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Identification of Chemosensory Sensilla Activating Antennular Grooming Behavior in the Caribbean Spiny Lobster, *Panulirus argus*

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Abstract

Crustaceans such as crabs and lobsters clean or 'groom' their olfactory organ, the antennule, by wiping it through a pair of mouthpart appendages, the third maxillipeds. In the lobster, only a few chemicals found in prey extracts, especially glutamate, elicit grooming. Chemosensory input driving grooming is likely to be mediated via sensilla located on antennules and third maxillipeds. Chemosensory antennular sensilla are innervated by neurons with central projections either to the glomerular olfactory lobe (*aesthetasc* sensilla) or to non-glomerular antennular neuropils (*nonaesthetasc* sensilla). By selectively ablating the chemosensory sensilla on the antennules and the third maxillipeds we have determined that the aesthetascs are necessary and sufficient to drive grooming behavior. Chemosensory activation of antennular grooming behavior likely follows a 'labeled-line' model in that aesthetasc neurons tuned to glutamate provide adequate input via the olfactory lobe to motor centers in the brain controlling antennular movements.

Introduction

An enduring question in chemosensory biology is how olfactory systems encode chemical signals. At one extreme, the labeled-line theory argues that discrete sensory neurons may encode specific qualities of a stimulus. In its simplest derivation, activation of this subset of neurons will drive a behavior. In contrast, the across-neuron pattern theory argues that stimulus quality is encoded by the pattern of activity across a population of neurons. Because many behaviorally relevant chemical signals are complex mixtures requiring activation of many neurons, the latter theory is often favored.

This is quite apparent in aquatic environments in which chemical signals allowing identification and location of food consist of mixtures that include amino acids, amines, nucleotides and organic acids (Carr *et al*., 1996). The chemosensory systems of aquatic predators and scavengers are adapted to sense and process this complex information. Lobsters possess a diversity of receptors tuned to specific chemicals in the mixture (Voigt and Atema, 1992; Daniel *et al*., 1994), including those located on the olfactory organ, the antennules (Figure 1). The result is that the peripheral olfactory system both encodes the identity of the mixture as a unique odor, through the response of the entire population of sensory neurons, and at the same time retains the identities of the individual elements making up the mixture, through the responses of subsets of neurons tuned to particular chemicals (Derby, 2000). Thus it is possible that both labeled-lined and across-neuron pattern codes may function, although most evidence argues for the latter.

Sensory neurons from the antennules appear to follow two independent pathways into the brain of the spiny lobster. The 'aesthetasc' pathway consists of afferents from the lateral antennular flagella, most likely innervating aesthetasc sensilla, which terminate on one or a few of the ~1100 columnar glomeruli within the paired olfactory lobes (OL) found in the deutocerebrum. The olfactory lobes also receive some mechanosensory input (Schmidt and Ache, 1992; Schmidt *et al*., 1992). The 'nonaesthetasc' pathway consists of nonaesthetasc chemosensory and mechanosensory afferents from medial and lateral antennular flagella, which terminate in the paired nonglomerular lateral antennal neuropils (LAN) of the deutocerebrum (Schmidt *et al*., 1992; Schmidt and Ache, 1996a). The LAN and associated median antennal neuropil have been identified as lower motor centers for the antennules (Roye, 1986; Roye and Bashor, 1991), initiating and controlling the antennular movements involved in antennular withdrawal, flick and grooming (Snow, 1973; Roye and Bashor, 1991; Roye, 1994).

One behavior in lobsters elicited in response to chemical signals is antennular grooming behavior or AGB (Barbato and Daniel, 1997; Daniel *et al*., 2001). The behavior consists of deflection of either one or both of the antennules downward, permitting the lateral flagella of the antennules (Figure 1A) to be grasped by the third maxillipeds (Figure 1B), the paired appendages on either side of the mouth. The

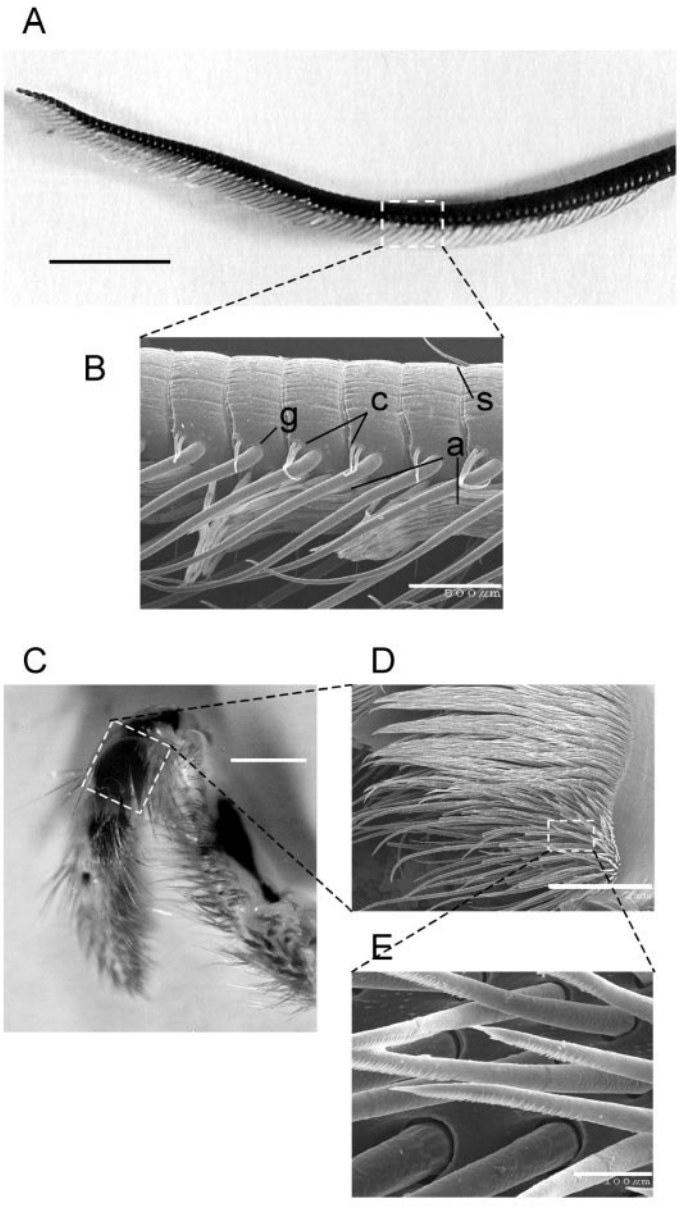

Figure 1 Appendages and setae involved in AGB. **(A, B)** Lateral flagellum of antennule of *P. argus* and associated setae. (A) Photograph of distal half of lateral flagellum in which the guard setae are visible. Scale bar = 5 mm. (B) SEM of aesthetascs-bearing (a) portion of lateral flagellum. Also visible are guard (g), companion (c) and simple (s) setae. Asymmetric setae are not visible but can be seen in the SEM of the flagellum with guard and companion setae excised (Figure 3). Scale bar = 500 μm. **(C–E)** Third maxilliped of *P. argus* and associated setae. (C) Photograph of medial side of maxilliped. Scale bar = 5 mm. (D) SEM of patch of setae used to groom antennules. Scale bar = 2 mm. (E) SEM of serrate setae in which the serrated edges are visible. Scale bar = 100 μm.

antennules are pulled repeatedly through the setal combs of the maxillipeds ('antennule wiping'). After a number of repetitions the antennules are returned to their original position and the entire sequence may be repeated. Usually, at the end of each sequence the maxillipeds are rubbed against each other repeatedly ('auto-grooming'). Auto-grooming is always preceded by a bout of antennule wiping (Barbato *et al*., 1996).

In this way the setae on the lateral flagella are scoured by the serrate setae of the maxillipeds, which results in the removal of debris accumulating on the setal surfaces. Prolonged accumulation of debris on the antennular surface results in breakage and loss of the aesthetascs (Bauer, 1977).

In lobsters, AGB is elicited almost exclusively by one chemical, L-glutamate (Glu), found in complex mixtures mimicking food (Barbato and Daniel, 1997; Daniel *et al*., 2001). In *Panulirus argus*, for example, the next best stimulus for activating AGB, glycine, elicits only 30% of the response elicited by an equimolar concentration of Glu. This suggests that chemosensory activation of AGB may follow a labeled-line pathway. In contrast, other behaviors associated with food stimuli, namely search and antennular flick, are elicited in response to many of the chemicals found in complex mixtures (Daniel and Derby, 1991; Fine-Levy and Derby, 1992; Lynn *et al*., 1994), making it likely that across-neuron pattern encoding is predominant. In effect, the neural processing leading to AGB must deviate considerably from the processing leading to search and flick behaviors, even though all three are elicited by the same odorant mixtures encountered in the natural environment.

A first step towards elucidating the neural processing leading to AGB requires the identification and characterization of the source of chemosensory input. Sensilla with known or putative chemosensory function have been identified on virtually all lobster appendages, including the antennules, antennae, maxillipeds and periopods, as well as the body surface (Derby, 1989; Voigt and Atema, 1992; Hallberg *et al*., 1997; Cate and Derby, 2001). Because AGB involves movements of the antennules and the third maxillipeds, sensilla on these appendages most likely provide chemosensory input for the behavior. The most obvious and best-studied chemosensory sensilla are the aesthetascs found on the distal half of the lateral flagellum of the antennules (Figure 1). The very dense patches of aesthetascs are the only sensilla known to have unimodal chemosensory function (Schmiedel-Jakob *et al*., 1986). Each of the ~1300 aesthetascs found on each antennule is innervated by ~300 olfactory receptor neurons—ORNs (Cate and Derby, 2001). Closely associated with the aesthetascs are three other types of setae: guard (~160 per antennule), companion (~200 per antennule) and asymmetric (~80 per antennule)— see Figures 1 and 3 (Cate and Derby, 2001). The four setae together comprise an easily visible 'tuft' on the distal half of the lateral flagellum. Evidence for mechano- or chemoreceptive function in the guard, companion and asymmetric setae is speculative (Derby, 1989). In addition to setae associated only with the tuft, a number of setae are dispersed throughout the lateral and medial flagella: hooded, plumose, short setuled and simple (Cate and Derby, 2001). Hooded setae and two of the three types of simple setae

(short and medium) are innervated by chemoreceptive and mechanoreceptive neurons (Cate and Derby, 2001). Setae on the third maxillipeds are largely composed of serrate setae, which are mechano- and chemoreceptive— see Figure 1 (Derby, 1989; Corotto *et al*., 1992).

Ablation techniques have been successfully used to examine the behavioral functions of chemosensory organs in lobsters (Steullet *et al*., 2001, 2002). We therefore used ablation techniques to examine the involvement of the antennules and maxillipeds in providing chemosensory input driving AGB. Selected appendages or specific setae on appendages were ablated by either immersion in distilled water or surgical excision. AGB responses towards Glu were measured before and after ablation.

Materials and methods

Source and maintenance of spiny lobsters

Caribbean spiny lobsters, *P. argus*, were obtained from the Florida Keys Marine Laboratory in Long Key, FL and maintained in separate 80 l aquaria (one lobster/aquarium), at 25–27°C with a 12 h:12 h light:dark cycle. Red light (25 W, ceramic-coated light bulbs) was provided during the dark period. Aquaria were equipped with a gravel bottom filter system, lined with crushed coral and filled with aerated, recirculating artificial seawater (ASW; Instant Ocean®). Spiny lobsters were fed *ad libitum* scallop and shrimp every other day. Uneaten food was removed after 1 h.

Chemical stimuli

Stock solutions (10 mM, pH 8.1) of Glu were prepared in ASW (Cavanaugh, 1964) and stored at –70°C until needed. On the day of an experiment, stock solutions were thawed and diluted to 0.05 or 0.5 mM with ASW.

Experimental design for ablation procedures

Two ablation techniques—distilled water (DW) ablation and excision—were used in this experiment (Table 1). In DW ablation, antennules are exposed to DW for at least 5 min. The osmotic shock results in destruction of the dendritic portion of chemosensory cells in contact with DW, but does not kill the cells (Gleeson *et al*., 1997). Excision removes the dendrites of sensory cells that extend into the setae and leads eventually to the death of ORNs (Harrison *et al*., 2001). Unlike DW ablation, the excision procedure allows removal of specific setae; however, it is a more laborious procedure and it also affects mechanosensilla in addition to chemosensilla.

DW ablation was performed separately on a whole appendage (maxillipeds or medial flagella of antennules) or a region of an appendage (distal halves of lateral flagella of antennules; Table 1). In the excision procedure (Figure 3), specific putative sensilla guard (G) and companion (C) setae (GC excision), and all tuft setae (guard, companion, aesthetasc and asymmetric setae) were excised (Table 1). Behavioral responses to chemical stimuli known to elicit AGB were determined before and after each of the ablation procedures.

Table 1 Experimental design for ablation procedures

Region ablated	*n*	Procedure
Lateral flagella of antennules (distal halves)	6	assay → sham ablation → assay after 3 h → DW ablation → assay after 3 h → assay after >24 h
Medial flagella of antennules	9	sham ablation → assay after 3 h → DW ablation → assay after 3 h
Maxillipeds	12	sham ablation → assay after 3 h → DW ablation → assay after 3 h → assay after >24 h
Guard and companion setae	6	sham ablation → assay after 3 h → excise setae → assay after 3 h → DW ablation of lateral flagella → assay after 3 h → assay after 24 h
All tuft setae	9	sham ablation → assay after 3 h → excise setae → assay after 3 h → assay after 24 h

Behavioral responses to chemical stimuli known to elicit AGB were measured.

DW ablation of whole appendages and region of appendages

Three regions (medial flagella, distal halves of lateral flagella of antennules, and maxillipeds) of spiny lobster sensory appendages were ablated, in separate experiments, to identify the source of chemosensory input to AGB. Six, nine and 12 spiny lobsters were used for ablations of distal halves of lateral flagella of antennules, medial flagella of antennules and maxillipeds, respectively. Each lobster was removed from its tank, wrapped in wet paper towels and restrained in an Instant Ocean® bath (during ablations of antennules) or hand-held in air (during ablations of maxillipeds). A sham ablation was performed in which appendages were placed for 15 min in a 20 ml vial containing ASW. Sham-ablated lobsters were tested with stimuli after a 3 h recovery period. After at least 24 h, the same ablation procedure was repeated, this time using DW. Lobsters were again allowed a 3 h recovery period and were tested with stimuli at least 3 h (DW+3-h) and 24–72 h (DW+24-h) after ablation.

Excision of selected putative sensilla

Different setae on the distal halves of lateral flagella were excised to identify the specific source of chemosensory input to AGB. Six and nine lobsters were used for GC excision and excision of all tuft setae, respectively. Each lobster was removed from its tank, wrapped in wet paper towels and restrained in an Instant Ocean® bath. Lateral flagella were

then sham-ablated as described above. Lobsters were tested for responses to chemical stimuli 3 h later. After at least 24 h, each lobster was again removed from its tank, wrapped in wet paper towels and restrained in an Instant Ocean® bath. Each lateral flagellum was placed on a dissecting tray and held in place with a staple pin. Selected setae were identified with the aid of a dissecting microscope and excised using a scalpel. At least 3 h later (excise+3-h), spiny lobsters were tested for responses to chemical stimuli. Finally, after another recovery period of 24–72 h, the lateral flagella were ablated with DW and the lobsters tested for responses 3 h (DW+3-h) and 24–72 h (DW+24-h) after excision.

Proper excision of setae was verified using scanning electron microscopy (SEM). Lateral flagella were removed from lobsters and rinsed with DW. The flagella were then air dried, coated with gold–palladium and examined with a Hitachi S-2460 N scanning electron microscope.

Presentation of stimuli

Spiny lobsters were tested with two chemical stimuli (0.5 and 0.05 mM Glu) and ASW. Stimuli were presented in triplicate and in random order. Chemical stimuli (5 ml) were presented using a hand-held pipette, which was placed near the antennules to minimize dilution of stimuli. At least 10 min were allowed to pass before introduction of the next stimulus in order to minimize residual responses to a previous stimulus and avoid desensitization. All trials were videotaped, beginning at least 15 s before each stimulus was presented and continuing for 2 min afterwards.

Data analysis

The magnitude of the AGB response was determined from videotapes. Antennule wipes were recorded for all ablation experiments. A wipe was defined as a single pull of either antennule through the setal combs of the third maxillipeds. In addition, auto-grooms were recorded for the experiment in which the third maxillipeds were ablated. A groom was defined as the rubbing back and forth of the third maxillipeds. A preliminary study indicated that ablation of maxillipeds resulted in a decrease in number of auto-grooms towards Glu and no change in the number of wipes (Barbato *et al.*, 1996). Pre-stimulus responses were determined by counting the number of auto-grooms (maxilliped ablation experiment only) or number of wipes that occurred during the 15 s preceding stimulus introduction. Post-stimulus responses were determined by counting the number of auto-grooms (maxilliped ablation experiment only) or number of wipes that occurred for the 2 min following stimulus introduction. Response rates were calculated (wipes/min) and pre-stimulus rates were subtracted from post-stimulus rates. Responses to the three presentations of a stimulus were averaged to obtain a mean response rate.

Differences in response to a given stimulus following ablation procedures were analyzed using either parametric statistical tests (if parametric assumptions were met) or non-parametric statistical tests (if parametric assumptions were not met). With the exception of ablation of medial flagella, one-way repeated measures analysis of variance (ANOVA) or Friedman's repeated measures ANOVA on ranks were used (Sigmastat^{TM}; Jandel Scientific) to compare responses to a given stimulus following each ablation or excision procedure in an experiment. For example, in the experiment in which the distal halves of lateral flagella were ablated, responses to 0.05 mM Glu after no ablation (None), sham ablation (Sham), at least 3 h recovery from DW ablation (DW+3-h) and at least 24 h recovery from DW ablation (DW+24-h) were compared. Where statistical differences were found, *post hoc* pairwise comparisons between responses to a stimulus following ablation procedures were performed using the Student–Newman–Keuls (S–N–K) test. For the experiment involving ablation of medial flagella, we used a paired *t*-test (parametric assumptions met) or a Wilcoxon signed ranks test (parametric assumptions not met) to compare responses to chemical stimuli after the two ablation procedures (sham and DW ablation).

Results

Suppression of wipe response in AGB occurred only following destruction of aesthetascs, either by DW ablation or excision of these setae. Removal of other setae did not affect the wipe rate.

DW ablation of whole appendages and regions of appendages

DW ablation of distal halves of the lateral flagella resulted in a loss of responsiveness to Glu (Figure 2A). This was most evident when lobsters were tested with the higher concentration of Glu. Responses to 0.5 mM Glu were significantly less when tested 3 h after DW ablation, compared to responses before ablation, after sham ablation, or 24–72 h after DW ablation [one-way repeated measures ANOVA, $n = 6$, $F(3,15) = 6.249$, $P = 0.006$, S–N–K tests, $P < 0.05$]. There were no significant differences between responses before ablation (None), after sham ablation (Sham) and after a 24–72 h recovery period (DW+24-h) for responses to 0.5 mM Glu (S–N–K tests, $P > 0.05$). Therefore, recovery of wipe responses was evident in spiny lobsters tested at least 24 h after DW ablation. The results were not quite as dramatic when the lobsters were tested with 0.05 mM Glu, although a similar pattern was apparent. At this stimulus concentration, responses measured 3 h after DW ablation were significantly less than those following sham ablation, but not significantly different to responses measured before ablation and 24–72 h after DW ablation [one-way repeated measures ANOVA, $n = 6$, $F(3,15) = 3.484$, $P = 0.043$, S–N–K tests, $P < 0.05$]. As expected, very few wipes were produced in response to ASW, which remained unchanged after each ablation technique [one-

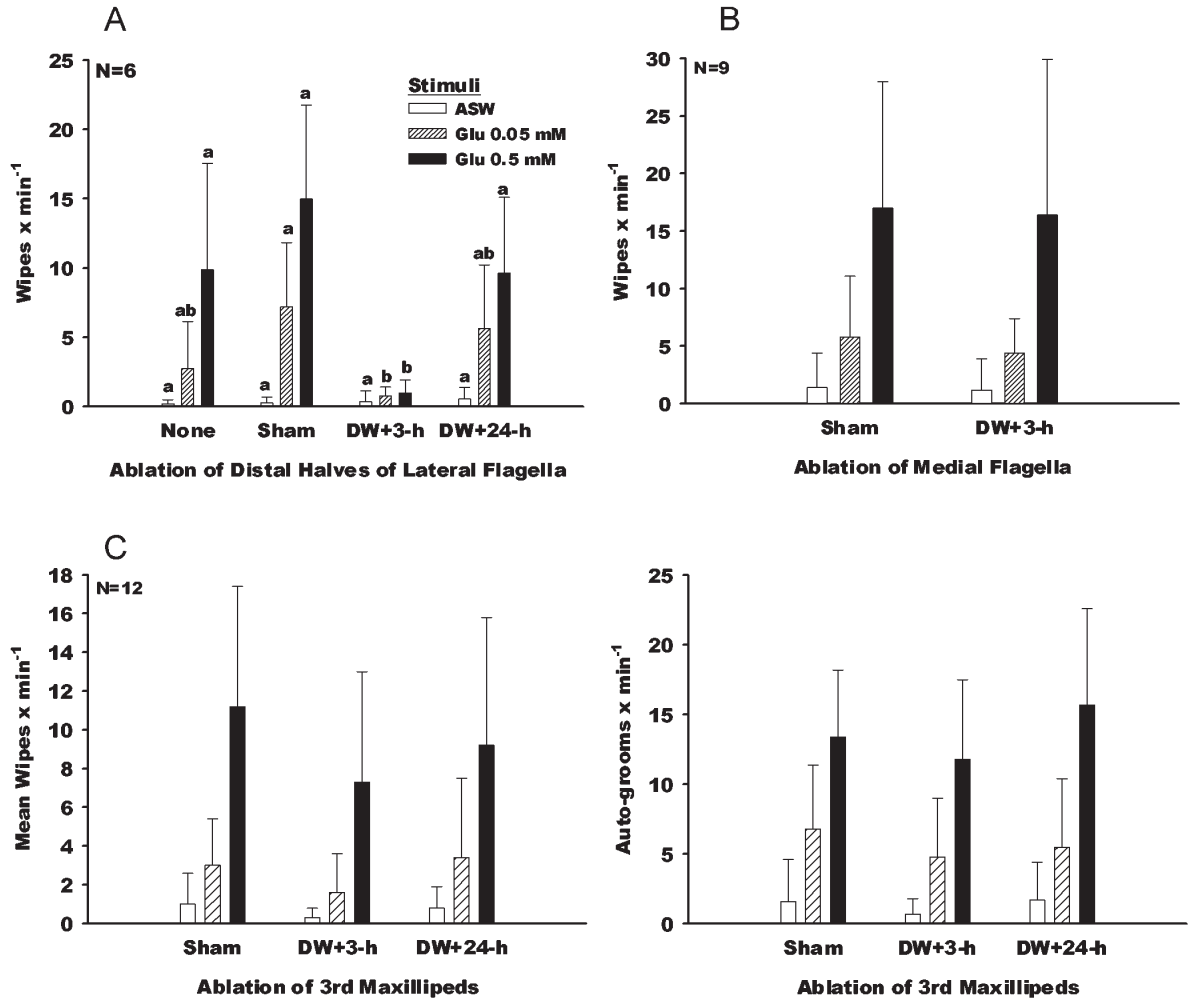

Figure 2 AGB responses to ASW and 0.05 and 0.5 mM Glu following DW ablation of the distal halves of the lateral flagella of the antennules **(A)**, medial flagella **(B)** and third maxillipeds **(C)**. (A) DW ablation of the distal halves of the lateral flagella of the antennules. Responses following no ablation (None), at least 3 h following sham ablation (Sham), at least 3 h (DW+3-h) and at least 24 h (DW+24-h) following ablation with DW are shown. Each bar shows the mean number of wipes/min or auto-grooms/min [(C) right graph only] and standard deviations (capped vertical lines). For (A), ablation procedures with same letter (a or b) do not have significantly different wipe rates, whereas ablation procedures with different letters have significantly different wipe rates (one-way repeated measures ANOVA and *post hoc* S–N–K tests, $P < 0.05$). For (B) and (C), none of the ablation procedures were significantly different, therefore no *post hoc* tests were performed (see text for details).

way repeated measures ANOVA, $n = 6$, $F(3,15) = 0.413$, $P = 0.746$].

No other DW ablation procedure attenuated wipe rates towards Glu. DW ablation of the medial flagella did not produce a statistically significant change in response to 0.5 mM Glu, 0.05 mM Glu or ASW, compared to responses following sham ablation (Figure 2B; Wilcoxon signed rank test, $n = 9$; 0.5 mM Glu, $W = -15.000$, $P = 0.426$; ASW, $W = 0.0000$, $P = 1.000$; 0.05 mM Glu, paired t-test, $n = 9$, $t = 0.727$, $P = 0.488$). Changes in responses (wipe rate and auto-groom rate) to chemical stimuli following DW ablation of the third maxillipeds were also not significant compared to responses following sham ablation (Figure 2C). Results for wipe rate were: one-way repeated measures ANOVA, $N = 12$, 0.5 mM Glu, $F(2,22) = 2.459$, $P = 0.109$; ASW, $F(2,22) = 1.446$, $P = 0.257$; 0.05 mM Glu, Friedman repeated measures ANOVA, $n = 12$, $\chi^2 = 1.319$, $P = 0.517$. Results for auto-groom rate were: one-way repeated measures ANOVA, $n = 12$, 0.5 mM Glu, $F(2,22) = 2.084$, $P = 0.148$; 0.05 mM Glu, $F(2,22) = 0.799$, $P = 0.463$; ASW, Friedman repeated measures ANOVA, $n = 12$, $\chi^2 = 2.3389$, $P = 0.303$.

Excision of selected putative sensilla

Excision of guard and companion setae (GC excision) had no effect on AGB responses. However, when all tuft setae were excised, AGB responses were extinguished.

While there were no decreases in wipe rates toward 0.05 and 0.5 mM Glu 3 h after GC excision compared to wipe rates following sham ablation, there were significant decreases in responses toward both 0.5 and 0.05 mM Glu 3 h after DW ablation compared to responses to 0.5 and

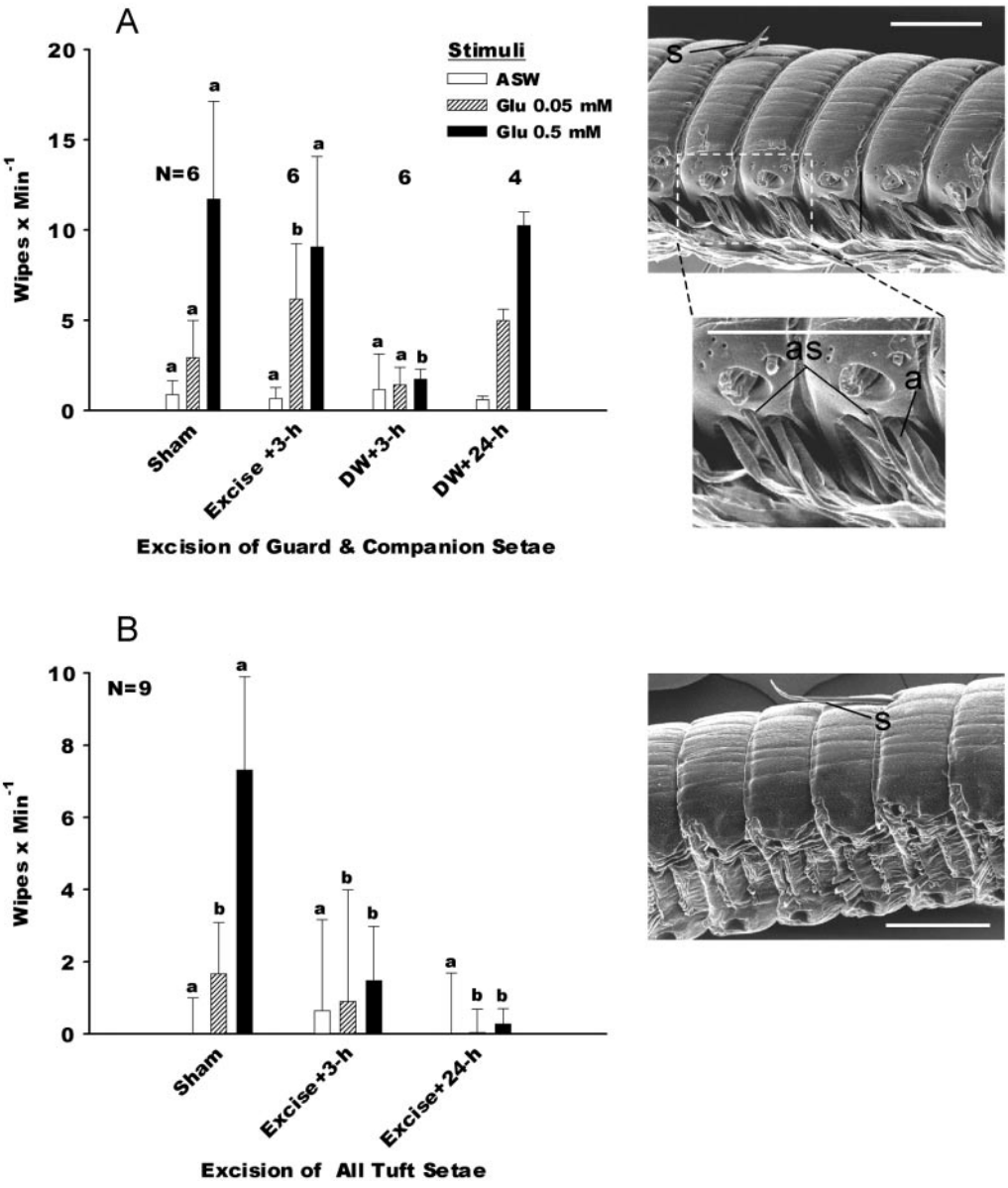

Figure 3 AGB responses to ASW and 0.05 and 0.5 mM Glu after excision of guard and companion setae followed by DW ablation of the distal halves of the lateral flagella of the antennules **(A)** and following excision of all tuft setae **(B)**. Also shown are SEMs of a lateral flagellum after each excision procedure. (A) Responses at least 3 h following sham ablation (Sham), at least 3 h following GC excision (excise+3-h) and at least 3 h (DW+3-h) and 24 h (DW+24-h) following ablation with DW are shown. Each bar shows the mean number of wipes/min and standard deviation (capped vertical lines). Ablation procedures with same letter (a or b) do not have significantly different wipe rates, whereas ablation procedures with different letters have significantly different wipe rates (one-way repeated measures ANOVA and *post hoc* S–N–K tests, $P < 0.05$). To the right of the bar graph is an SEM of a lateral flagellum after excision of guard and companion setae, showing remaining simple (s), asymmetric (as) and aesthetasc setae (a). Scale bar = 500 μm. (B) Responses at least 3 h following sham ablation (Sham), at least 3 h (excise+3-h) and at least 24 h (excise+24-h) following excision of all tuft setae are shown. Bars, capped vertical lines and letters represent same statistics as in (A). To the left of the bar graph is an SEM of a lateral flagellum after excision of guard, companion, aesthetasc and asymmetric setae, showing a remaining simple setae on the dorsal side. Scale bar = 500 μm.

0.05 mM Glu following GC excision [Figure 3A, one-way repeated measures ANOVA, 0.5 mM Glu, $n = 6$, $F(2,10) = 12.044$, $P = 0.002$; 0.05 mM Glu, $n = 6$, $F(2,10) = 3.484$, $P = 0.043$, S–N–K tests for either 0.5 or 0.5 mM, $P < 0.05$]. Recovery of behavior (DW+24-h) was tested on only four out of six lobsters and therefore was not included in the statistical tests. However, AGB appeared to return to previous magnitudes following at least a 24 h recovery period.

Excision of all tuft setae produced a significant decrease in wipe rate in response to 0.5 mM Glu compared to responses following sham ablation [Figure 3B, one-way repeated measures ANOVA, $n = 9$, $F(2,16) = 41.374$, $P < 0.001$; S–N–K multiple comparison tests, $P < 0.05$].

Table 2 Summary of results for antennule ablation experiments: setae affected and AGB responses to Glu

Setal type	Region ablated			
	Lateral flagella of antennules (distal halves)	Medial flagella of antennules	Guard and companion setae	All tuft setae
Non-tuft				
On lateral flagella	partial removal	functional	functional	functional
On medial flagella	functional	nonfunctional	functional	functional
Tuft				
Guard	nonfunctional	functional	nonfunctional	nonfunctional
Companion	nonfunctional	functional	nonfunctional	nonfunctional
Aesthetasc	nonfunctional	functional	functional	nonfunctional
Asymmetric	nonfunctional	functional	functional	nonfunctional
AGB response to Glu	none	full	full	none

No AGB recovery was observed even after 24 h following excision of setae (S–N–K tests, $P > 0.05$). Decreases in wipe rates toward 0.05 mM Glu were not evident, probably due to the overall lower sensitivity toward less concentrated stimuli [one-way repeated measures ANOVA, $n = 9$, $F(2,16) = 1.542$, $P = 0.244$].

Excision techniques were effective in removing specific setae as determined by visual inspection of scanning electron micrographs. GC excision removed guard and companion setae as expected, leaving intact aesthetascs as well as asymmetric setae and simple setae (Figure 3A). Excision of all tuft setae removed all setae on the ventral side of the distal half of the lateral flagellum, leaving intact the simple setae found dorsally (Figure 3B).

Discussion

These results provide further evidence that AGB is initiated via a labeled-line pathway. Of the four chemosensory sensilla that have been identified on the antennules—aesthetascs, hooded, and short and medium simple setae—only the aesthetascs appear to provide the necessary and sufficient chemosensory input driving AGB (Table 2). Functional removal of aesthetascs and other tuft setae by DW ablation or surgical excision resulted in complete attenuation of AGB towards Glu. DW ablation of the medial flagella, which reduced the number of non-tuft setae available for chemosensory detection, had no effect on the ability to respond to Glu. In excision experiments, attenuation of AGB occurred only with removal of all tuft setae but not guard and companion setae alone. It should be noted that removal of aesthetascs along with guard and companion setae also resulted in removal of asymmetric setae. While it is not possible unequivocally to rule out the contribution of asymmetric setae to chemosensory input, there is no evidence at present that asymmetric setae are chemosensory. Furthermore, aesthetascs outnumber asymmetric setae (16:1) and are most likely more heavily innervated: ~300 per aesthetasc versus <15 per asymmetric seta if same as other nonaesthetasc sensilla (Cate and Derby, 2001). Finally, DW ablation of maxillipeds, which bear chemosensory serrate setae, had no effect on either the wiping or auto-grooming components of Glu-induced AGB.

The Role of ORNs in chemosensory mediation of AGB

Because of the stimulus specificity of chemosensory activation of AGB and the localization of that input to one specific type of sensilla, it is likely that chemosensory activation of AGB follows a labeled-line model. In effect, ORNs tuned to Glu and located in the aesthetascs provide adequate input to motor centers in the brain controlling antennule and 3rd maxilliped movements.

While ORNs express multiple excitatory receptor types (Cromarty and Derby 1997), one receptor type appears to predominate, either due to a higher density or affinity. In electrophysiological studies, cells best for a particular chemical, such as Glu or taurine, have been identified. Equivalent responses to the best and next-best chemicals can be activated by concentrations that are several orders of magnitude apart (Derby *et al.*, 1991; Daniel *et al.*, 1994; Cromarty and Derby, 1997). Biochemical ligand binding assays of cell membranes isolated from aesthetascs have identified independent olfactory receptor sites for adenosine-5′-monophosphate, taurine, D- and L-alanine and Glu (Michel *et al.*, 1993; Olson and Derby, 1995; Burgess and Derby, 1997). The narrow tuning of AGB clearly reflects these characteristics of ORNs.

The aesthetasc pathway mediates odorant activation of AGB

In an earlier paper we proposed that chemosensory processing leading to AGB most likely followed the nonaesthetasc pathway because this provided the most direct route leading to activation of AGB (Barbato and Daniel, 1997). In this

hypothesis, chemosensory input from neurons tuned narrowly to Glu and innervating nonaesthetasc sensilla project to the LAN. However, the results of the present study argue strongly against this hypothesis and suggest instead activation through the aesthetasc pathway.

Further processing of chemosensory input by the OL leading to AGB must fulfill several criteria. First, the integrity of the response properties of ORNs tuned to Glu must be retained within the OL. This could occur if specific functional ORN types terminate on specific glomeruli. In effect, ORNs most responsive to Glu might converge on a specific glomerulus or glomeruli. The olfactory system of vertebrates appears to behave in this manner. Considerable evidence now exists to conclude that the individual glomeruli in the olfactory bulb receive input only from ORNs expressing a specific olfactory receptor type (Hildebrand and Shepherd, 1997; Mombaerts, 1999). In spiny lobsters, neuroanatomical and physiological studies indicate that most of the chemosensory neurons terminate on single glomeruli, while at least 10% project to multiple glomeruli (Schmidt and Ache, 1992). It has not been determined whether glomeruli receive convergent input from ORNs with specific response properties.

Secondly, output from the OL mediating AGB activation must go through a narrow band filter allowing mostly Glu responses to pass. This could be accomplished by projection neurons innervating only glomeruli receiving input from Glu-best ORNs. While projection neurons are multiglomerular, dense innervation is observed in only three to four glomeruli (Wachowiak and Ache, 1994; Schmidt and Ache, 1996b). In one study of the crayfish, identified OL projection neurons were each responsive to a number of the single chemicals tested (Arbas *et al*., 1988). In spiny lobsters, extracellular recordings from interneurons further downstream from the OL, namely interneurons in the optic tract ascending from the brain and interneurons in the circumesophageal connectives descending from the brain, were found to be generally more responsive to a broader spectrum of chemicals than chemosensory afferents (Derby and Ache, 1984; Derby *et al*., 1984). In both crayfish and spiny lobsters, a small number of interneurons were characterized by narrow response spectra.

Thirdly, output from the OL mediating AGB activation must project monosynaptically or polysynaptically to the antennular neuropils. In crayfish, two classes of interneurons have been identified with dendritic branches in both the OL and the LAN (Arbas *et al*., 1988; Mellon and Alones, 1994). While these may provide a monosynaptic connection between the two regions, they do not display characteristics consistent with a labeled-line pathway. Arborization in the OL is extensive, impinging on many glomeruli, and a broad range of chemical stimuli elicit activity (Mellon and Allones, 1994). Alternatively, a polysynaptic pathway from the OL to the LAN might exist via the protocerebrum. Projection neurons associated with the OL ascend into the protocerebrum via the olfactory globular tract (Schmidt and Ache, 1996b). Chemosensitive projection neurons ascending and descending the protocerebral tract to the protocerebrum have been identified (Derby and Blaustein, 1988; Schmidt and Ache, 1996b). Other, as yet undefined pathways are also possible.

Aesthetasc and nonaesthetasc pathways mediate search behavior

There is considerable evidence that search behavior, in which spiny lobsters orient and move towards sources of food odorants, requires chemosensory input from a number of types of sensilla. Early studies indicated that the lateral flagella played a major role in search behavior, although the medial flagella appeared to provide a minor role (McLeese, 1973; Reeder and Ache, 1980; Devine and Atema, 1982). Recent ablation studies suggest that aesthetasc and nonaesthetasc sensilla provide sensory input mediating search behavior. Ablation of aesthetascs must be accompanied by ablation of at least one other group of nonaesthetasc sensilla (i.e. ablation of all tuft sensilla, ablation of aesthetascs and medial flagella, ablation of all tuft sensilla and medial flagella) in order to observe attenuation of search behavior (Steullet *et al*., 2001). The ability to orient towards and locate successfully an upstream odor source and to discriminate between different odorants following aversive conditioning procedures is diminished only slightly in spiny lobsters in which either aesthetascs or nonaesthetasc antennular sensilla have been ablated (Derby *et al*., 2001; Steullet *et al*., 2002). Thus, processing leading to search behavior likely utilizes both aesthetasc (via the olfactory lobe) and nonaesthetasc pathways (via the LAN). These results, coupled with evidence that a large number of chemicals in odorant mixtures can activate search behavior, provide further argument for an across-neuron pattern of encoding for this behavior. It is becoming apparent that both models of stimulus encoding exist concurrently in lobster olfactory systems.

Recovery of AGB from osmotic shock

Within 24–72 h following DW ablation of antennules, spiny lobsters recovered behavioral sensitivity to Glu. This cannot be a result of replacement of damaged ORNs within this time. Normal turnover of aesthetasc sensilla and associated ORNs occurs over a period of three to six molts (Steullet, 2000). New aesthetascs proliferate at the proximal part of the flagellum and are not functionally active for several weeks after emergence. Damage to specific regions of the antennule via excision of aesthetascs can also cause death of ORNs and their replacement by new ORNs (Harrison *et al*., 2001). Over a period of several weeks, damaged cells degenerate and are replaced by new cells which must reconnect to the olfactory lobe. Thus, quick replacement of damaged ORNs with functional ORNs is unlikely. This is consistent

with the lack of recovery observed after excising all tuft setae (Figure 3B).

It is more likely that the damage caused by osmotic shock is reversible. Studies on blue crabs reveal that osmotic shock vesiculates the outer dendritic segments of ORNs (Gleeson *et al*., 1997). Physiological recordings from the antennular nerve of freshwater-acclimated blue crabs showed greatly diminished olfactory responses. Olfactory responses begin to show recovery within 24–48 h after transferring these crabs to saltwater. Recovery of olfactory function is correlated with lengthening of the outer dendritic segment, the location of receptors. Thus it is likely that the recovery of chemosensory-mediated AGB in spiny lobster is due to regeneration of the outer dendritic segments following DW ablation.

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