2001). This corresponds to 1–2 intact aesthetascs per animal for the animals that we used in this study.

#### *Control*

Control animals were immobilized in the plastic restraining device in the same manner as ablated animals, but no sensilla were removed or inactivated.

#### *All antennular flagellar chemoreceptors ablated*

Aesthetasc and non-aesthetasc chemosensory neurons on both antennules were chemically ablated by immersing the lateral and medial flagella of each antennule in a tube of distilled water for 15 min. Distilled water functionally inactivates chemosensory neurons by disrupting the osmotic balance of the outer dendrites (Derby and Atema, 1982; Gleeson et al., 1997). The ablation is temporary and reversible, lasting only ~24 h before the neurons once again respond to chemical stimuli (Derby and Atema, 1982; Steullet et al., 2001). Because of the ephemeral nature of this ablation, it was performed within 24 h of each experimental trial. Distilled water effectively inactivates chemosensory neurons but may also affect the function of some types of mechanosensory neurons. Mechanosensory neurons with dendrites projecting up the length of the sensillum may be exposed to and inactivated by the distilled water environment (Derby and Atema, 1982; Garm et al., in press).

#### *Aesthetascs ablated*

All aesthetasc sensilla on both lateral flagella were surgically removed at the base using a hand-tooled blade. Asymmetric setae, which are located laterally to the aesthetasc rows (Gleeson et al., 1993; Cate and Derby, 2001), were also removed during this ablation. Removal of aesthetascs in this manner obliterates the chemosensory dendrites of the sensillum, which results first in unresponsiveness to odors, followed by death and degradation of the receptor neurons (Harrison et al., 2001).

#### *Non-aesthetasc chemo- and mechanoreceptors ablated*

All visible non-aesthetasc sensilla were surgically removed from the entire length of the lateral and medial flagella of both antennules. The flagella were then coated with a thin layer of cyanoacrylate glue (Super Glue Corp., Rancho Cucamonga, CA, USA) to prevent stimulus access to any remaining, unseen non-aesthetasc sensilla. Covering the antennules with cyanoacrylate glue effectively prevents stimulation of both non-aesthetasc chemosensory neurons and mechanosensory neurons that are responsive to hydrodynamic and some tactile stimuli (Derby and Atema, 1982).

#### *Non-aesthetasc chemoreceptors ablated*

This ablation was designed to specifically eliminate the

function of non-aesthetasc chemoreceptors while maintaining the integrity of at least some non-aesthetasc mechanoreceptors. Non-aesthetasc sensilla were surgically removed from annuli located within the aesthetasc region of each lateral flagellum. The shaved region was then coated with a thin layer of cyanoacrylate glue to prevent stimulus access to any remaining non-aesthetasc sensilla. The rest of the antennule (medial flagellum and proximal region of lateral flagellum) was then immersed in distilled water for 15 min to ablate non-aesthetasc chemoreceptor neurons in these regions. The aesthetasc region on each lateral flagellum was maintained in seawater during this process. Although the shaving and gluing inactivated mechanoreceptor neurons within the aesthetasc region, at least some of the mechanoreceptors along the medial flagellum and proximal portion of the lateral flagellum probably remained intact and functional (see above – All antennular flagellar chemoreceptors ablated – for explanation).

#### *Odor stimuli*

Three different odor stimuli were used in the experiments. Control stimuli consisted of artificial seawater (Instant Ocean<sup>®</sup>) taken directly from the flume before the start of trials, and experimental stimuli consisted of two concentrations of shrimp extract. Shrimp extract is a potent feeding stimulus for spiny lobsters (Carr, 1988; Derby, 2000) and was prepared by homogenizing frozen shrimp in artificial seawater with a blender and then collecting and freezing the raw extract in 10-ml aliquots. The final concentration of the raw extract was approximately  $300 \text{ g l}^{-1}$ . We then made dilutions of this stimulus by mixing raw shrimp extract in artificial seawater taken directly from the flume. Each stimulus was thoroughly mixed by shaking and filtered through Whatman #5 filter paper to remove large pieces of shrimp material. Preliminary experiments showed that shrimp concentrations of 3 and  $0.3 \text{ g l}^{-1}$  were effective in attracting lobsters to the odor source, so they were used in subsequent trials and were called ‘high’ and ‘low’ concentrations, respectively.

#### *Experimental setup*

To simulate semi-natural flow conditions where fluid flow and boundary layer conditions could be controlled, all trials were conducted in a recirculating 5000-liter flume housed at Georgia Institute of Technology (Fig. 2). (See Webster and Weissburg, 2001; Weissburg et al., 2003; Keller et al., 2003 for descriptions of the flume and its use in examining chemosensory behavior of other animals.) The flume measured 12.5 m long, 0.75 m wide and 0.35 m high, and the 2 m working section for this study began 10 m downstream of the entry way and ended 0.5 m upstream of the reservoir (Fig. 2). The floor of the flume was covered with a 1 cm-deep layer of fine-grained quartz sand, and the side walls were covered with black panels to eliminate confounding visual cues. The flume was filled with artificial seawater (Instant Ocean<sup>®</sup>) at  $\sim 22^\circ\text{C}$ . Flow velocity was  $4.9 \pm 0.08 \text{ cm s}^{-1}$  (mean  $\pm$  S.D.), as measured by an acoustic-Doppler flow meter, with a water depth of  $23.0 \pm 0.348 \text{ cm}$  (mean  $\pm$  S.D.) controlled by a vertical tailgate.



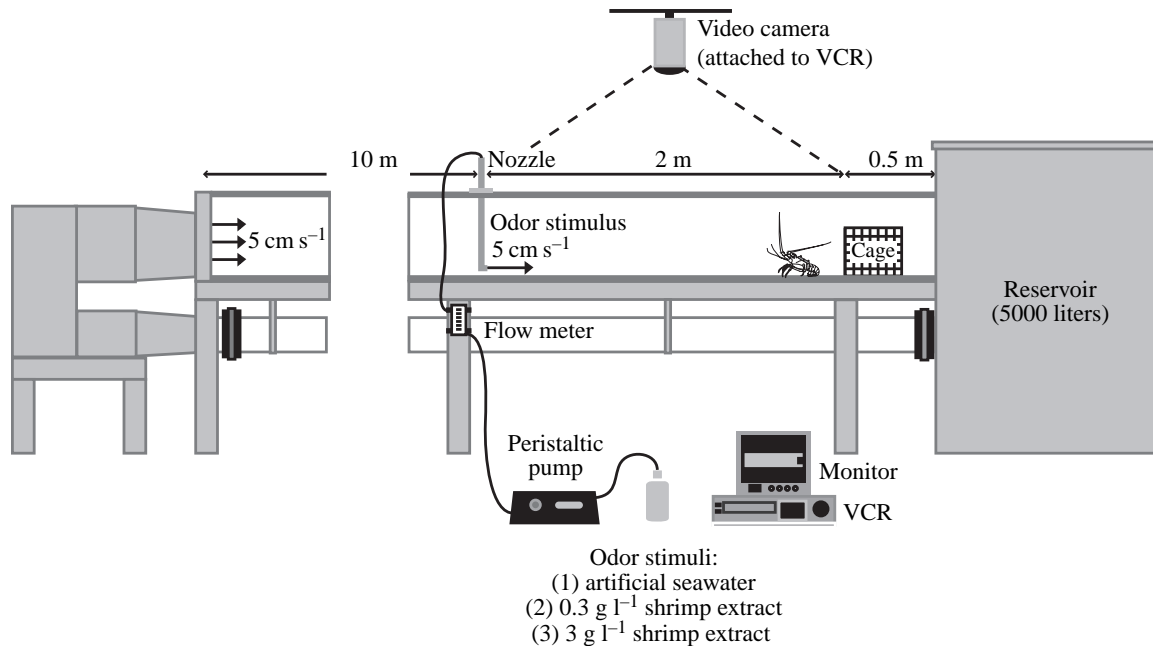


Fig. 2. Diagram of the flume setup at Georgia Institute of Technology. See text for explanation.

At this flow speed, the boundary layer shear velocity,  $u^*$ , calculated using the law-of-the-wall equation, and boundary layer structure conformed well to expectations for turbulence in open channel flows (Keller et al., 2003). The near-bed flow was smooth (Reynold's number = 2.65) with a  $u^*$  of 3.1 mm s<sup>-1</sup>. A cage (0.32 m long × 0.31 m wide × 0.18 m high) constructed out of plastic grating (1 × 1 cm grate size) was placed at the downstream end of the working section. Odor stimuli were released parallel to the flow 2 m upstream from the cage and 2.5 cm above the bed from a 4.7 mm-diameter brass nozzle with a fairing to minimize the flow perturbation. Control and experimental odors were introduced into the flume by a peristaltic pump, which pushed the stimuli through the nozzle at approximately the same velocity as the background flow (i.e. isokinetic release of the stimulus).

All trials were conducted during the day under low light conditions. Although *P. argus* is nocturnal in the natural environment, in the laboratory spiny lobsters will search when presented with food odors during the day if light levels are low enough. A video camera mounted above the flume was used to track the two-dimensional movements of the animals. Prior to the start of a trial, each animal was fitted with a watertight silicone (Sylgard) backpack containing two red light emitting diodes to facilitate tracking (Weissburg et al., 2002; Keller et al., 2003). The backpack was attached to the animal by a strip of Velcro® that had been glued to the carapace. The presence of the backpack had no apparent effect on the behavior of the animal.

Fifteen minutes before the start of each trial, the lobster was fitted with the backpack and placed in the cage. This was done in order to acclimate the animal to the flume conditions and to provide a constant starting point for each trial. At the end of

this acclimation period, the odor stimulus was introduced into the flow, and 30 s later the cage door was opened, allowing the animal to exit and move freely around the flume. The task was for the animal to exit the cage, track the odor plume to its source and physically grab the nozzle.

Each trial lasted a maximum of 10 min. Each spiny lobster had 5 min to completely exit the cage. If the animal did not exit the cage within 5 min, the trial was terminated immediately. If the animal did exit the cage within the first 5 min, it was then given an additional 5 min to locate the odor source and grab the nozzle. A trial ended either when the animal successfully located the odor source and held onto the nozzle or when the additional 5-min period had expired. Lobsters were offered a piece of shrimp at the conclusion of every trial as a test of motivational state. The lobster was removed from the experiments and was not included in the final data set if it failed to take the shrimp. This was done to ensure that an unsuccessful search attempt was due to sensory deficits rather than lack of interest in food.

Each animal was tested a total of three times over the course of 3 days (once each day in one of the three stimulus concentrations). The order of stimulus presentation was randomly determined for each animal prior to the start of the experiment. The three trials were not necessarily run on consecutive days, but all were conducted within a two-week period so that no animal was housed at Georgia Institute of Technology for more than two weeks.

#### *Immobilization of second antennae*

When spiny lobsters search for the source of an odor stimulus, they typically walk with their second antennae positioned perpendicularly to the long axis of the body. During

the course of our experiments, we observed that some lobsters walked towards the source with one antenna in constant contact with the side wall of the flume. We were concerned that this additional contact might enhance search efficiency and mask any possible deficits caused by antennular ablations. To identify any possible confounding effects of physical contact between the flume walls and second antennae, we conducted a series of trials using animals with and without their second antennae immobilized. We chose to immobilize rather than remove the second antennae because immobilization was a less severe treatment that retains some sensory function of the antennae and limits non-specific effects. The second antennae of five lobsters were positioned above and parallel to the long axis of the body and secured in this position by binding the two antennae together and then to the horns above each eye with plastic-coated wire. This arrangement restricted the movement of the second antennae and thus prevented the animals from extending them perpendicularly from the body. If the animals were relying on physical contact with the wall to move towards the source, then we would expect that those with restrained antennae would have less direct search paths because they would have to move further from the center of the flume in order to bring their antennae in contact with the wall.

## Results

### *Success rate for locating the odor source*

Antennular sensilla are necessary for locating the odor source, but either the aesthetascs alone or the non-aesthetascs alone are sufficient to mediate this behavior (Fig. 3). Control animals successfully located the source regularly when challenged with both the high (68%;  $N=22$ ) and low (45%;  $N=22$ ) concentrations of shrimp stimulus (Fig. 3). However, when tested under control conditions with seawater as an

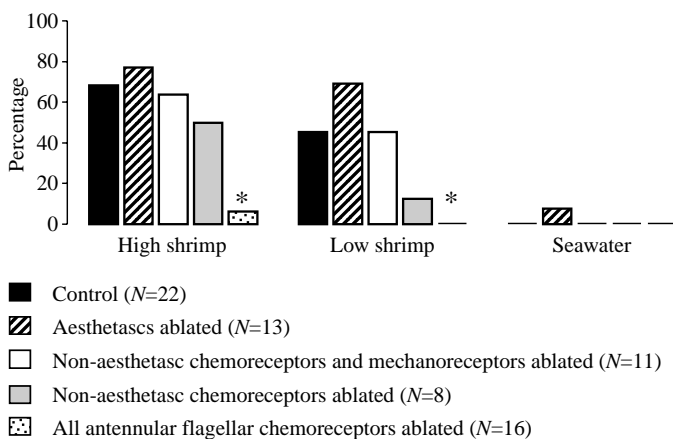


Fig. 3. Percentage of spiny lobsters that successfully located the odor source. In each of the three stimulus groups, only those ablation conditions with an asterisk differed significantly from the control (unablated) animals (Fisher exact test with Bonferroni correction,  $P < 0.0125$ ).

odorant, none of the animals located the odor source (Fig. 3). Thus, the presence of chemical stimuli is necessary for spiny lobsters to locate and grab the nozzle.

In contrast to control animals, animals with all antennular flagella chemoreceptors ablated generally did not locate the odor source when exposed to either of the shrimp extract stimuli or the seawater control (Fig. 3). Post-test feeding responses to shrimp showed that the lack of response in this group of animals was not due to low motivation. Less than 16% of the animals in this treatment group failed to respond to the post-test shrimp. Similar response levels were observed with the other treatment groups, and there was no difference in the percentage of animals that did not respond to the post-test shrimp between the five treatment groups [ $\chi^2_{0.05,4} = 5.48001$ ;  $P > 0.05$ ]. Thus, functional antennular chemosensilla are necessary for locating the odor source. However, neither the aesthetascs alone nor the non-aesthetascs alone are required to mediate this behavior.

Aesthetasc-ablated animals responded similarly to control animals (Fig. 3) and there were no significant differences between the percentage of control *versus* aesthetasc-ablated animals finding the source. Aesthetasc-ablated animals found the source frequently when high (77%;  $N=13$ ) and low (69%;  $N=13$ ) concentrations of shrimp extract were used as an odorant and rarely left the cage, let alone found the source, when seawater was used as an odorant. Thus, aesthetascs alone are not necessary, and non-aesthetascs alone are sufficient to drive this behavior.

Non-aesthetasc chemo- and mechanoreceptor-ablated animals also responded similarly to the control animals (Fig. 3). They also located the source regularly in response to both the high (64%,  $N=11$ ) and low (45%,  $N=11$ ) concentrations of shrimp stimulus and they did not locate the source with seawater. The success rate of non-aesthetasc chemoreceptor-ablated animals was not significantly different from that of the non-aesthetasc chemo- and mechanoreceptor-ablated animals (Fig. 3), although there were fewer animals in this treatment ( $N=8$ ).

The combination of these results suggests that antennular flagellar chemoreceptors are necessary for spiny lobsters to locate an odor source but that either the aesthetascs alone or the non-aesthetascs alone are sufficient to accomplish this task. Additionally, over the short time frame of these experiments, we saw no evidence that non-antennular chemoreceptors may be able to compensate for the loss of antennular chemoreceptors, as has been shown over a longer time period for other species (Hazlett, 1971b).

### *Search efficiency*

In addition to recording the overall success rate of animals in each treatment group, we also examined the efficiency of successful searches to identify more subtle influences of the ablations. Search efficiency was quantified using four parameters that are commonly used in orientation experiments (Devine and Atema, 1982; Moore et al., 1991; Moore and Grills, 1999; Kraus-Epley and Moore, 2002; Keller et al.,

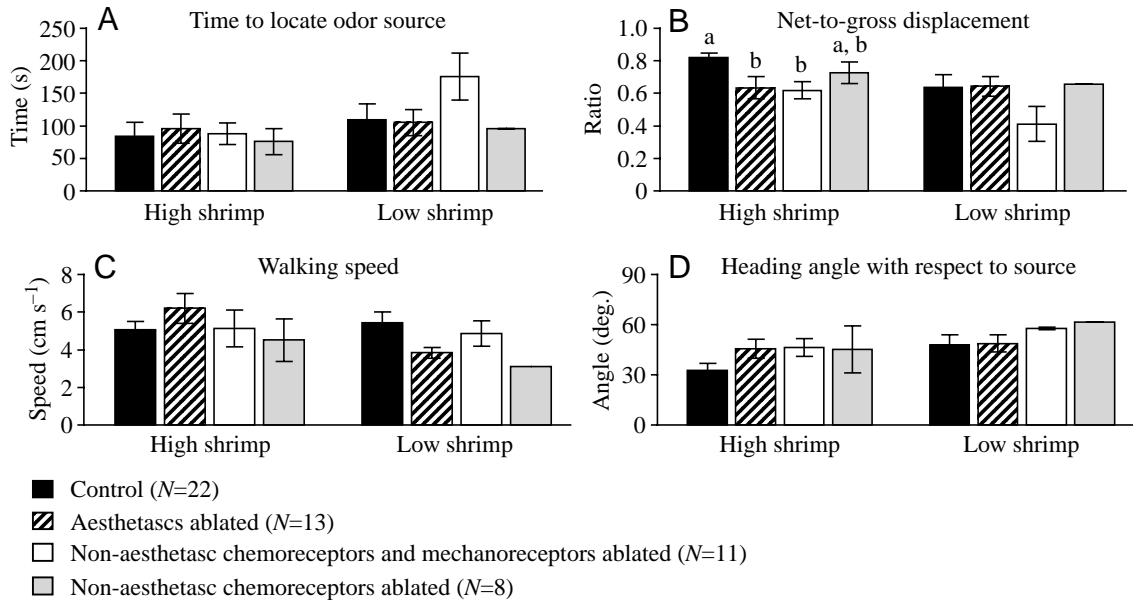


Fig. 4. Efficiency of successful searches. Mean  $\pm$  S.E.M. of time to find the odor source (A), net-to-gross displacement ratio (B), walking speed (C) and heading angle with respect to odor source (D). For behaviors in A, C and D, there were no differences in responses of the ablated animals at either shrimp concentration (A,C: ANOVA,  $P>0.05$ ; D: Watson-Williams test,  $P>0.05$ ; Zar, 1996). For B, at high shrimp concentration only, there was a significant ablation effect (ANOVA,  $F_{3,30}=3.50$ ,  $P=0.027$ ); ablation conditions whose bars have different letters are significantly different (LSD test,  $P<0.05$ ).

2003). The parameters were mean time to locate the odor source (Fig. 4A), net-to-gross displacement ratio (NGDR; Fig. 4B), mean walking speed (Fig. 4C) and mean heading angle with respect to the source (Fig. 4D). Because our analysis of efficiency is limited only to successful searches, we did not include completely ablated animals or searches with seawater as a stimulus (since animals did not locate the source under these conditions).

#### Mean time to locate the source

The mean time to locate the odor source was calculated as the average time difference between exiting the cage and grabbing the source for all animals in the treatment group. For animals tested in both the high and low shrimp stimulus concentrations, the time to locate the odor source was not different for control and ablated groups (Fig. 4A). All four groups of animals found the source within 96 s in the high concentration and 176 s in the low concentration (Fig. 4A).

#### Net-to-gross displacement ratio

The NGDR was used to describe the directness of a search path. The ratio was calculated as the Euclidean distance from the cage to the nozzle divided by the total distance traveled by the animal. Ratios approaching 1 represent more direct paths to the source whereas values approaching 0 represent increasingly more tortuous paths to the source. The NGDR values of the control and the three ablated groups were not different when the animals were tested in the low concentration of shrimp extract (Fig. 4B). By contrast, when tested in the high stimulus concentration, there was a significant difference

in the NGDR between control and ablated groups (aesthetascs ablated, non-aesthetasc chemo- and mechanoreceptors ablated) (Fig. 4B). Control animals took very direct paths to the source (NGDR=0.82;  $N=14$ ). Compared with control animals, aesthetasc-ablated and non-aesthetasc chemo- and mechanoreceptor-ablated animals took significantly more tortuous paths to the source, with NGDR values of 0.63 ( $N=10$ ) and 0.62 ( $N=6$ ), respectively (Fig. 4B). Interestingly, although these ablated groups differed from the control group, they did not significantly differ from one another. Thus, the different ablations produced a similar deficit in this measure of search efficiency.

#### Mean walking speed

The mean walking speed for each orientation path was calculated by averaging the speed of the animal over 1-s intervals. There were no significant differences between the walking speed of control and ablated animals in either of the stimulus concentrations (Fig. 4C). In fact, the mean walking speed of all the groups remained relatively constant over all trials regardless of stimuli being tested. Animals tested with seawater as a stimulus walked at similar speeds (in the range of 3–6  $\text{cm s}^{-1}$ ) to those tested with the shrimp extracts.

#### Heading angle with respect to the odor source

Heading angle with respect to the odor source was determined using the methodology of Moore et al. (1991). Heading angle was calculated as the absolute value of the angle between a straight line connecting the lobster's current position on the search path (based on the location of the first light-

emitting diode of the backpack) and the nozzle, and a straight line connecting the lobster's current position on the search path and the lobster's next position on the search path. Values ranged between 0 and 180°, with 0° heading directly towards the source and 180° heading directly away from the source. There were no significant differences between the heading angles of control and ablated animals in either of the stimulus concentrations (Fig. 4D).

#### Effects of stimulus concentration

The behavior of the lobsters was somewhat dependent on the concentration of shrimp odor extract. Animals tended to find the odor source more successfully in high than in low shrimp concentration (Fisher exact test,  $P=0.06$ ). Animals performed more efficient searches in the high compared with the low concentration of shrimp for two of the behaviors, as suggested by the higher NGDR and lower heading angles in high vs low plumes (Fig. 4B, ANOVA,  $F_{1,57}=5.42$ ,  $P=0.023$  for NGDR; Fig. 4D, Watson-Williams test,  $F_{1,57}=6.909$ ,  $P=0.025$  for heading angle). Additionally, there was a strong trend for animals in the high vs low plumes to locate the odor source more quickly (Fig. 4A, ANOVA,  $F_{1,57}=3.45$ ,  $P=0.068$ ) and a weak trend for them to walk faster (Fig. 4C, ANOVA,  $F_{1,57}=1.95$ ,  $P=0.168$ ).

#### Effects of mechanical stimulation of the second antennae

There was no difference between the percentages of animals locating the odor source with free or immobilized antennae (Fig. 5A). Both groups of animals found the source regularly when tested with the high concentration of shrimp extract, and

neither group located the source with seawater as an odorant (Fig. 5A). Additionally, in all measures of search efficiency (mean time to source, NGDR, mean walking speed and mean heading angle), the two groups of animals did not differ (Fig. 5B–E). Thus, contact between the second antennae and the wall of the flume does not significantly influence the success or efficiency of search in our flume.

#### Discussion

The purpose of this study was to investigate the roles of the dual antennular chemosensory pathways in the spiny lobster during orientation to a 2 m-distant food odor source. Our results show that, although antennular sensilla in general are necessary for food localization, either the aesthetasc pathway or the non-aesthetasc pathway alone is sufficient to drive the behavior. Food localization was mediated equally well by either the aesthetasc or the non-aesthetasc pathway in our assay, indicating a high degree of functional overlap in the pathways for this task.

#### *Either aesthetasc or non-aesthetasc chemosensory neurons can mediate food localization behavior*

Several previous studies have demonstrated that distance chemoreception in decapod crustaceans is mediated primarily by antennular chemoreceptors (Reeder and Ache, 1980; Devine and Atema, 1982; Hazlett, 1971a; Kraus-Epley and Moore, 2002), and the results of the present study also support this conclusion. When all antennular flagellar chemoreceptors were ablated, spiny lobsters lost the ability to locate the source of a 2 m-distant food odor stimulus (Fig. 3). They did, however, respond to a piece of shrimp brought into contact with their legs, indicating that the impairment was due to sensory deficit rather than lack of motivation to feed. Thus, chemosensory input from antennular sensilla in general is necessary for orientation. However, the presence of only a subset of functional chemoreceptors is sufficient to

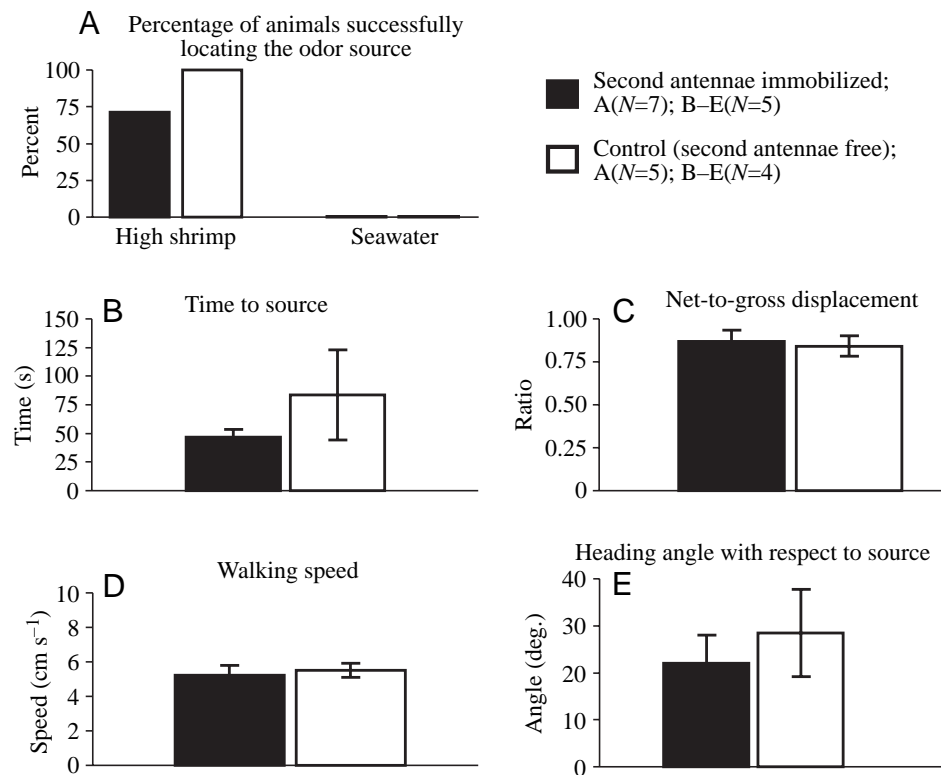


Fig. 5. Success rate and search efficiency of animals with free and immobilized second antennae. (A) Percentage of animals finding the odor source with shrimp and seawater as odorants. Mean  $\pm$  S.E.M. of (B) time to find the odor source with shrimp as an odorant, (C) net-to-gross displacement ratio, (D) walking speed and (E) heading angle with respect to odor source. For each of the four behaviors, there were no differences in responses of the ablation conditions (A–D, ANOVA,  $P>0.05$ ; E: Watson-Williams test,  $P>0.05$ ).



enable orientation. Aesthetasc-ablated lobsters were as successful as control animals in locating the odor source (Fig. 3). The same pattern of behavior was seen in non-aesthetasc-ablated animals (Fig. 3). Thus, either of the two chemosensory pathways – the aesthetasc pathway or the non-aesthetasc pathway – is alone sufficient to allow orientation. Although it did not affect the percentage of animals that successfully located the odor source, ablation of a single pathway did affect search efficiency in some cases. For example, when the aesthetasc pathway was ablated, animals took more circuitous paths to the odor source than control animals in the high shrimp stimulus condition (Fig. 4B). Interestingly, the same deficit was seen when the non-aesthetasc pathway alone was ablated (Fig. 4B), further suggesting an overlapping role for the pathways in our assay.

The results of this study strongly suggest that the two antennular chemosensory pathways are equally able to mediate the behavior under the current experimental conditions. There were no statistically significant differences in the percentage of animals locating the source (Fig. 3) or in the search efficiency (Fig. 4A–D) between aesthetasc-ablated and non-aesthetasc-ablated animals. Thus, the function of the aesthetasc and non-aesthetasc pathways in this behavioral assay appears to overlap. Possible reasons for the observed overlap and potentially unique roles for each pathway in odor-mediated behaviors are discussed in later sections.

*Non-antennular sensors and non-odor stimuli are not sufficient to mediate food localization behavior*

In addition to the antennules, chemosensilla are concentrated on several other body regions of the spiny lobster, including the walking legs, mouthparts and second antennae (Derby and Atema, 1982; Cate and Derby, 2002a; Garm et al., 2003). Work on other decapod crustaceans has shown that leg chemoreceptors in particular can aid in orientation as the animal approaches the source of an odor stimulus (Devine and Atema, 1982; Moore et al., 1991; Keller et al., 2003). In our experiments, however, inputs from chemosensilla on the legs or other regions of the body were not sufficient to allow the lobster to overcome the sensory deficits caused by antennular ablation. Spiny lobsters with all antennular chemoreceptors ablated did not locate the odor source even though all other non-antennular chemoreceptors were intact (Fig. 3). Although our results suggest that non-antennular chemosensory inputs are not sufficient to drive orientation behavior, they do not suggest that these inputs are unimportant or unnecessary. In the natural environment, spiny lobsters are likely to use a combination of receptor inputs to efficiently locate prey in order to avoid unnecessary exposure to predators.

Additionally, visual, hydrodynamic and tactile cues were not sufficient to allow the lobsters to locate the odor source in the absence of chemical stimulation of the antennules. Spiny lobsters did not locate the nozzle when seawater was used as a stimulus, even though visual and hydrodynamic cues would have been comparable between seawater and shrimp odorant trials. Thus, the lobsters in our study were not simply locating

the nozzle by moving upstream in the flow; the presence of a chemical signal was necessary.

Although flow cues alone were not sufficient for lobsters to locate an odor source in our assay, lobsters may use these cues in combination with chemical cues to orient efficiently to the source of an odorant. Hydrodynamic stimuli can provide potentially valuable information about the direction and spatial arrangement of stimuli in the environment, and crustaceans are known to respond to strong local flows and also to more general cues like wave surge (Breithaupt et al., 1995; Nevitt et al., 1995; Wilkens et al., 1996). However, crustaceans do not rely exclusively on flow cues to locate the source of an odor stimulus; they also extract important information directly from the spatial or temporal properties of the chemical signal (Weissburg and Dusenbery, 2002). Blue crabs (*Callinectes sapidus*), for instance, employ a search strategy that incorporates both chemical and flow cues (odor-gated rheotaxis) to locate the source of an odor (Weissburg and Zimmer-Faust, 1993, 1994; Weissburg 2000; Webster and Weissburg, 2001; Weissburg et al., 2002; Weissburg and Dusenbery, 2002; Keller et al., 2003). The concurrent use of both hydrodynamic and chemical cues results in more efficient searches with more direct paths and fewer course corrections (Weissburg and Dusenbery, 2002; Keller et al., 2003). The spiny lobsters in our experiments may have also used flow cues to orient efficiently to the odor source after the chemical signal had been detected. However, because this experiment was designed specifically to examine the chemosensory pathways involved in odor guidance, we cannot definitively identify the searching strategy employed by the animals in our assay.

Tactile stimulation resulting from physical contact between the second antennae and the side walls of the flume also did not alter the ability of spiny lobsters to locate the odor source. The overall success rate and search efficiency of animals with immobilized antennae was not different from that of animals with free antennae (Fig. 5). Lobsters with immobilized antennae generally walked straight down the center of the flume without attempting to contact the side wall, suggesting that physical contact with the side wall does not necessarily enhance their success rate or search efficiency.

*Why have multiple chemosensory pathways?*

The results of our experiments strongly suggest that there is a high degree of functional overlap between the dual antennular pathways for food localization behavior. Functional overlap is an important feature of many sensory systems and can benefit an organism in several important ways (Derby and Steullet, 2001). Possession of multiple, overlapping sensors allows an animal to continue to function normally in the event of loss or damage to a subset of sensors (Derby and Steullet, 2001). Lobsters missing part or the entire aesthetasc region occur in both the field and laboratory (Harrison et al., 2001). Because the acquisition of food is crucial for survival, it is not surprising that lobsters can use other chemosensory structures besides the delicate aesthetascs to mediate this important behavior.

A multiplicity of receptors can also extend the range of stimuli that a lobster is able to detect and increase the sensitivity and resolution of the system (Derby and Steullet, 2001). Electrophysiological studies have demonstrated that aesthetasc and non-aesthetasc chemoreceptor neurons respond to the same types of odorants and have similar response thresholds (Fuzessery, 1978; Thompson and Ache, 1980; Cate and Derby, 2002b). The combination of inputs from these two pathways may allow for much greater sensitivity than either pathway alone could provide, as suggested by some of the results of this study. When tested in the high stimulus concentration, intact animals took more direct paths to the source than either group of partially ablated animals (aesthetascs ablated and non-aesthetasc chemo- and mechanoreceptors ablated), suggesting that the combined input of both chemosensory pathways provides more information than either pathway alone. Although each pathway alone is sufficient to drive the behavior in this instance, the performance of the lobster is enhanced by their combined activity.

Although functional overlap can have important benefits, it is likely that the aesthetasc and non-aesthetasc chemosensory pathways also have specialized roles that would emerge under different experimental conditions. Despite the lack of experimental demonstrations of specific roles for each pathway in complex behaviors, both the organization of the pathways and the results of behavioral studies with other species of decapod crustaceans provide some possibilities.

The aesthetasc pathway originates in the olfactory receptor neuron innervating each aesthetasc on the antennule. The axons of these neurons synapse onto olfactory interneurons within the olfactory lobes of the deutocerebrum (Schmidt and Ache, 1992, 1996b; Sandeman and Mellon, 2002). The paired olfactory lobes have a glomerular organization and are structurally analogous to the olfactory bulbs of vertebrates and the antennal lobes of insects (Sandeman and Denburg, 1976; Mellon and Munger, 1990; Sandeman et al., 1992; Schmidt and Ache, 1992, 1996b). Glomeruli are typical features of first-order olfactory processing centers (Hildebrand, 1995; Eisthen, 2002) and are thought to play an important role in determining odor quality. Indeed, behavioral experiments show that the aesthetascs are sufficient to mediate olfactory discrimination of relevant food odor mixtures (Steullet et al., 2002). Although they are not necessary for analyzing food odors (at least at the concentrations tested), aesthetascs may be important in determining the quality of other types of odor stimuli. In the male blue crab, aesthetascs are essential for mediating the response to courtship and mating signals (Gleeson, 1982, 1991). It is possible that the aesthetasc pathway also functions in spiny lobster intraspecific communication, perhaps by mediating the response to aggregation signals.

By contrast, the organization of the non-aesthetasc pathway suggests that it may play a role in detecting spatial aspects of a chemical stimulus. The non-aesthetasc pathway contains both chemosensory and mechanosensory afferents, including those from bimodal non-aesthetasc sensilla on the antennular flagella. Although this pathway is thought to be involved

primarily in driving sensory-motor reflexes and movements of the antennules (Maynard, 1966; Schmidt and Ache, 1993), more recent work indicates that it also functions in a variety of odor-mediated behaviors (Steullet et al., 2001, 2002; present study). It has been hypothesized that the bimodal non-aesthetasc sensilla, which allow spiny lobsters to detect both chemical and hydrodynamic characteristics of an odor stimulus, may provide the animal with information about the location of stimulation on the antennule. Additionally, the stratified organization of the lateral antennular neuropils (one pair of target neuropils in this pathway) has been hypothesized to represent a spatial map of sensory inputs on the antennule (Schmidt and Ache, 1996a). Although it has not been demonstrated experimentally, the non-aesthetasc pathway may detect spatial aspects of an odor stimulus through the integration of chemosensory and mechanosensory cues. The fact that the output interneurons from the lateral antennular neuropils and from the olfactory lobes project to distinctly different regions of the protocerebrum (Sullivan and Beltz, 2001) supports the notion that these pathways have some divergent functions.

Possession of multiple chemosensory pathways with redundant as well as complementary functions may allow a lobster to detect and discriminate over a much broader range of chemical stimuli than would be possible with only a single chemosensory pathway. Although unique behavioral roles for either chemosensory pathway in the Caribbean spiny lobster have not yet been conclusively demonstrated, several possibilities for specialized functions exist. Ongoing experiments in our laboratory are focused on these possibilities in order to understand the functional significance of the dual chemosensory pathways of the Caribbean spiny lobster.

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