The Journal of Experimental Biology 208, 233-248
Published by The Company of Biologists 2005
doi:10.1242/jeb.01357



# Non-olfactory chemoreceptors in asymmetric setae activate antennular grooming behavior in the Caribbean spiny lobster *Panulirus argus*

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*Accepted 25 October 2004*

## Summary

**In the spiny lobster *Panulirus argus* the antennules carrying olfactory sensilla called aesthetascs and several types of other non-olfactory sensilla accompanying them are frequently groomed by the third maxillipeds in a stereotyped behavioral pattern. This behavior can be elicited by chemical stimulation with L-glutamate. Using selective sensillar ablations, we tested whether this behavior is driven by the numerous aesthetascs, which have been implicated as mediating this chemically elicited antennular grooming behavior in a previous investigation, or other, less numerous sensilla called asymmetric setae, which are tightly associated with aesthetascs. The selective sensilla ablations showed that the asymmetric setae are necessary and sufficient for driving chemically elicited antennular grooming. Bilateral elimination of the ca. 160 asymmetric setae almost completely abolished the behavior, whereas bilateral elimination of the ca. 2600 aesthetascs or of another type of sensilla associated with them (guard setae) did not cause a reduction in chemically elicited antennular grooming. Microscopical analysis of the morphological properties of the asymmetric setae revealed the presence of a terminal pore at the tip of the seta and a phalloidin-positive scolopale below its base. Since these structures have been identified in decapod crustaceans as modality-specific structures of bimodal chemo- and mechanosensory sensilla, we conclude that the asymmetric setae belong to this type of sensilla and thus have the appropriate features to function as chemoreceptors in the elicitation of antennular grooming. The identification of asymmetric setae and not aesthetascs as the drivers of chemically elicited antennular grooming suggests that it is not the olfactory pathway in the brain but a parallel pathway, constituted mainly by the lateral antennular neuropils, that is the neuronal substrate of this behavior. The lateral antennular neuropils receive non-olfactory sensory input from the antennule and contain the major arborizations of antennular motoneurons, allowing that direct sensory-motor coupling is involved in mediating the chemical elicitation of antennular grooming behavior.**

Key words: chemical senses, antennule, Crustacea, olfaction, spiny lobster, *Panulirus argus*.

## Introduction

Chemoreception in multicellular animals is generally organized into two or more ‘chemical senses’ or chemosensory pathways, differing in the physiology and location of the receptor neurons, the way the sensory information is processed in the central nervous system, and the behavioral output that is generated. One goal in the study of chemical senses is to functionally link these chemosensory pathways to behavioral responses. In aquatic animals, including decapod crustaceans, this problem is made more challenging by the fact that all of their chemical senses are tuned to water-soluble substances, like amino acids, organic acids, bile acids, sugars, nucleotides and peptides (Carr, 1988). In decapod crustaceans, an additional problem is the fact that the major chemosensory organ, the paired first antennae or antennules, contains more than one chemosensory pathway, each of which is activated by many of the same chemicals. Each antennule comprises three basal segments and two flagella, a lateral and a medial one, which are organized into numerous repeating units called annuli (Laverack, 1964; Steullet et al., 2000b). The basal segments and all annuli bear numerous and diverse types of sensilla, which are small cuticular setae innervated by sensory neurons.

The most obvious and usually most numerous type of antennular chemosensory sensilla are the aesthetascs (AE; Laverack, 1964; Spencer and Linberg, 1986; Grünert and Ache, 1988; Cate and Derby, 2001). Aesthetascs are long, slender, tube-like setae with an extremely thin cuticle. They are unique to the antennule, and on the antennule they are restricted to the lateral flagellum. Aesthetascs are classified as olfactory sensilla, based on their similarities with olfactory sensilla of insects (Laverack and Ardill, 1965; Spencer and Linberg, 1986; Grünert and Ache, 1988; Gleeson et al., 1996).

In the spiny lobster *Panulirus argus*, aesthetascs occur on each annulus in the distal third of the lateral flagellum, except for some annuli at the very tip of the flagellum (Laverack, 1964; Cate and Derby, 2001). A mature annulus bears two parallel rows of aesthetascs with 8–12 sensilla each. Each aesthetasc is innervated by ca. 300 chemosensory receptor neurons, but no mechanosensory receptor neurons (Laverack and Ardill, 1965; Grünert and Ache, 1988; Steullet et al., 2000b). Aesthetascs respond to a wide range of chemical stimuli that are typical for aquatic chemoreceptors (Anderson and Ache, 1985; Schmiedel-Jakob et al., 1989; Michel et al., 1991, 1993; Steullet et al., 2000b).

Besides the aesthetascs, numerous other sensilla of different types are located on the lateral and the medial flagella and the basal segments of the antennule. Most if not all of these sensillar types represent bimodal, chemo- and mechanoreceptive sensilla (Laverack, 1964; Derby, 1982; Spencer and Linberg, 1986; Cate and Derby, 2001) and are called 'non-olfactory' (Schmidt and Ache, 1996a) or 'non-aesthetasc' (Steullet et al., 2001; Cate and Derby, 2001) sensilla. Among the non-olfactory sensilla, two groups can be differentiated: sensilla that occur on both flagella and on the basal segments, and sensilla that are restricted to the lateral flagellum and, together with the aesthetascs, form a conspicuous 'tuft' of setae (Laverack, 1964; Spencer and Linberg, 1986; Cate and Derby, 2001). In spiny lobsters, the first group includes simple smooth setae (of different lengths), plumose setae, short setuled setae, and hooded setae (Cate and Derby, 2001). The non-olfactory 'tuft' sensilla comprise guard setae (GS), companion setae (CS), and asymmetric setae (AS; Laverack, 1964; Spencer and Linberg, 1986; Gleeson et al., 1993; Cate and Derby, 2001). On each mature aesthetasc-bearing annulus reside two guard setae, 2–4 companion setae, and one asymmetric seta. The guard setae flank the distal row of aesthetascs, 1 or 2 companion setae are located laterally to each guard seta, and the asymmetric seta is located between the end of the aesthetasc rows and the lateral guard seta (Laverack, 1964; Spencer and Linberg, 1986; Gleeson et al., 1993; Cate and Derby, 2001). None of the tuft setae except for aesthetascs has been analyzed in its ultrastructure or electrophysiological properties.

Sensory neurons of the aesthetascs and the non-olfactory sensilla project to two almost entirely separated pathways in the brain. Aesthetascs selectively innervate the olfactory lobe (OL), a glomerular neuropil of the deutocerebrum (Sandeman and Denburg, 1976; Mellon and Munger, 1990; Schmidt and Ache, 1992; Sandeman and Sandeman, 1994). The axons of the olfactory lobe projection neurons form a common fiber tract (olfactory globular tract, OGT) projecting to protocerebral neuropils in the eyestalk ganglia (Mellon et al., 1992; Schmidt and Ache, 1996b; Sullivan and Beltz, 2001a,b). These criteria define the aesthetasc–OL–OGT axis as the olfactory pathway of decapod crustaceans. Chemo- and mechanosensory neurons of the non-olfactory sensilla on both flagella project to a stratified but non-glomerular, bilobed neuropil of the deutocerebrum, the lateral antennular neuropil (LAN) (Schmidt et al., 1992; Schmidt and Ache, 1996a), which also contains the major arborizations of antennular motoneurons (Maynard, 1965; Schmidt and Ache, 1993; Roye, 1994). Thus the non-olfactory sensilla-LAN pathway represents a second antennular chemosensory pathway that parallels the olfactory pathway and also functions as the antennular motor center.

Linking specific, chemically elicited behavioral responses to particular sensilla types and hence to one of the two pathways has been challenging. Sensillar ablation experiments in *P. argus* led to the conclusion that detection of, orientation to, and associative learning of food-related chemicals can be mediated by either the olfactory or non-olfactory antennular pathway (Steullet et al., 2001, 2002; Horner et al., 2004). The only specific behavior definitively linked to one of the pathways is elicitation of courtship behavior in male blue crabs *Callinectes sapidus* by a female pheromone (Gleeson, 1982), which is elicited only by the aesthetasc pathway.

Recently, another behavior also has been attributed to activation of aesthetascs: antennular grooming behavior (AGB), produced by many decapod crustaceans spontaneously without any obvious sensory stimulation and occurring with an increased frequency after feeding (Maynard and Dingle, 1963; Snow, 1973; Farmer, 1974; Bauer, 1977, 1981; Alexander et al., 1980; Zimmer-Faust et al., 1984; Barbato and Daniel, 1997; Daniel et al., 2001; Wroblewska et al., 2002). AGB is a very distinctive and stereotyped behavior consisting of two major components. In the first component, called 'antennule wiping', both antennular flagella are repeatedly brought down towards the last pair of mouthpart appendages, the third maxillipeds. Then they are grabbed by their endopodites equipped with pad-like structures consisting of densely packed specialized setae (Farmer, 1974; Bauer, 1977; Alexander et al., 1980; Wroblewska et al., 2002) and are repeatedly pulled through these pads. The second component, called 'auto grooming', consists of rubbing movements of the two third maxillipeds against each other, and usually occurs after a bout of wipes. In several species of decapod crustaceans, including *P. argus*, both components of AGB can be elicited by chemical stimulation, but normally by only one chemical: L-glutamate (Barbato and Daniel, 1997; Daniel et al., 2001). In a series of sensillar ablation experiments on *P. argus*, Wroblewska et al. (2002) attempted to identify the sensilla responsible for the chemical elicitation of AGB. After establishing that 'tuft' sensilla on the lateral flagellum are solely responsible for the elicitation of AGB by L-glutamate, guard and companion setae were selectively ablated. From the result of this experiment, in which the elicitation of AGB by L-glutamate was unaffected, it was concluded that chemical elicitation of AGB is mediated by aesthetascs. This conclusion was based on the argument that aesthetascs are far more numerous than asymmetric setae, the only other sensilla not eliminated by the ablations (Wroblewska et al., 2002).

Here we report further ablation experiments in the spiny lobster *Panulirus argus* aimed at scrutinizing the conclusion that the aesthetascs, and hence the olfactory pathway, mediate the chemical elicitation of AGB. Selective removal of aesthetascs or asymmetric setae showed that asymmetric setae

are necessary and sufficient for the elicitation of AGB and that aesthetascs do not contribute. Based on scanning electron microscopy and confocal microscopy, we provide morphological evidence that asymmetric setae represent typical bimodal chemo- and mechanosensitive sensilla. From these findings, we conclude that AGB is mediated by the non-olfactory sensilla-LAN pathway.

## Materials and methods

### *Animals*

Caribbean spiny lobsters *Panulirus argus* Latreille 1804 (carapace length: range, 46–79 mm; mean ± S.D., 61.7±6.0 mm; *N*=42) were obtained from the Florida Keys Marine Laboratory, shipped to Georgia State University, and held in communal 800 liter aquaria containing aerated, recirculated, filtered artificial seawater (ASW) (Instant Ocean, Aquarium Systems: Mentor, OH, USA). Animals were maintained in a 12 h:12 h light:dark cycle and fed shrimp or squid 3 times a week. Only intermolt animals with fully intact antennules were used. About 2–3 weeks before the start of the experiments, test animals were individually placed into 16 liter aquaria (20 cm wide×20 cm tall×40 cm long) containing aerated, recirculated, filtered ASW, because pilot experiments showed that this length of time was needed for animals to acclimatize to the experimental situation and reach sufficient responsiveness.

### *Chemical stimuli*

Stock solutions (0.1 mol $l^{-1}$, pH 7.9) of L-sodium glutamate were prepared in ASW and stored in aliquot samples at –20°C until needed. Prior to an experiment, the samples of stock solution were thawed and diluted to 0.5 mmol $l^{-1}$ with ASW.

All chemicals were obtained from Sigma (St Louis, MO, USA) unless otherwise noted.

### *Behavioral assays*

Animals were tested in individual 16 liter aquaria for elicitation of antennular grooming behavior (AGB) by application of 3 ml of 0.5 mmol $l^{-1}$ L-glutamate (in ASW) using a hand-held syringe with a thin polyethylene tube attached to its tip. The opening of the tube was placed close to the antennules to minimize dilution of the stimulus. All experiments were performed at about the same time of the day, in the early afternoon, with illumination by fluorescent lighting. On each experimental day, each animal was stimulated 3 times, allowing about 1 h between stimulations. In the first set of experiments (Exp. 1), we counted the total number of complete wipes of both antennules for 2 min before introduction of the stimulus and for another 2 min after stimulation in order to subtract baseline activity from activity induced by the stimulation. Since the number of spontaneous wipes in the 2 min before stimulation was extremely small (in a total of 90 trials on 18 animals, only 1 wipe occurred in the pre-stimulation period) compared to the induced activity, we only counted the number of wipes in the 2 min post-stimulation period. The numbers given in the Results section are based on these counts. A 'complete wipe' is defined as a sequence of movements, in which at least one flagellum of the lowered antennule touched the third maxillipeds and was actively pulled forward while the third maxillipeds moved backwards. This excluded occasionally occurring 'incomplete wipes', in which the third maxillipeds executed grasping and backwards pulling movements typical of wiping, but without actually being in touch with an antennule. The number of wipes was counted by direct observation during the experiments. About 10% of the animals that were initially tested for responsiveness did not respond to the stimulation with L-glutamate even after some days in the test tanks. These animals were eliminated from the experiments and replaced by others.

### *Ablations*

To address whether aesthetascs or asymmetric setae are responsible for the chemical elicitation of AGB, we performed selective bilateral ablations of the sensilla in question and control experiments, in which other sensilla not implicated in AGB (the guard setae) were ablated. All ablations were performed on animals immobilized on a plastic retaining device within a shallow container of ASW with the proximal region of the lateral flagella stapled to a Sylgard®-coated platform. Sensilla were removed surgically under a dissecting scope (SZ40, Olympus: Melville, NY, USA) by cutting them off at their base using microblades (Fine Science Tools: North Vancouver, Canada) or with hand-honed minuten pins held by a blade holder. In all cases, the tip of the lateral flagellum comprising ca. 15 slender annuli was cut off, since it was not possible to reliably eliminate the sensilla in question from this region.

Since aesthetascs and asymmetric setae are located within 2 rows of comparatively massive and stout guard setae, it is not possible to surgically remove one of the former sensilla populations without removing at least one row of guard setae. Thus for experimental animals, either the lateral guard setae were removed together with the asymmetric setae (AS-ablation) or the medial guard setae were removed together with the aesthetascs (AE-ablation). To control for a possible effect of the ablation of guard setae and to serve as a general control for possible effects of the surgery itself, either the lateral or medial guard setae were selectively removed in two groups of experimental animals (GS-ablation).

In one experiment, a group of 8–14 spiny lobsters was tested in the above detailed way for 3 consecutive days. On the following day (or the following 2 days in the case of AE-ablation in Exp. 2, see below), the sensilla in question were surgically removed. In those experiments in which the animals were subdivided into two subgroups that were subjected to removal of different sensilla (Exp. 3 and 4, see below), care was taken to match animals according to their previous responsiveness to avoid a possible bias in one of the treatment groups that might obscure the treatment effect. This 'matched selection' was used because of the substantial variation in the baseline responsiveness between individuals. After at least 1

day of rest after surgery, animals were retested for 3 consecutive days. In some experiments, animals were retested in further test periods of 3 consecutive days following the initial retest period over longer time intervals.

In Exp. 1, animals were subjected to two consecutive surgeries. In the first surgery, lateral guard setae were ablated, and after a retest period of 3 consecutive days, asymmetric setae were removed. In Exp. 2, the medial guard setae and the aesthetascs were removed simultaneously. In Exp. 3, two subgroups of animals were treated differently: in one subgroup, lateral guard setae and asymmetric setae were removed simultaneously; in the other subgroup, medial guard setae and aesthetascs were removed simultaneously. In Exp. 4, two subgroups of animals were treated differently: in one subgroup, medial guard setae were removed; in the other subgroup, medial guard setae and aesthetascs were removed simultaneously.

To evaluate the completeness of selective sensilla removal, lateral flagella of all experimental animals were collected and fixed in 4% paraformaldehyde (+ 15% sucrose) for several hours. After rinsing in 0.1 mol $l^{-1}$ Soerensen phosphate buffer (SPB), the flagella were viewed under a high-power dissecting scope (MZ 16, Leica: Wetzlar, Germany), and the number of sensilla that had escaped removal, as well as the number of sensilla that were removed unintentionally (AS, in the case of AE-ablation), were counted. To document the completeness of sensilla removal, lateral flagella were imaged using a high-resolution digital camera (DC 500, Leica: Wetzlar, Germany) attached to the dissecting scope.

### *Data analysis and statistics*

The number of wipes counted in each 2 min post-stimulation period represents the wipe rate (wipes/2 min). Responses to the three daily presentations of the standard stimulus were averaged for each individual to obtain its mean daily wipe rate. The individual mean daily wipe rates were averaged over the entire population of experimental animals. Differences in the mean daily wipe rates of the population throughout the duration of the respective experiment were analyzed using one-way repeated-measures analysis of variance (RM-ANOVA). Since for the blocks of 3 consecutive days, in which the test conditions were constant, the mean daily wipe rates of the population usually did not differ significantly (with only two exceptions: Day 07 and Day 09 in Exp. 1 and Day 08 and Day 10 in AE-ablations of Exp. 3), we averaged the mean daily wipe rates of the population for each of these blocks. Differences in the 3-day population means were analyzed using one-way RM-ANOVA and paired *t*-tests where appropriate. All statistical tests were performed with statistics software (GraphPad Prism, GraphPad Software: San Diego, CA, USA).

### *Scanning electron microscopy*

For scanning electron microscopy, lateral flagella of intermolt specimens of *Panulirus argus* were cut off proximal to the tuft region under *Panulirus* saline (460 mmol $l^{-1}$ NaCl, 13 mmol $l^{-1}$ KCl, 13.6 mmol $l^{-1}$ $CaCl_2$, 10 mmol $l^{-1}$ $MgCl_2$, 14 mmol $l^{-1}$ $Na_2SO_4$, 3 mmol $l^{-1}$ Hepes, 1.7 mmol $l^{-1}$ glucose, pH 7.4 adjusted with NaOH, 950 mOsmol) and cleaned by sonication for ca. 10 min (VWR Model 50T, VWR International: West Chester, PA, USA). The tuft region was then cut into pieces that were fixed by immersion in 5% glutaraldehyde (in 0.1 mol $l^{-1}$ SPB + 15% sucrose) followed by 2% osmium tetroxide (in 0.1 mol $l^{-1}$ SPB) as described in detail previously (Gnatzy et al., 1984). After rinsing with SPB for 4× 30 min, the pieces were dehydrated in an ascending ethanol series (50%, 70%, 85%, 95%, 100%) for 2× 30 min at each concentration. Then the pieces were incubated for 1 h in hexamethyldisilazane and air dried (Nation, 1983). The pieces were glued to holders, sputtered with palladium (Vacuum Desk II, Denton: Moorestown, NJ, USA), and viewed in a scanning electron microscope (Stereoscan 420, Leica: Wetzlar, Germany) equipped with digital image capturing (LEO-32, Leica: Wetzlar, Germany).

### *Confocal microscopy*

For confocal microscopy, lateral flagella of late premolt and early postmolt specimens of *Panulirus argus* were cut off proximal to the tuft region under *Panulirus* saline and divided into pieces that were fixed in 4% paraformaldehyde (in 0.1 mol $l^{-1}$ SPB + 15% sucrose) for at least 4 h. After rinsing with SPB, the pieces were embedded in gelatin as described in detail previously (Schmidt et al., 1992). The gelatin-blocks were hardened with 4% paraformaldehyde overnight at 4°C, rinsed briefly with SPB and then cut into 50 µm or 70 µm thick sagittal sections on a vibrating microtome (VT 1000S, Leica: Wetzlar, Germany). The free-floating sections were incubated overnight in AlexaFluor568-labeled phalloidin (Molecular Probes: Eugene, OR, USA) at a dilution of 1:200 in SPB containing 0.3% Triton-X-100. Afterwards the sections were rinsed 3× 30 min in SPB, then incubated for 20 min in Hoechst 33258 diluted 1:100 in SPB from a stock solution of 1 mg $ml^{-1}$, and after a final rinse in SPB, coverslips were placed on top of the sections in (1:1) glycerol/SPB containing 5% diazabicyclol[2.2.2]octane (DABCO) to prevent bleaching. The sections were viewed and imaged on a confocal microscope (LSM 510, Zeiss: Jena, Germany) using the associated software package. Stacks of 0.5–1.0 µm thick optical sections covering the entire section thickness were collected, and the stacks were then collapsed to produce single two-dimensional images.

### *Image processing*

All digital images were processed by a graphics program (Paint Shop Pro 5, Jasc Software: Eden Prairie, MN, USA) before they were arranged to the final figures using a presentation program (PowerPoint, Microsoft: Redmond, WA, USA).

## Results

### *General observations on antennal grooming behavior*

Pre-ablation animals reliably (in 361 of 378, or 95.5%, of

the trials) responded with AGB to stimulation with L-glutamate. The magnitude of AGB varied considerably between animals. The mean wipe rate over the 9 trials of the initial 3-day test period ranged from 2.0 to 32.6 wipes/2 min (mean ± S.D. = 15.1±7.5; median=13.8). In general, AGB was organized in a well-defined temporal pattern, such that wipes almost always occurred in bouts separated by pauses. The first bout of wiping usually began within a few seconds after application of the stimulus and typically lasted between 10 and 50 s. Then a pause of 30–60 s occurred before the second bout of wiping, which was shorter and contained fewer wipes than the first bout. In some very responsive animals, another pause occurred after the second bout, followed by a third bout of wiping.

In bilateral coordination of wiping as well as the way in which the antennules were grasped by the third maxillipeds, animals showed individually different 'styles', to which they adhered over many trials. In the bilateral coordination of wiping, two different patterns could be clearly differentiated: either both antennules were wiped simultaneously, or a bout of wiping of one antennule was followed by a bout of wiping of the other antennule (usually with about the same number of wipes). Alternating wiping of both antennules also occurred but was much rarer. In almost all animals, a typical response contained elements of both major patterns, but the relative proportion of both varied substantially between individuals constituting typical 'styles' of wiping patterns. Individual animals differed greatly in the way in which the antennules were grasped: usually both flagella were pulled through the grooming brushes on the third maxillipeds, but sometimes only one was in contact with them (wipes were counted). Rarely (but in some individuals quite frequently) the antennule was not in touch with the maxillipeds but still pulled forward in the typical fashion (wipes not counted); at other times, the antennules were grabbed correctly by the maxillipeds but not pulled while the maxillipeds also failed to execute their normal backward movement (wipes not counted).

As described previously, AGB in the spiny lobster, as in other decapod crustaceans, is a stereotyped behavior and consists of a sequence of movements that can readily be distinguished from all other behaviors (Maynard and Dingle, 1963; Snow, 1973; Farmer, 1974; Bauer, 1977, 1981; Alexander et al., 1980; Zimmer-Faust et al., 1984; Barbato and Daniel, 1997; Daniel et al., 2001; Wroblewska et al., 2002). Clearly, the antennules and third maxillipeds are the main appendages involved in this behavior, and their movements define it. However, according to our observations, AGB is more complex than that. Frequently, AGB was 'assisted' by coordinated movements of the first 1 or 2 pairs of legs, helping to bring the antennular flagella down to the maxillipeds. Furthermore, AGB also was usually accompanied by a change in body posture. In the resting state, the body of the animals had a horizontal to slightly frontally raised orientation, which upon stimulation changed to a posture in which the frontal part of the body was raised considerably to accommodate the backward and downward movement of the antennules towards the mouthparts. In their resting posture and activity, the experimental animals also adhered to individually different styles. The resting posture reached from lying flat on the bottom to standing high on maximally extended legs, and the resting activity reached from no obvious locomotor activity (ca. 80% of the animals) to continuous walking and probing with the legs (ca. 10% of the animals). Both aspects seemed to have a systematic influence on the magnitude of AGB: animals that had a flat resting posture showed less intense AGB and much more often failed to respond at all than animals with a raised resting posture. Animals that were very active during rest seemed to respond less than more inactive animals with similar resting posture.

### *Effect of selective sensilla removal*

#### *Experiment 1*

Exp. 1 examined the effect of the removal of asymmetric setae (AS) on chemically elicited AGB (Fig. 1). It started with

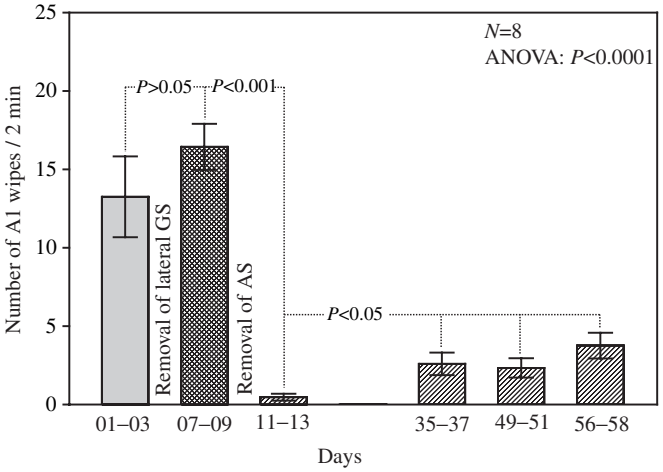


Fig. 1. Experiment 1. Effect of the bilateral selective removal of the lateral guard setae (GS) and the following bilateral selective removal of the asymmetric setae (AS) on the mean wipe rate of the antennules after stimulation with 3 ml of 0.5 mmol $l^{-1}$ L-glutamate. Wipe rate values are means ± S.E.M. of *N*=8 experimental animals (*Panulirus argus*) over the 9 trials of an experimental 3-day block; experimental days are numbered chronologically. Note that selective removal of lateral GS does not cause a decrease in the mean wipe rate (Days 07–09) compared to the mean wipe rate measured in the unoperated animals in the initial 3-day block (Days 01–03). Note further the almost total loss of antennular wiping after selective removal of the AS (Days 11–13) and the small but constant functional recovery of wiping after longer time periods (Days 35–37, Days 49–51, Days 56–58). ANOVA shows an overall statistically significant difference between the columns ($P$<0.0001). Pair-wise comparisons by Newman–Keuls *post* tests between the means of the 3-day blocks reveal no significant difference between the pre-ablation (Days 01–03) and the post-GS-ablation (Days 07–09) blocks ($P$>0.05), whereas the post-AS-ablation block (Days 11–13) differs significantly ($P$<0.001) from the pre-AS-ablation block (Days 07–09) and from the retest blocks following after longer time periods ($P$<0.05 for all three blocks). Wipe rates of all 3-day blocks (means ± S.E.M.): Days 01–03, 13.3±2.6; Days 07–09, 16.4±1.5; Days 11–13, 0.5±0.2; Days 35–37, 2.6±0.7; Days 49–51, 2.3±0.6; Days 56–58, 3.8±0.8.

a period of 3 consecutive days in which the baseline responsiveness of the animals ($N$=8) was determined. During these 3 days, the mean wipe rates increased slightly and continuously, from 11.5±2.3 to 15.1±3.4 wipes/2 min (mean ± S.E.M.), but these differences were not statistically significant ($P$>0.05, RM-ANOVA, Newman–Keuls *post*-test). Removal of the lateral guard setae (GS; Fig. 2A), which was required to access the AS for their later removal but also served as a control for possible non-specific effects of the surgery itself, did not significantly change the wipe rate on the day following the surgery (12.2±1.7 wipes/2 min) compared to the last day before surgery. In the 2 following days, the wipe rate again increased continuously, surpassing the maximum pre-surgery wipe rate on both days (day 2, 16.1±1.4 wipes/2 min; day 3, 20.7±2.7 wipes/2 min). The difference in wipe rate between the post-surgery days 1 and 3 was statistically significant ($P$<0.01, RM-ANOVA, Newman–Keuls *post*-test). Comparing the mean wipe rates between the 3-day pre- and post-surgery blocks revealed no statistically significant difference ($P$>0.05; RM-ANOVA, Newman–Keuls *post*-test). Collectively these data demonstrate that (1) AGB is not influenced by removal of the lateral GS (confirming previous observations by Wroblewska et al. 2002), (2) under the experimental conditions used, surgical trauma at most has a small, non-significant negative effect on AGB, and (3) independent of sensilla removal, the mean wipe rate tends to increase over the 3 consecutive days of an experimental block.

The subsequent removal of the AS (Fig. 2B) caused a dramatic and statistically significant reduction of the mean wipe rate. From 1.0±0.6 wipes/2 min on post-surgery day 1, the wipe rate dropped further and actually reached 0 on post-surgery day 3. Comparing the mean wipe rates between the 3 day pre- and post-AS-ablation blocks showed that this difference is significant statistically ($P$<0.001, RM-ANOVA, Newman–Keuls *post*-test). This result demonstrates that AS are necessary for the chemical elicitation of AGB.

Retesting ablated animals after longer time intervals (ca. 3 weeks, Days 35–37; 5 weeks, Days 49–51; 6 weeks, Days

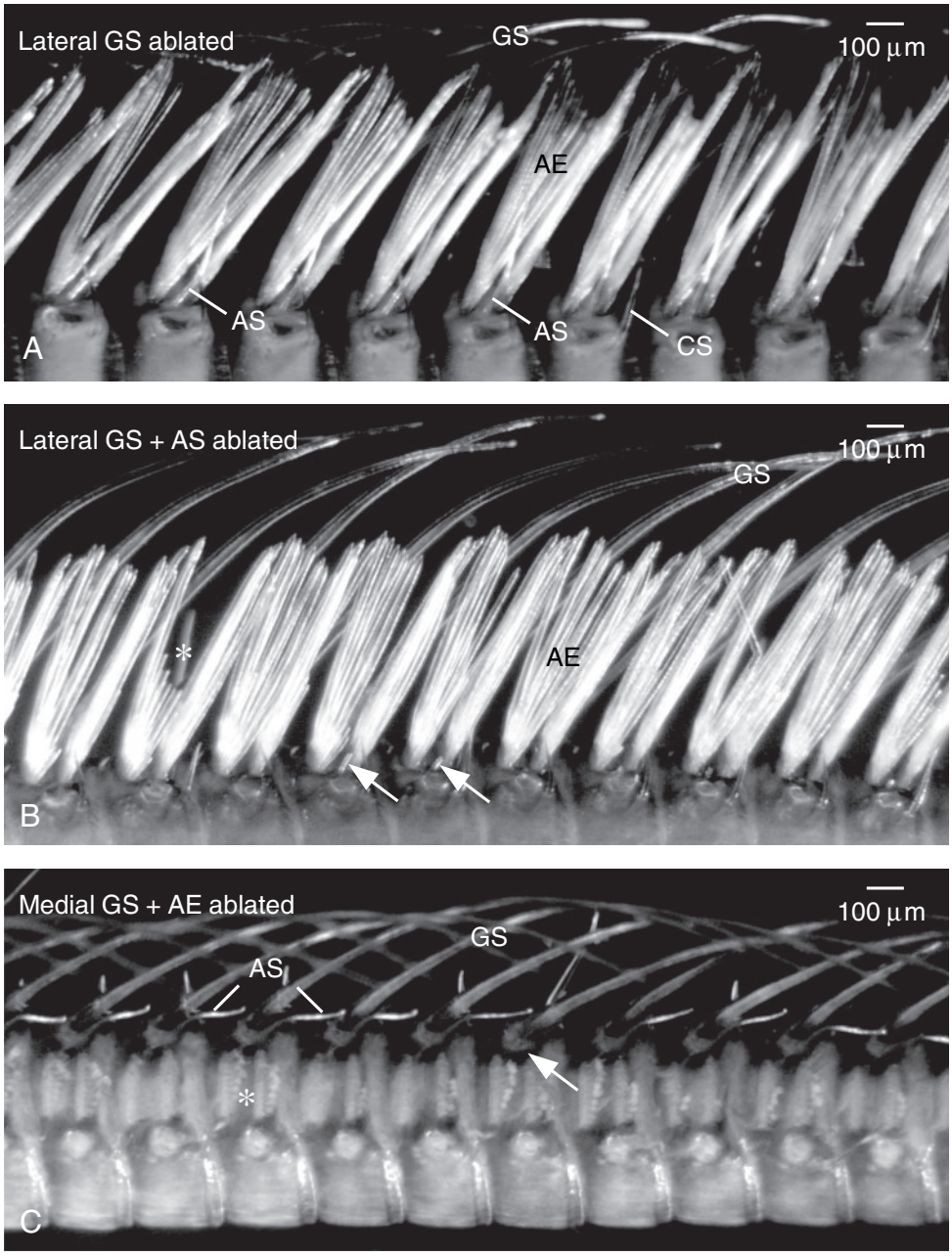


Fig. 2. Examples of the efficiency of selective sensillar shaving. Light micrographs. (A) Lateral guard setae selectively removed. Every annulus bears two rows of aesthetascs (AE) and one asymmetric seta (AS) at their lateral edge. Medial guard setae (GS); companion setae (CS). (B) Lateral guard setae and asymmetric setae selectively removed (animal from Exp. 1). Every annulus bears two rows of aesthetascs (AE), some of which are damaged (asterisks) either by the shaving procedure or due to natural causes. Of asymmetric setae, only some stumps remain (arrows). Medial guard setae (GS). (C) Medial guard setae and aesthetascs selectively removed (animal from Exp. 2). Of the aesthetascs, only bases remain (asterisk). Every annulus bears one asymmetric seta (AS) with some exceptions where the AS either has been eliminated accidentally (arrow) when shaving the aesthetascs or was lost by natural causes. Lateral guard setae (GS).

Table 1. *Shaving efficiency and percentage of accidentally removed asymmetric setae in aesthetasc ablations*

| Exp. no. | Ablation | *N* | Number of tuft annuli | Sensilla escaping removal | | Shaving efficiency | AS accidentally removed in AE-ablations | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean (± S.D.) | Range | Mean (± S.D.) | (%) | Mean (± S.D.) | (%) |
| 1 | AS– | 14 | 70.7±10.1 | 0–7 | 2.3±2.1 | 96.7 | n.a. | n.a. |
| 2 | AE– | 14 | 66.6±8.1 | 0–13 | 1.9±3.4 | 99.9 | 14.5±11.5 | ca. 29 |
| 3 | AS– | 12 | 70.6±6.8 | 0–2 | 0.9±0.8 | 98.7 | n.a. | n.a. |
| 3 | AE– | 12 | 74.0±12.8 | 0–5 | 1.0±1.4 | 99.9 | 14.6±5.7 | ca. 20 |
| 4 | AE– | 12 | 73.2±4.5 | 0–4 | 1.5±1.3 | 99.9 | 11.1±3.2 | ca. 15 |

Ablation, type of sensillum that was selectively removed, either Aesthetascs (AE–) or Asymmetric Setae (AS–); *N*, number of lateral flagella analyzed (note that in Exp. 1, 2, 4, two lateral flagella were lost in processing); Number of tuft annuli, number of annuli bearing aesthetascs and an asymmetric seta after removal of some distal annuli; Sensilla escaping removal, number of sensilla missed in the selective ablation; Shaving efficiency, percentage of removed sensilla relative to entire population (in case of AS: number of tuft annuli set as total number of AS; in case of AE: 1300 taken as estimate of total number of AE according to Laverack, 1964; Gleeson et al., 1993; Cate and Derby, 2001); AS accidentally removed in AE-ablations: number and percentage of asymmetric setae removed unintentionally in aesthetasc ablations.

56–58, after the post-AS-ablation block; none of the animals molted during this time) showed a small and sustained increase in the mean wipe rates with respect to the 3-day post-AS-ablation period; nonetheless, wipe rates remained substantially lower than in the pre-AS-ablation period (maximum mean wipe rate as measured on the last test day: 4.3±0.8 wipes/2 min). Comparing the mean wipe rates between the 3-day test blocks revealed that at all three longer time intervals, the mean wipe rate was significantly different from the mean wipe rate of the 3-day pre-AS-ablation block ($P<0.001$ for all three time intervals; RM-ANOVA, Newman–Keuls *post*-tests) but also from the mean wipe rate of the 3-day post-AS-ablation block ($P<0.05$ for all three time intervals; RM-ANOVA, Newman–Keuls *post*-tests). These results suggest that chemically elicited AGB functionally recovers to some extent after longer time intervals even without any possible regeneration of the ablated AS. Since the wipe rates in this time do not increase systematically but stay at a rather constant and substantially lower level than before removal of the AS, this functional recovery represents a partial one at best.

Counting the number of AS that had escaped selective removal after the end of the experiments, showed a shaving efficiency of about 97% (Table 1, Fig. 2B).

*Experiment 2*

Exp. 2 examined the effect of the removal of aesthetascs (AE) on chemically elicited AGB (Fig. 3). It also started with a period of 3 consecutive days in which baseline responsiveness of animals ($N=8$) was determined. In contrast to Exp. 1, the mean wipe rates stayed rather constant during this time period. Since Exp. 1 had shown that removal of one row of GS has no significant effect on the elicitation of AGB, we selectively removed the medial row of GS guard setae together with the aesthetascs (AE) in a single procedure (Fig. 2C). Retesting animals on 3 consecutive days after this surgery showed responses that were very similar to those obtained during the pre-AE-ablation block. Comparing the mean wipe rates between the 3-day pre- and post-AE-ablation

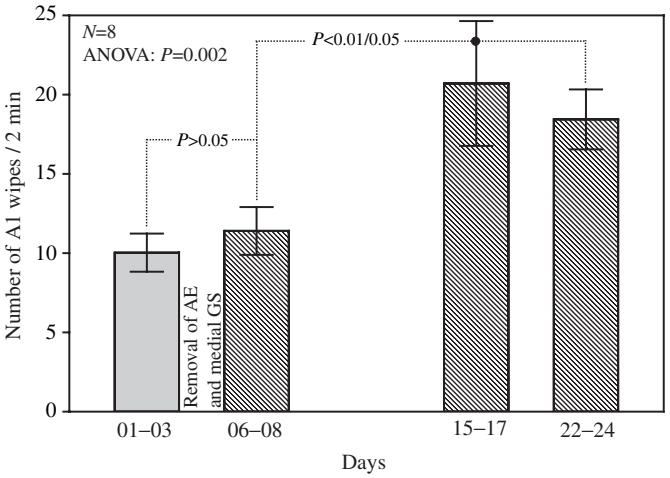


Fig. 3. Experiment 2. Effect of the bilateral selective removal of the aesthetascs (AE) and the medial guard setae (GS) on the mean wipe rate of the antennules after stimulation with 3 ml of 0.5 mmol $l^{-1}$ L-glutamate. Wipe rate values are means ± S.E.M. of $N=8$ experimental animals (*Panulirus argus*) over the 9 trials of an experimental 3-day block; experimental days are numbered chronologically. Note that selective removal of the aesthetascs (together with the medial GS) does not cause a decrease in the mean wipe rate (Days 06–08) compared to the mean wipe rate measured in the unoperated animals in the initial 3-day block (Days 01–03). Note further that the mean wipe in the re-test blocks following after longer time intervals (Days 15–17, Days 22–24) is substantially higher than in the first two 3-day blocks of the experiment. ANOVA shows an overall statistically significant difference between the columns ($P=0.002$). Pair-wise comparisons by Newman–Keuls *post* tests between the means of the 3-day blocks reveal no significant difference between the pre-AE-ablation (Days 01–03) and the post-AE-ablation (Days 06–08) blocks ($P>0.05$), whereas the retest blocks following after longer time periods differ significantly from the post-AE-ablation block ($P<0.01$ for Days 15–17, $P<0.05$ for Days 22–24). The two retest blocks do not differ significantly from each other ($P>0.05$). Wipe rates of all 3-day blocks (means ± S.E.M.): Days 01–03, 10.0±1.2; Days 06–08, 11.4±1.5; Days 15–17, 20.7±3.9; Days 22–24, 18.4±1.9.

blocks revealed no statistically significant difference ($P>0.05$; RM-ANOVA, Newman–Keuls *post*-test). Within the post-AE-

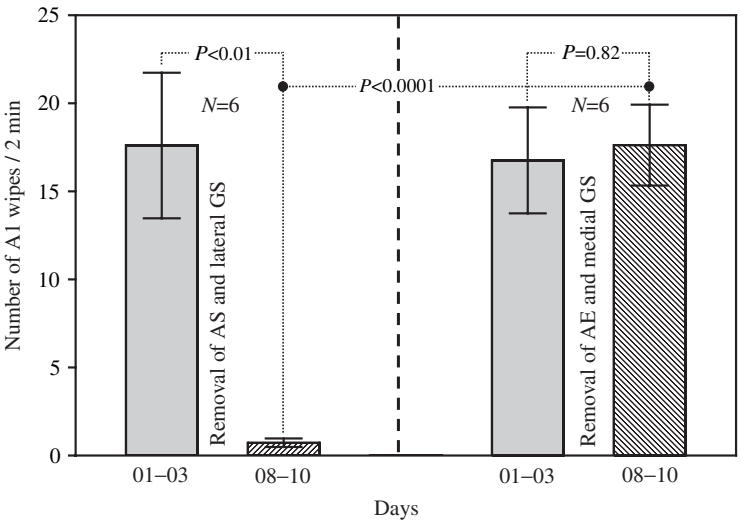


Fig. 4. Experiment 3. Direct comparison of the effect of the bilateral selective removal of the asymmetric setae (AS) and the lateral guard setae (left side) *vs* the bilateral selective removal of the aesthetascs (AE) and the medial guard setae (right side) on the mean wipe rate of the antennules after stimulation with 3 ml of 0.5 mmol $l^{-1}$ L-glutamate. Wipe rate values are means ± S.E.M. of *N*=6 experimental animals (*Panulirus argus*) in each experimental group over the 9 trials of an experimental 3-day block; experimental days are numbered chronologically. Note that selective removal of the AS (Days 08–10, left) is followed by an almost total loss of antennular wiping, whereas selective removal of the AE (Days 08–10, right) causes no decrease in the mean wipe rate compared to the mean wipe rate measured in the unoperated animals in the initial 3-day block (Days 01–03). Pair-wise comparisons by paired *t*-tests between the means of the 3-day blocks reveal a significant difference between the pre-AS-ablation (Days 01–03, left) and the post-AS-ablation (Days 08–10, left) blocks ($P<0.01$), but not between the pre-AE-ablation block (Days 01–03, right) and the post-AE-ablation block (Days 08–10, right) ($P=0.82$). Comparison between the post-AS-ablation and the post-AE-ablation block by an unpaired *t*-tests reveals a significant difference between them ($P<0.0001$). Wipe rates of all 3-day blocks (means ± S.E.M.): Days 01-03 AS-ablation, 17.7±4.1; Days 08–10 AS-ablation, 0.7±0.2; Days 01–03 AE-ablation, 16.8±3.0; Days 08-10 AE-ablation, 17.6±2.3.

ablation block, the mean wipe rate showed a small, statistically non-significant increase. This result demonstrates that AE are not necessary for the chemical elicitation of AGB.

Retesting ablated animals after longer time intervals (1 week, Days 15–17; 2 weeks, Days 22–24, after the post-AS-ablation block; none of the animals molted during this time) showed a substantial and sustained increase in the mean wipe rates with respect to the 3-day post-AE-ablation period. Comparing the mean wipe rates between the 3-day test blocks revealed that at both longer time intervals, the mean wipe rate was significantly different from the mean wipe rate of the 3-day post-AE-ablation block (1 week, $P<0.01$; 2 weeks, $P<0.05$; RM-ANOVA, Newman–Keuls *post*-test), whereas it did not differ significantly between the two longer time intervals ($P>0.05$; RM-ANOVA, Newman–Keuls *post*-test). This unexpected result suggests that removal of the AE might have a long-term positive effect on chemically elicited AGB. Since no proper controls (sham-ablated or GS-ablated animals) were run in parallel in this experiment, we did an additional experiment (Exp. 4, see below) to specifically address this question.

Counting the number of AE that had escaped selective removal after the end of the experiments, showed a shaving efficiency of about 99.9% (Table 1, Fig. 2C). Since shaving the AE carries a relatively high risk of accidentally removing some AS, we also counted the number of missing AS and found that on average the experimental animals had lost about 29% of the entire AS population in the shaving (Table 1, Fig. 2C).

*Experiment 3*

Exp. 3 addressed the concern that the different effects of selective AS and AE removal on chemically elicited AGB as seen in Exp. 1 and 2, could be partially due to uncontrolled factors since these experiments were performed sequentially on two different groups of animals. Therefore in Exp. 3, we did the selective ablations in parallel on different members of a third group of animals. To avoid any bias due to the substantial inter-individual differences in responsiveness (see above), we did not assign the animals (*N*=12) to one of the two treatments before the experiment but based this decision on their performance during the initial period of 3 consecutive days, in which the baseline responsiveness was determined. After this 3-day block, we paired similarly responsive animals and subjected one arbitrarily chosen member of each pair to the selective removal of AS (and lateral GS) and the other member to the selective removal of AE (and medial GS).

Exp. 3 started with a period of 3 consecutive days in which the baseline responsiveness of the animals was determined (Fig. 4). As in Exp. 1, the mean wipe rates increased continuously slightly but non-significantly during these 3 days in both groups of animals, from 13.6±4.3 to 21.1±4.1 wipes/2 min in the animals that got their AS shaved afterwards, and from 14.2±2.1 to 18.9±4.9 wipes/2 min in the animals that got their AE shaved afterwards.

Subsequent removal of AS (together with lateral GS) in one group of animals (*N*=6) and of AE (together with medial GS) in the other group of animals (*N*=6) had drastically different effects on chemically elicited AGB. In animals without AS, the mean wipe rate dropped substantially to reach 0 on post-surgery day 2, with a small rebound on post-surgery day 3 where the mean wipe rate increased to 1.9±0.6 wipes/2 min. Comparing the mean wipe rates between the 3-day pre- and post-AS-ablation blocks showed that this difference is statistically significant ($P<0.01$, paired *t*-test). In contrast, the mean wipe in the animals without AE dropped only slightly on the first post-surgery day (the difference between this day and the last day before the surgery was not statistically significant) and then rebounded to reach a value on post-AE-ablation day 3, slightly but not significantly higher than on the last day before the surgery. Comparing the mean wipe rates between the 3-day pre- and post-AE-ablation blocks showed no

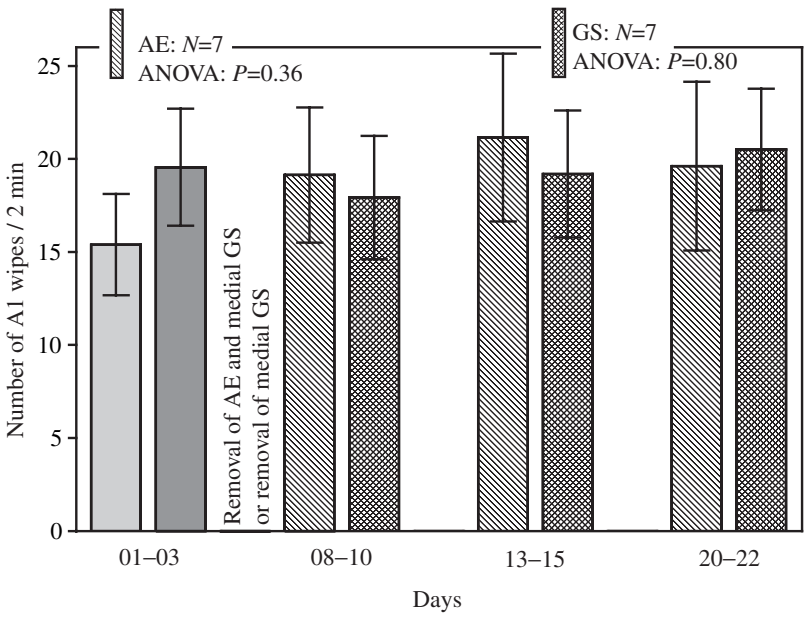


Fig. 5. Experiment 4. Direct comparison of the effect of the bilateral selective removal of the aesthetascs (AE) and the medial guard setae (lighter gray and hatched columns) *vs* the bilateral selective removal of only the medial guard setae (GS) (darker gray and cross-hatched columns) on the mean wipe rate of the antennules after stimulation with 3 ml of 0.5 mmol $l^{-1}$ L-glutamate. Wipe rate values are means ± S.E.M. of *N*=7 experimental animals (*Panulirus argus*) in each experimental group over the 9 trials of an experimental 3-day bock; experimental days are numbered chronologically. Note that neither the selective removal of the AE (together with the medial GS) nor the selective removal of only the medial GS has any consistent, long-term effect on the mean wipe compared to the mean wipe rate measured in the unoperated animals in the initial 3-day block (Days 01–03). ANOVA shows no overall statistically significant difference between the columns in either experimental group (*P*=0.36 for AE+GS-ablated animals, *P*=0.80 for GS-ablated animals). Pair-wise comparisons by unpaired *t*-tests between the means of the respective 3-day blocks also reveal no significant differences between both treatments (*P*=0.81 for Days 08–10, *P*=0.74 for Days 13–15, *P*=0.88 for Days 20–22). Wipe rates of all 3-day blocks (means ± S.E.M.): Days 01–03, AE-ablation, 15.4±2.7; Days 01–03, GS-ablation, 19.6±3.1; Days 08–10, AE-ablation, 19.1±3.1; Days 08–10, GS-ablation, 17.9±3.3; Days 13–15, AE-ablation, 21.2±4.5; Days 13–15, GS-ablation, 19.2±3.4; Days 20–22, AE-ablation, 19.6±4.5; Days 20–22, GS-ablation, 20.5±3.3.

statistically significant difference between them (*P*=0.82, paired *t*-test). Comparing the post-ablation mean wipe rates between the two groups of animals revealed a significant difference between them (*P*<0.0001, unpaired *t*-test). In summary, the results obtained in Exp. 3 fully support the main results from Exp. 1 and 2: selective removal of AS causes a dramatic decrease in chemically elicited AGB (to almost zero), whereas selective removal of AE has no detectable effect on AGB. This supports the conclusion that AS are necessary and sufficient for driving chemically elicited AGB and that AE have no role in eliciting this behavior.

Counting the number of AS and AE, respectively, that had escaped selective removal after the end of the experiments revealed shaving efficiencies of about 99% for the AS-ablated animals and 99.9% for the AE-ablated animals (Table 1). Counting the number of AS that had been removed accidentally in the AE-ablated animals revealed that on average they had lost about 20% of the entire AS population in the shaving (Table 1).

*Experiment 4*

Exp. 4 examined the possibility that removal of AE has a long-term positive effect on chemically elicited AGB as indicated by the results of Exp. 2. To test this possibility, we set up a new group of 14 spiny lobsters, half of which were subjected to the same treatment as in Exp. 2 in that their AE were specifically removed (together with the medial GS) whereas the other half served as a control and received a selective shaving of only the medial GS. To avoid any bias due to the substantial inter-individual differences in responsiveness, we assigned the animals to one of these treatments based on their baseline responsiveness in the same way as described for Exp. 3.

Exp. 4 started with a period of 3 consecutive days in which the baseline responsiveness of the animals was determined (Fig. 5). As in Exp. 1 and 3, the mean wipe rates increased slightly but non-significantly during these 3 days in both groups of animals from 12.1±2.6 to 19.2±3.8 wipes/2 min in the animals that got their AE shaved afterwards, and from 14.1±2.6 to 20.8±3.7 wipes/2 min in the animals that got their medial GS shaved afterwards.

The subsequent removal of AE (together with medial GS) in one group of animals (*N*=7) and of only medial GS in the other group of animals (*N*=7) had no effect on chemically elicited AGB, including retests of the ablated animals after longer time intervals (3 days: Days 13–15, and 10 days: Days 20–22, after the post-ablation block). This observation is supported by statistical analyses. Comparing the mean daily wipe rates or the mean wipe rates averaged over the 3-day blocks revealed no statistically significant differences for either group of animals (*P*=0.36 for AE-ablated animals; *P*=0.80 for GS-ablated animals; RM-ANOVA). There also was no statistically significant difference between the mean post-ablation wipe rates (averaged over the 3-day blocks) of both groups of animals (*P*=0.81, Days 08–10; *P*=0.73, Days 13–15; *P*=0.88, Days 20–22; unpaired *t*-tests). From this result, we conclude that removal of AE has no effect, also in particular not any positive effect, on chemically elicited AGB, as was suggested by the result of Exp. 2. This in turn indicates that the increased mean wipe rate observed in Exp. 2 at long time intervals after removal of the AE is most likely the result of an uncontrolled factor (see Discussion for a possible explanation).

Counting the number of AE that had escaped selective removal after the end of the experiments showed a shaving efficiency of about 99.9% (Table 1). Counting the number of AS that had been removed accidentally in AE-ablated animals

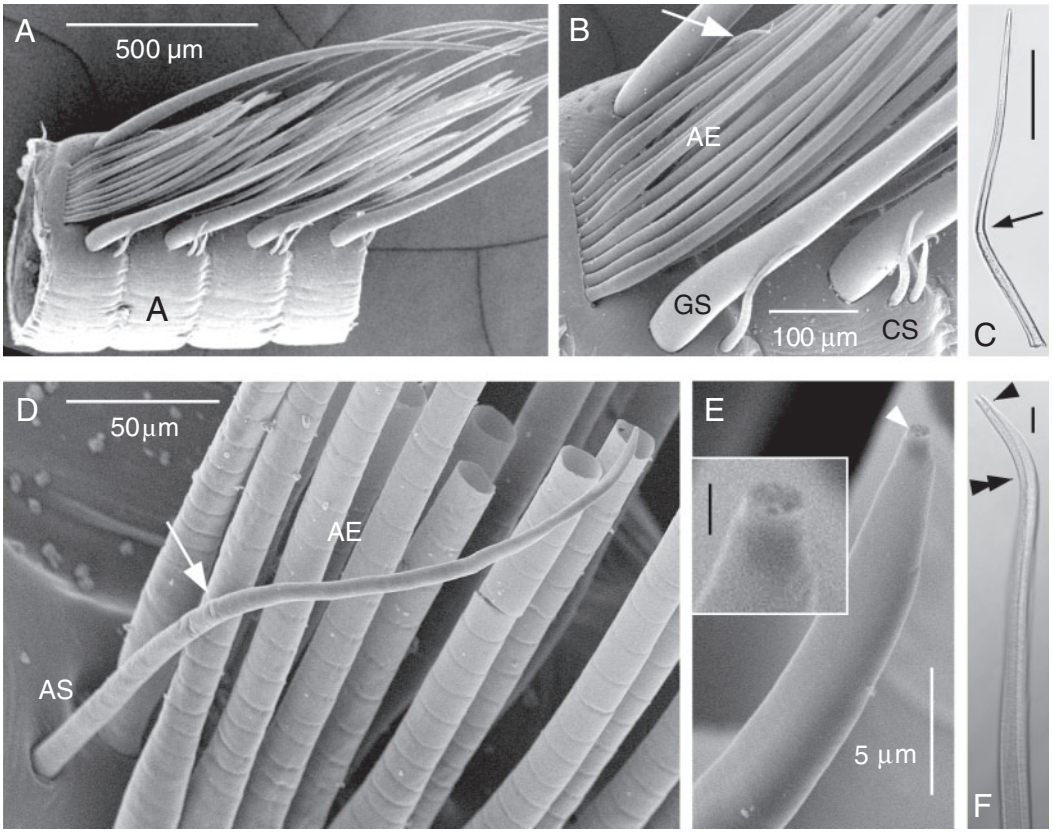


Fig. 6. Outer morphology of asymmetric setae. Scanning electron micrographs (A,B,D,E) or light micrographs (C,F). (A,B) Overview of sensilla arrangement in the 'tuft' region of the lateral flagellum of *Panulirus argus*. Each annulus (A) bears two rows of aesthetascs (AE) flanked by two guard setae (GS). Guard setae are accompanied by companion setae (CS) at their lateral margins. One asymmetric seta (arrow) is located between the lateral guard seta and the aesthetascs. (C–F) Structure of asymmetric setae. Asymmetric setae have a smooth, slender setal shaft with two kinks and a terminal pore. (C) The proximal kink in the shaft (arrow) occurs at about one third of the length and has an angle of about 13°. Scale bar, 100 µm. (D) The shaft of the asymmetric seta (AS) inserts in a narrow socket and protrudes within the rows of aesthetascs (AE), many of which are intentionally broken off in this preparation to reveal their extremely thin cuticle. Proximal kink in the shaft (arrow). (E,F) At the tip of the shaft, a terminal pore is present (arrowhead), positioned at the base of a shallow cuticular rim. (E) Terminal pore at higher magnification. Scale bar in inset, 1 µm. (F) Distal kink in the shaft (double-arrow). Scale bar, 10 µm.

showed that on average they had lost about 15% of the entire AS population in the shaving (Table 1). In the GS-ablated animals, AS were missing on some annuli (maximally 6), supposedly due to natural wear and tear, since the lateral side of the flagella was not touched in the shaving.

### *Morphological features of asymmetric setae relevant for sensory transduction*

Our behavioral experiments lead to the conclusion that in *P. argus*, the asymmetric setae are solely responsible for the elicitation of AGB by chemical stimulation. To understand the sensory basis of AGB in more depth, we analyzed the morphology of asymmetric setae to search for modality-specific features, which have been described for diverse sensilla types of decapod crustaceans (Schmidt and Gnatzy, 1984). Confocal fluorescence microscopy, which revealed the cuticular apparatus of the asymmetric setae due to its wide-spectrum autofluorescence (excitation wavelength 450 nm; recorded emission >500 nm), in conjunction with bright field light microscopy and scanning electron microscopy, showed that the asymmetric setae have a smooth, slender shaft tapering only very gradually towards the tip (Figs 6C,D,F, 7A,B,D). The setal shaft is about 400 µm long (384±23 µm, mean ± S.D., *N*=8) and its maximum diameter at the base is about 15 µm (15.5±2.2 µm, mean ± S.D., *N*=8). A morphological feature distinguishing the setal shaft of AS from other sensilla with smooth shafts (GS, CS) is that it is not straight or gradually curved but has two noticeable kinks, one at about one third of the total length (Figs 6C,D, 7A) and one close to the tip, at about 95% of the total length (Figs 6F, 7A). The angle of the first kink is about 13°; the angle of the second kink is about 30°. Distal to the second kink the shaft changes its shape from cylindrical to laterally flattened. Both kinks are in roughly the same plane and cause the shaft to project mesially in between the two rows of aesthetascs. The setal base of AS is located within a socket structure that lies in a slight depression of the cuticle (Figs 6D, 7B,E). The cuticular depression forms a tight bulge around the lateral and distal aspects of setal base, but is flatter and thus leaves a larger gap at its proximal aspect. The setal shaft inserts in the bulge with an angle of approximately 70° pointing distally.

A central canal passes through the shaft on its entire length ending in a terminal pore (Fig. 6E,F). The terminal pore has a diameter of ca. 0.1 µm and is located at the bottom of a cuticular ring with a diameter of ca. 1 µm. A terminal pore has been identified as a modality specific structure of bimodal chemo- and mechanosensory sensilla in decapod crustaceans, and it is thought that the pore allows chemical stimuli to access the tips of the dendrites ending below it (Schmidt and Gnatzy, 1984).

Labeling vibrating-microtome sections cut sagittally through the lateral flagellum with the f-actin marker phalloidin–AlexaFluor568 revealed a short, phalloidin-positive tube-like structure associated with the base of AS (Fig. 7) and other non-olfactory sensilla types of the tuft region (GS, CS) but lacking at the base of aesthetascs. Since in insect sensilla phalloidin strongly labels the scolopale (Wolfrum, 1990), a tube-like supporting structure within the innermost sheath cell surrounding the transitional region of the dendrites, and because the phalloidin-positive structures associated with the base of AS, GS and CS have the size and position known for the scolopales of other sensilla of decapod crustaceans (Schmidt and Gnatzy, 1984), we conclude that the phalloidin-positive structures identified here represent scolopales. The scolopale of AS is 30–40 µm long and has a maximum diameter of 10–15 µm. It consists of relatively fine longitudinal strands of phalloidin-positive material that only in the middle region form an almost closed tube-like structure (Fig. 7C,F,H). Analyzing animals in different molt stages (from late premolt to early postmolt; intermolt animals could not be analyzed since the hardened cuticle cannot be cut with a vibrating microtome) revealed that the position of the scolopale in the setal base region changes systematically with the molt stage. In late premolt animals, in which a new cuticle is already formed below the old one ($N$=2), the scolopale was located in the base of the shaft above the socket (Fig. 7G,H). In very early postmolt animals (1 day after molting, $N$=2), the scolopale was also located in the base of the shaft, but sometimes closer to the socket than in the late premolt animals (Fig. 7E,F). In animals that were about 1 week postmolt ($N$=2), the scolopale was consistently located below the socket (Fig. 7B–D). A scolopale has been identified as a modality specific structure of mechanosensory and of bimodal chemo- and mechanosensory sensilla in decapod crustaceans (Schmidt and Gnatzy, 1984).

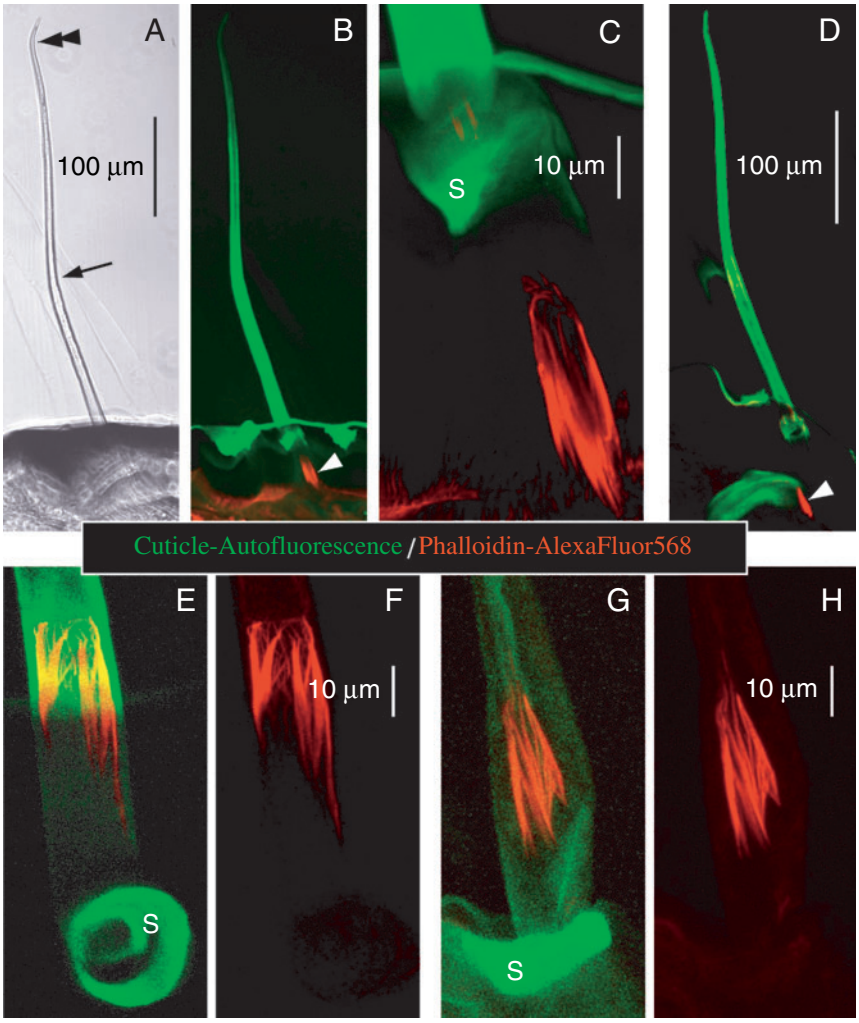


Fig. 7. Identification of a scolopale at the base of asymmetric setae. Confocal micrographs representing collapsed stacks of optical sections (green, autofluorescence of cuticle; red, phalloidin–AlexaFluor568). (A) Asymmetric seta of a spiny lobster several days after molting as seen in the transmitted light channel. Proximal kink in the shaft (arrow); distal kink in the shaft (double-arrow). (B) The same asymmetric seta as seen in the fluorescence channels. Cuticle autofluorescence clearly delineates the shaft and the socket of the asymmetric seta. Below the socket a phalloidin-positive tube-like structure, the scolopale, is located (arrowhead). Scale as in A. (C) Base region of the same AS at higher magnification. Note tight socket structure and strand-like substructure of scolopale. (D) Another AS of the same animal. Note the generally identical arrangement and the larger distance of the scolopale (arrowhead) to the socket. (E,F) Base of an AS of an animal 1 day after molting. The scolopale is located within the shaft ca. 20 µm distal to the socket (S). (F) Note strand-like substructure of scolopale. (G,H) Base of an AS on the new cuticle of an animal shortly before molting. The scolopale is located within the shaft ca. 20 µm distal to the socket (S). (H) Note strand-like substructure of scolopale.

## Discussion

### *Identity of sensilla eliciting AGB*

Our study was designed as a follow-up to the study of Wroblewska et al. (2002), in which it was demonstrated by ablation studies that chemically elicited AGB in the spiny lobster *Panulirus argus* is mediated by sensilla in the tuft region of the lateral flagellum of the antennules. From the finding that

elimination of guard and companion setae did not diminish chemically elicited AGB, the authors concluded that the aesthetascs are sufficient and necessary to drive AGB, although they had not eliminated the asymmetric setae (AS) from the tuft. This conclusion therefore was not based on direct experimental evidence but on a conjecture derived from the dramatically different numbers of sensilla in question: there are about 1300 aesthetascs in the tuft region of the lateral flagellum compared to maximally about 80 AS (Laverack, 1964; Gleeson et al., 1993; Cate and Derby, 2001; this study). In our study, we used selective sensilla ablations to directly address the question whether AGB in *P. argus* is mediated by aesthetascs or by asymmetric setae.

Collectively, the results of Exp. 1, 2 and 3 clearly show that chemically elicited AGB is driven exclusively by the asymmetric setae and that aesthetascs do not contribute to the elicitation of this behavior. Selective elimination of the AS as achieved in Exp. 1 and 3 with a completeness of about 97% and 99%, respectively, while leaving the vast majority of aesthetascs intact, almost completely eliminated chemically elicited AGB. This demonstrates that the asymmetric setae are necessary for driving AGB. Conversely, selective elimination of the aesthetascs, as was achieved in Exp. 2, 3 and 4 with 99.9% efficiency while leaving the majority of AS (ca. 70%, 80%, 85%, respectively) intact, had no detectable effect on chemically elicited AGB (at least during the first 3-day post-ablation block). This demonstrates that, contrary to the previous conclusion (Wroblewska et al., 2002), the aesthetascs are not necessary for driving chemically elicited AGB. Since in the extensive ablation experiments performed by Wroblewska et al. (2002) and in the experiments presented here (selective ablation of guard setae) no other sensilla types have been found that are required for elicitation of AGB, it follows that the AS are not only necessary but also sufficient for driving AGB.

The results of the long-term observations in Exp. 2 indicated a positive influence of selective asymmetric setae removal on AGB, which might be interpreted as an inhibition of AGB mediated by aesthetasc input. Testing this possibility directly in Exp. 4, however, showed no effect of selective aesthetasc removal compared to the previous responses of these animals or compared to the responses of control animals in which only the medial guard setae had been removed. From this finding, we conclude that the aesthetascs are also not mediating any kind of inhibition of AGB – and thus seem not to be involved in the elicitation of AGB at all. This poses the question of how the long-term increase in wipe-rate after selective aesthetasc removal in Exp. 2 can be explained. Comparing the responses obtained in all four experiments it becomes obvious that the responsiveness in the initial 3-day test period remained almost constant in Exp. 2, whereas it systematically increased in the other experiments, resulting in an overall reduced level of responsiveness in the initial 3-day test period of Exp. 2 compared to the others. The animals in Exp. 2 had the shortest acclimatization period in the test tanks before the start of the experiments, and we assume that their unusually low and constant responsiveness during the initial test period could result from this. Then the considerably higher (almost doubled) responsiveness after longer time intervals might merely represent a delayed full acclimatization to the test situation. This interpretation is strengthened by the fact that already during the first post-ablation test period the responsiveness rises systematically as it did in the pre-ablation test period in the other experiments. Thus the long-term increase in wipe-rate after selective aesthetasc removal in Exp. 2 likely represents an experimental artifact.

Another unexpected observation was that weeks after an initial almost total loss of responsiveness following the selective removal of asymmetric setae, chemical elicitation of AGB showed a partial functional recovery. To attribute this functional recovery to a possible regeneration of the ablated AS seems highly unlikely since sensilla formation in decapod crustaceans is only possible through molting (e.g. Sandeman and Sandeman, 1996; Steullet et al., 2000a), which did not occur in the test animals during the experiment. Thus, two other explanations are more probable. The first is that very few remaining AS (ca. 2 or 1 per lateral flagellum in Exp. 1 and 3, respectively) could strengthen their connections in the CNS and thereby achieve a much higher effectiveness in eliciting AGB than they had prior to and immediately after selective AS removal. The second is that other sensillar types on the lateral flagellum, which project to the same neuropil as AS (most likely the LAN, see below) but normally do not connect to the interneurons and/or motoneurons that receive AS input and mediate AGB, start to establish such connections when the sensory axons of the AS fall silent or begin to degenerate. We assume that the second possibility is the more likely one, since the partial recovery of the chemical elicitation of AGB occurred in all experimental animals, including those in which re-checking confirmed that no AS were left on both flagella (two animals). Since responses to L-glutamate are quite common in extracellular recordings from sensory axons in the lateral flagellum of *P. argus* (Laverack, 1964; Johnson and Ache, 1978; Derby et al., 1991), it is conceivable that not only the AS (see below) but also other non-olfactory sensillar types respond to L-glutamate.

To our knowledge, the findings reported here represent the second case in which in decapod crustaceans a chemically mediated behavior can be attributed to only one identified type of sensilla. The other case is the courtship behavior of male blue crabs *Callinectes sapidus*, elicited by a sex pheromone released by pre-molt females and driven by aesthetascs but not by asymmetric setae (Gleeson, 1982). Several studies on decapod crustaceans have aimed to identify sensillar types driving specific chemically mediated behaviors (Steullet et al., 2001, 2002; Wroblewska et al., 2002; Keller et al., 2003; Horner et al., 2004). In all of these studies (except Wroblewska et al., 2002), it was found that several sensillar types, including those providing input to different sensory pathways in the brain (see below), contribute to the analyzed behaviors, food search, orientation and associative learning. This large degree of redundancy and functional overlap of sensilla contributions may be due to the complexity of the observed behaviors and/or the complexity of the stimulants eliciting them. In contrast, AGB and the courtship dance of *Callinectes sapidus* are more stereotyped behaviors elicited by one key compound (L-glutamate and female sex

pheromone, respectively) making it more conceivable that one sensillum type suffices to drive them. This conforms to one principle of chemosensory coding known as 'labeled line' (e.g. Ache, 1991): one key compound leads to the activation of specific sensory neurons, which causes the activation of specific interneurons in the CNS, which in turn leads to the elicitation of a specific, often stereotyped behavior.

While it is surprising that the most numerous sensilla on the lateral flagellum, the aesthetascs, have been shown only once (Gleeson, 1982) to be necessary for driving specific behaviors, it may be equally unexpected to find the least numerous sensilla, the asymmetric setae (ca. 80 per flagellum in *P. argus*), to be not only necessary but also sufficient for driving a prominent and specific behavior, AGB. Due to their exposed position, the lateral flagella are often damaged distally and also lose sensilla, including AS, more proximally by wear and tear (Harrison et al., 2001; this study), raising the question of how reliable the elicitation of AGB by AS is, or how many AS are sufficient to drive it. Although we did not study this question directly, the accidental removal of some AS in the specific shaving of the aesthetascs (Exp. 2, 3 and 4) is informative in this context. In Exp. 2 the highest percentage of accidentally removed AS occurred (on average ca. 30% of the entire population), but this had no negative effect on the wipe-rate observed after the sensilla ablation indicating that the remaining 70% of AS are sufficient to drive AGB. On the other extreme, the maximal average number of AS escaping selective removal was 3.3% (Exp. 1), and as the result of Exp. 1 clearly demonstrates this number was not sufficient to drive AGB. Thus we conclude that the minimal number of AS sufficient for driving AGB must lie between ca. 3 and 70% of the entire population.

### *Sensory pathway mediating AGB*

The identification of the asymmetric setae as driving AGB, leads to a revision of the hypothesis developed by Wroblewska et al. (2002) that AGB is mediated by the central olfactory pathway. It instead strongly suggests that AGB is mediated by the second antennular sensory pathway in the deutocerebrum, which parallels the olfactory pathway and is comprised of the lateral and medial antennular neuropils (LAN, MAN; Maynard, 1965; Schmidt et al., 1992; Schmidt and Ache, 1996). Several lines of evidence support this new interpretation, which was originally proposed by Barbato and Daniel (1997).

(1) The available evidence from labeling sensilla with radioactive leucine and backfilling axon bundles in the antennular nerve with neuronal tracers strongly suggests that the axons of the aesthetascs specifically and exclusively target the ipsilateral olfactory lobe, whereas the afferent axons from the non-olfactory sensilla on the antennular flagella project to the ipsilateral LAN (Sandeman and Denburg, 1976; Mellon and Munger, 1990; Schmidt and Ache, 1992; Schmidt et al., 1992; Sandeman and Sandeman, 1994). As there is no reason to suggest that the afferents of the AS deviate from this scheme, we conclude that most likely they also project to the LAN.

(2) The LAN and MAN contain the major arborizations of the antennular motoneurons, whereas the olfactory lobe is completely devoid of them (Maynard, 1965; Schmidt and Ache, 1993; Roye, 1994). This means that LAN and MAN represent the motor center for the antennules, the main appendages executing AGB. Therefore the presumptive afferent projections of the AS to the LAN, together with the close spatial juxtaposition of sensory afferents and motoneuron arborizations in the LAN (Schmidt et al., 1992; Schmidt and Ache, 1993), allow that a direct sensory-motor coupling underlies the activation of antennular wiping movements by the chemical stimulation of the AS. This would represent a far simpler pathway than that proposed by Wroblewska et al. (2002), involving the olfactory lobe activated by aesthetasc input, higher brain centers in the lateral protocerebrum innervated and activated by ascending OL projection neurons (Mellon et al., 1992; Schmidt and Ache, 1996b; Sullivan and Beltz, 2001a,b), and finally the antennular motoneurons residing in the LAN/MAN activated by descending projection neurons from these protocerebral neuropils.

(3) AGB, although representing a highly stereotyped behavior, cannot be considered as a simple reflexive behavior since it involves the coordinated movements of not only the antennules but also other appendages like the third maxillipeds and the anterior walking legs. To achieve this coordination, the LAN/MAN neuropils have to be neuronally connected with the motor centers controlling the movements of the third maxillipeds and the walking legs, which reside in the suboesophageal ganglion and the thoracic ganglia, respectively (e.g. Wiens, 1976). Descending as well as ascending projection neurons, with axons in the circumoesophageal connectives and arborizations in LAN/MAN that could provide the neuronal substrate for the observed coordination, have been identified in *P. argus* (Schmidt and Ache, 1996a) and other decapod crustaceans (Glantz et al., 1981; Arbas et al., 1988), whereas such neurons are very rare for the olfactory lobe (Schmidt and Ache, 1996b).

(4) In addition to the coordination with the third maxillipeds and the anterior walking legs, both antennules also show coordination with one another in AGB, which is most obvious when both antennules are wiped simultaneously. The most likely explanation for this bilateral coordination is a direct neuronal connection between both sides of the brain. Such connections are indeed numerous between the LAN/MAN of both sides, since several antennular motoneurons have minor arborizations on the contralateral side (with respect to their axon and main arborizations), and many descending and ascending projection neurons have bilateral arborizations in the LAN/MAN (Hamilton and Ache, 1983; Arbas et al., 1988; Schmidt and Ache, 1993; Schmidt and Ache, 1996a). By contrast, only a few neurons have been found that directly connect the olfactory lobes of both sides of the brain (Schmidt and Ache, 1996b).

### *Functional morphology of asymmetric setae*

Our analysis of the morphological features of the asymmetric setae led to the identification of two modality specific structures, a terminal pore at the tip of the setal shaft and a scolopale below its base. With these two prominent features, the AS can be classified as bimodal chemo- and mechanosensory sensilla

typical of decapod crustaceans (Schmidt and Gnatzy, 1984). The terminal pore presumably provides access for chemical stimuli to the tips of the dendrites located below, and the scolopale is interpreted as a rigid structural element that is involved in mechanosensory transduction likely taking place in the transitional region of the dendrites (Moran et al., 1977; Crouau, 1982; Eberl et al., 2000).

Since a terminal pore is indicative of chemosensory function, its presence in the AS corroborates the conclusion from the ablation experiments that the chemical stimulus eliciting AGB is detected by these sensilla. L-Glutamate is by far the most potent substance eliciting AGB (Barbato and Daniel, 1997). AS are therefore expected to contain chemosensory neurons responding to L-glutamate. While it is known that the lateral flagellum of *P. argus* contains numerous L-glutamate-sensitive units (Laverack, 1964; Johnson and Ache, 1978; Derby et al., 1991) and with biochemical assays specific, independent binding sites for L-glutamate have been identified (Burgess and Derby, 1997), it will require selective recordings from AS to characterize the glutamate sensitivity of their chemosensory neurons, including why such a high concentration of L-glutamate (>0.1 mmol $l^{-1}$) is necessary for eliciting AGB (Barbato and Daniel, 1997).

The presence of a scolopale in the base region of the asymmetric setae indicates an additional mechanosensory function. The general notion is that simple setae of decapod crustaceans do not respond to subtle stimuli such as water movements, but are rather insensitive and require direct touch to be stimulated (Garm et al., 2004). The very tight articulation of the setal shaft of the AS in the socket and the surrounding cuticular bulge is consistent with this scheme and suggests that they need direct touch for activation. Given that the AS are located within the rows of very stout guard setae, they are well shielded from touching larger outside objects when the antennule moves, suggesting that they are activated in other ways. The execution of AGB appears to be one likely way in which the AS could be activated since the antennular flagella are forcefully pulled through the third maxillipeds during this behavior, which likely causes a deflection of the AS. Another possible mode of direct mechanical stimulation of the AS is suggested by the direction in which their setal shaft faces. The shaft of the AS is articulated and kinked in such a way as to project 'inwards' towards the rows of aesthetascs and to come into direct contact with about half of the aesthetascs in one row. This construction indicates that the AS are loosely mechanically coupled to the shafts of the aesthetascs and that they could be activated when several aesthetascs move simultaneously. Such simultaneous movements of the aesthetascs occur regularly not only in AGB but also in antennular flicking (Gleeson et al., 1993; Koehl et al., 2001). Thus the AS seem to be well suited to monitor the displacement of the aesthetascs during flicking and possibly other antennular movements, a function that appears to be quite useful given that the aesthetascs themselves lack mechanosensory innervation (Grünert and Ache, 1988). It is conceivable that in this indirect way the AS might also be able to detect 'abnormal' movement patterns of the aesthetascs caused, for instance, by epibiotic organisms growing on them or by larger objects lodging between them. If this is so, then AS could elicit AGB not only upon chemical but also upon this kind of mechanical stimulation. The possibility of a mechanical elicitation of AGB has not been studied in detail so far, but has been reported to occur in crabs (Snow, 1973).

Changes in the position of the scolopale related to the molt stage, as we have found for the asymmetric setae, have not been previously reported in decapod crustaceans. The position of the scolopale within the cuticular apparatus of a sensillum can provide clues as to which mechanical parameter is the adequate stimulus. In sensilla equipped with a scolopale, sensory transduction probably occurs in the transition region of the dendrites surrounded by it (Moran et al., 1977; Crouau, 1982; Eberl et al., 2000). Thus, the transformation of the outside mechanical stimulus into a mechanical deformation of the dendrites can only occur distal to the scolopale. In all cuticular sensilla of decapod (and other) crustaceans in which a scolopale has been identified, it is always located below the socket (e.g. Schöne and Steinbrecht, 1968; Ball and Cowan, 1977; Altner et al., 1983; Espeel, 1985; Cate and Roye, 1997), allowing that displacement of the shaft within the socket acts as the adequate stimulus. Consequently this mode of stimulus transformation is believed to represent the general principle in mechanosensitive setae of crustaceans (Crouau, 1982), as well as other arthropods (Barth and Dechant, 2003). In contrast, a position of the scolopale within the shaft, which has never been reported before in sensilla of decapod crustaceans, would not be consistent with this model; it rather suggests that bending of the shaft serves as an adequate stimulus, as has been speculated previously (Crouau, 1982). We interpret the finding that, several days after molting the scolopale of the asymmetric setae is located proximal to the socket region, as reflecting the intermolt, physiologically mature situation. Thus in the functional state of the AS, displacement of the shaft within the socket could serve as adequate stimulus. The position of the scolopale in the basal region of the shaft, on the other hand, seems to be solely a molt-related phenomenon, since we observed this position only in late premolt and very early postmolt animals. In premolt animals, the dendrites of sensory neurons pass through the newly formed cuticular apparatus to the old one, which continues to represent the functional sensillum until ecdysis occurs (e.g. Kouyama and Shimozawa, 1984; Espeel, 1986). Therefore a scolopale position within the newly formed shaft is still proximal to the old, mechanically active cuticular apparatus and would continue to allow that displacement of the shaft within the socket serves as adequate stimulus. Only during the first days after ecdysis, when the scolopale remains within the base of the shaft, would displacement of the shaft within the socket not be an adequate stimulus.

### *Function of antennular grooming*

Antennular grooming is widespread among marine decapod crustaceans and often, like in *P. argus*, the antennules are the most frequently groomed appendages or body parts, suggesting that AGB plays a prominent role in maintaining the function of the aesthetascs (Maynard and Dingle, 1963; Snow, 1973;

Farmer, 1974; Bauer, 1977, 1981; Alexander et al., 1980; Zimmer-Faust et al., 1984; Barbato and Daniel, 1997; Daniel et al., 2001). Two likely complementary functions of AGB have been proposed, both based on the idea that pulling the flagella through the grooming pads of the third maxillipeds leads to a mechanical combing of the aesthetascs. One proposed function is the removal of food particles trapped between aesthetascs during feeding (Barbato and Daniel, 1997; Daniel et al., 2001). This interpretation is supported by the observation that in many decapod crustaceans, including *P. argus*, long bouts of AGB occur after feeding, whereas at other times the frequency of spontaneous AGB is rather low (Maynard and Dingle, 1963; Snow, 1973; Zimmer-Faust et al., 1984; Barbato and Daniel, 1997; Daniel et al., 2001). It also is consistent with the robust and selective elicitation of AGB by chemical stimulation (Zimmer-Faust et al., 1984; Barbato and Daniel, 1997; Daniel et al., 2001; Wroblewska et al., 2002; this study). The second proposed function of the mechanical combing caused by AGB is the removal of epibiotic organisms from the aesthetascs, which otherwise would cause fouling. Supporting evidence for this interpretation is the observation that epibiotic organisms indeed occur on the aesthetascs (Shelton, 1974; Bauer, 1977, 1978) and that their number increases dramatically when AGB is prevented by ablation of the third maxillipeds (Bauer, 1977, 1978; P. Daniel, personal communication). In one experiment on shrimps, in which AGB was prevented for several weeks, all aesthetascs were destroyed; however no explanation as to how epibiotic fouling could have caused this dramatic effect was provided (Bauer, 1977). We propose another possible function of AGB in the maintenance of aesthetascs. In diverse decapod crustaceans, including spiny lobsters, large openings of exocrine epithelial glands have been identified at the base of the guard setae (Derby, 1982; Gnatzy, 1984; Spencer and Linberg, 1986). Furthermore, smaller pores, which likely represent gland openings as well, frequently occur at the base of the aesthetascs (Derby, 1982; Fontaine et al., 1982; Gleeson, 1982), suggesting that epithelial glands of a different type are additionally present in the tuft region of the lateral flagellum. We hypothesize that the excretions produced by these 'tuft glands' provide chemical antifouling agents and/or substances that aid in the stabilization/protection of the extremely delicate cuticle of the aesthetascs. We further hypothesize that AGB not only causes the mechanical removal of food particles and/or epibiotic organisms but also provides the means by which the excretions of the 'tuft glands' are distributed over the entire length of the aesthetascs. This hypothesis would more easily explain why prevention of AGB caused the total loss of aesthetascs in a relatively short period of time in the experiments on shrimps (Bauer, 1977), and it is supported by the observation that in *P. argus* a layer of electron-dense material of hitherto unknown origin covers the aesthetasc cuticle (Grünert and Ache, 1988).

This study was supported by NSF IBN 0077474 and NIH DC00312. We thank Vivian Ngo for technical support and Dr Peter Daniel for interesting discussions and for sharing unpublished observations.

## References

**Ache, B. W.** (1991). Phylogeny of Smell and Taste. In *Smell and Taste in Health and Disease* (ed. T. V. Getchell, R. L. Doty, L. M. Bartoshuk and J. B. Snow, Jr), pp. 3-18. New York: Raven Press.

**Alexander, C. G., Hindley, J. P. R. and Jones, S. G.** (1980). Structure and function of the maxilipeds of the banana prawn *Penaeus merguiensis*. *Mar. Biol.* **58**, 245-249.

**Altner, I., Hatt, H. and Altner, H.** (1983). Structural properties of bimodal chemo- and mechanosensitive setae on the pereiopod chelae of the crayfish, *Austropotamobius torrentium*. *Cell Tissue Res.* **228**, 357-374.

**Anderson, P. A. V. and Ache, B. W.** (1985). Voltage- and current-clamp recordings of the receptor potential in olfactory receptor cells in situ. *Brain Res.* **338**, 273-280.

**Arbas, E. A., Humphreys, C. J. and Ache, B. W.** (1988). Morphology and physiological properties of interneurons in the olfactory midbrain of the crayfish. *J. Comp. Physiol. A* **164**, 231-241.

**Ball, E. E. and Cowan, A. N.** (1977). Ultrastructure of the antennal sensilla of *Acetes* (Crustacea, Decapoda, Natantia, Sergestidae). *Phil. Trans. R. Soc. Lond. B* **277**, 429-457.

**Barbato, J. C. and Daniel, P. C.** (1997). Chemosensory activation of an antennular grooming behavior in the spiny lobster, *Panulirus argus*, is tuned narrowly to L-glutamate. *Biol. Bull.* **193**, 107-115.

**Barth, F. G. and Dechant, H.-E.** (2003). Arthropod cuticular hairs: Tactile sensors and the refinement of stimulus transformation. In *Sensors and Sensing in Biology and Engineering* (ed. F. G. Barth, J. A. C. Humphrey and T. W. Secomb), pp. 159-171, Wien, New York: Springer-Verlag.

**Bauer, R. T.** (1977). Antifouling adaptations of marine shrimp (Crustacea: Decapoda: Caridea): Functional morphology and adaptive significance of antennular preening by the third maxillipeds. *Mar. Biol.* **40**, 261-276.

**Bauer, R. T.** (1978). Antifouling adaptations of caridean shrimps: cleaning of the antennal flagellum and general body cleaning. *Mar. Biol.* **49**, 69-82.

**Bauer, R. T.** (1981). Grooming behavior and morphology in the dacapod crustacea. *J. Crust. Biol.* **1**, 153-173.

**Burgess, M. F. and Derby, C. D.** (1997). Two novel types of L-glutamate receptors with affinities for NMDA and L-cysteine in the olfactory organ of the Caribbean spiny lobster *Panulirus argus*. *Brain Res.* **771**, 292-304.

**Carr, W. E. S.** (1988). The molecular nature of chemical stimuli in the aquatic environment. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 3-27. New York: Springer-Verlag.

**Cate, H. S. and Derby, C. D.** (2001). Morphology and distribution of setae on the antennules of the Caribbean spiny lobster *Panulirus argus* reveal new types of bimodal chemo-mechanosensilla. *Cell Tissue Res.* **304**, 439-454.

**Cate, H. S. and Roye, D. B.** (1997). Ultrastructure and physiology of the outer row statolith sensilla of the blue crab *Callinectes sapidus*. *J. Crust. Biol.* **17**, 398-411.

**Crouau, Y.** (1982). Primary stages in the sensory mechanism of the setulate sensilla, external mechanoreceptors of a cavernicolous mysidacea. *Biol. Cell* **44**, 45-56.

**Daniel, P. C., Shineman, M. and Fischetti, M.** (2001). Comparison of chemosensory activation of antennular grooming behavior in five species of decapods. *Mar. Freshwater Res.* **52**, 1333-1337.

**Derby, C. D.** (1982). Structure and function of cuticular sensilla of the lobster *Homarus americanus*. *J. Crust. Biol.* **2**, 1-21.

**Derby, C. D., Girardot, M.-N. and Daniel, P. C.** (1991). Responses of olfactory receptor cells of spiny lobsters to binary mixtures. I. Intensity mixture interactions. *J. Neurophysiol.* **66**, 112-130.

**Eberl, D. F., Hardy, R. W. and Kernan, M. J.** (2000). Genetically similar transduction mechanisms for touch and hearing in Drosophila. *J. Neurosci.* **20**, 5981-5988.

**Espeel, M.** (1985). Fine structure of the statocyst sensilla of the mysid shrimp *Neomysis integer* (Leach, 1814) (Crustacea, Mysidacea). *J. Morphol.* **186**, 149-165.

**Espeel, M.** (1986). Morphogenesis during molting of the setae in the statocyst sensilla of the mysid shrimp *Neomysis integer* (Leach, 1814) (Crustacea, Mysidacea). *J. Morphol.* **187**, 61-68.

**Farmer, A. S.** (1974). The functional morphology of the mouthparts and pereiopods of *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). *J. Nat. Hist.* **8**, 121-142.

**Fontaine, M. T., Passelecq-Gerin, E. and Bauchau, A. G.** (1982). Structures chemoreceptrices des antennules du crab *Carcinus maenas* (L.) (Decapoda, Brachyura). *Crustaceana* **43**, 271-283.

**Garm, A., Derby, C. D. and Høeg, J. T.** (2004). Mechanosensory neurons with

bend- and osmo-sensitivity in mouthpart setae from the spiny lobster *Panulirus argus*. *Biol. Bull.* (in press).

**Glantz, R. M., Kirk, M. and Viancour, T.** (1981). Interneurons in the crayfish brain: The relationship between dendrite location and afferent input. *J. Neurobiol.* **12**, 311-328.

**Gleeson, R. A.** (1982). Morphological and behavioral identification of the sensory structures mediating pheromone reception in the blue crab, *Callinectes sapidus*. *Biol. Bull.* **163**, 162-171.

**Gleeson, R. A., Carr, W. E. S. and Trapido-Rosenthal, H. G.** (1993). Morphological characteristics facilitating stimulus access and removal in the olfactory organ of the spiny lobster, *Panulirus argus*: insight from the design. *Chem. Senses* **18**, 67-75.

**Gleeson, R. A., McDowell, L. M. and Aldrich, H. C.** (1996). Structure of the aesthetasc (olfactory) sensilla of the blue crab, *Callinectes sapidus*: Transformations as a function of salinity. *Cell Tissue Res.* **284**, 279-288.

**Gnatzy, W.** (1984). 'Campaniform sensilla' on lobster antennae are dermal glands. *Cell Tissue Res.* **236**, 729-731.

**Gnatzy, W., Schmidt, M. and Römbke, J.** (1984). Are the funnel-canal organs the 'campaniform sensilla' of the shore crab *Carcinus maenas* (Crustacea, Decapoda)? I. Topography, external structure and basic organization. *Zoomorphol*. **104**, 11-20.

**Grünert, U. and Ache, B. W.** (1988). Ultrastructure of the aesthetasc (olfactory) sensilla of the spiny lobster, *Panulirus argus*. *Cell Tissue Res.* **251**, 95-103.

**Hamilton, K. A. and Ache, B. W.** (1983). Olfactory excitation of interneurons in the brain of the spiny lobster. *J. Comp. Physiol.* **150**, 129-140.

**Harrison, P. J. H., Cate, H. S., Steullet, P. and Derby, C. D.** (2001). Structural plasticity in the olfactory system of adult spiny lobsters: postembryonic development permits life-long growth, turnover and regeneration. *Mar. Freshwater Res.* **52**, 1357-1365.

**Horner, A. J., Weissburg, M. J. and Derby, C. D.** (2004). Dual antennular pathways can mediate orientation by Caribbean spiny lobsters in naturalistic flow conditions. *J. Exp. Biol.* **207**, 3785-3796.

**Johnson, B. R. and Ache, B. W.** (1978). Antennular chemosensitivity in the spiny lobster, *Panulirus argus*: amino acids as feeding stimuli. *Mar. Behav. Physiol.* **5**, 145-157.

**Keller, T. A., Powell, I. and Weissburg, M. J.** (2003). Role of olfactory appendages in chemically mediated orientation of blue crabs. *Mar. Ecol. Prog. Ser.* **261**, 217-231.

**Koehl, M. A. R., Koseff, J. R., Crimaldi, J. P., McCay, M. G., Cooper, T., Wiley, M. B. and Moore, P. A.** (2001). Lobster sniffing: Antennule design and hydrodynamic filtering of information in an odor plume. *Science* **294**, 1948-1951.

**Kouyama, N. and Shimozawa, T.** (1984). The ecdysis of hair mechanoreceptors in crayfish. *Cell Tissue Res.* **236**, 339-343.

**Laverack, M. S.** (1964). The antennular sense organs of *Panulirus argus*. *Comp. Biochem. Physiol.* **13**, 301-321.

**Laverack, M. S. and Ardill, D. J.** (1965). The innervation of the aesthetasc hairs of *Panulirus argus*. *Q. J. Microsc. Sci.* **106**, 45-60.

**Maynard, D. M.** (1965). Integration in crustacean ganglia. *Symp. Soc. Exp. Biol.* **20**, 111-149.

**Maynard, D. M. and Dingle, H.** (1963). An effect of eyestalk ablation on the antennular function in the spiny lobster, *Panulirus argus*. *Z. vergl. Physiol.* **46**, 515-540.

**Mellon, D., Jr, Alones, V. and Lawrence, M. D.** (1992). Anatomy and fine structure of neurons in the deutocerebral projection pathway of the crayfish olfactory system. *J. Comp. Neurol.* **321**, 93-111.

**Mellon, D., Jr and Munger, S. D.** (1990). Nontopographic projection of olfactory sensory neurons in the crayfish brain. *J. Comp. Neurol.* **296**, 253-262.

**Michel, W. C., McClintock, T. S. and Ache, B. W.** (1991). Inhibition of lobster olfactory receptor cells by an odor-activated potassium conductance. *J. Neurophysiol.* **65**, 446-453.

**Michel, W. C., Trapido-Rosenthal, H. G., Chao, E. T. and Wachowiak, M.** (1993). Stereoselective detection of amino acids by lobster olfactory receptor neurons. *J. Comp. Physiol. A* **171**, 705-712.

**Moran, D., Varela, F. and Rowley, J.** (1977). Evidence for active role of cilia in sensory transduction. *Proc. Natl. Acad. Sci. USA* **74**, 793-797.

**Nation, J. L.** (1983). A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Stain Technol.* **58**, 347-351.

**Roye, D. B.** (1994). Antennular withdrawal motoneurons in the lateral antennular neuropil of *Callinectes sapidus*. *J. Crust. Biol.* **14**, 484-496.

**Sandeman, D. C. and Denburg, J. L.** (1976). The central projections of chemoreceptor axons in the crayfish revealed by axoplasmic transport. *Brain Res.* **115**, 492-496.

**Sandeman, D. C. and Sandeman, R. E.** (1994). Electrical responses and synaptic connections of giant serotonin-immunoreactive neurons in crayfish olfactory and accessory lobes. *J. Comp. Neurol.* **341**, 130-144.

**Sandeman, R. E. and Sandeman, D. C.** (1996). Pre- and postembryonic development, growth and turnover of olfactory receptor neurons in crayfish antennules. *J. Exp. Biol.* **199**, 2409-2418.

**Schmidt, M. and Ache, B. W.** (1992). Antennular projections to the midbrain of the spiny lobster. II. Sensory innervation of the olfactory lobe. *J. Comp. Neurol.* **318**, 291-303.

**Schmidt, M. and Ache, B. W.** (1993). Antennular projections to the midbrain of the spiny lobster. III. Central arborizations of motoneurons. *J. Comp. Neurol.* **336**, 583-594.

**Schmidt, M. and Ache, B. W.** (1996a). Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. I. Non-olfactory chemosensory and mechanosensory pathway of the lateral and median antennular neuropils. *J. Comp. Physiol. A* **178**, 579-604.

**Schmidt, M. and Ache, B. W.** (1996b). Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. II. The olfactory pathway. *J. Comp. Physiol. A* **178**, 605-628.

**Schmidt, M. and Gnatzy, W.** (1984). Are the funnel-canal organs the 'campaniform sensilla' of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? II. Ultrastructure. *Cell Tissue Res.* **237**, 81-93.

**Schmidt, M., Van Ekeris, L. and Ache, B. W.** (1992). Antennular projections to the midbrain of the spiny lobster. I. Sensory innervation of the lateral and medial antennular neuropils. *J. Comp. Neurol.* **318**, 277-290.

**Schmiedel-Jakob, I., Anderson, P. A. V. and Ache, B. W.** (1989). Whole cell recording from lobster olfactory receptor cells: Responses to current and odor stimulation. *J. Neurophysiol.* **61**, 994-1000.

**Schöne, H. and Steinbrecht, R. A.** (1968). Fine structure of statocyst receptor of *Astacus fluviatilis*. *Nature* **220**, 184-186.

**Shelton, R. G. J.** (1974). Observations on the occurrence of an epizooic, blue-green alga on the chemoreceptor setae of the brown shrimp, *Crangon crangon* (L.). *J. Mar. Biol. Assn. UK* **54**, 301-307.

**Snow, P. J.** (1973). The antennular activities of the hermit crab, *Pagurus alaskensis* (Benedikt). *J. Exp. Biol.* **58**, 745-765.

**Spencer, M. and Linberg, K. A.** (1986). Ultrastructure of aesthetasc innervation and external morphology of the lateral antennule setae of the spiny lobster *Panulirus interruptus* (Randall). *Cell Tissue Res.* **245**, 69-80.

**Steullet, P., Cate, H. S. and Derby, C. D.** (2000a). A spatiotemporal wave of turnover and functional maturation of olfactory receptor neurons in the spiny lobster *Panulirus argus*. *J. Neurosci.* **20**, 3282-3294.

**Steullet, P., Cate, H. S., Michel, W. C. and Derby, C. D.** (2000b). Functional units of a compound nose: Aesthetasc sensilla house similar populations of olfactory receptor neurons on the crustacean antennule. *J. Comp. Neurol.* **418**, 270-280.

**Steullet, P., Dudar, O., Flavus, T., Zhou, M. and Derby, C. D.** (2001). 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. *J. Exp. Biol.* **204**, 4259-4269.

**Steullet, P., Krützfeldt, D. R., Hamidani, G., Flavus, T., Ngo, V. and Derby, C. D.** (2002). Dual antennular chemosensory pathways mediate odor-associative learning and odor discrimination in the Caribbean spiny lobster *Panulirus argus*. *J. Exp. Biol.* **205**, 851-867.

**Sullivan, J. M. and Beltz, B. S.** (2001a). Development and connectivity of olfactory pathways in the brain of the lobster *Homarus americanus*. *J. Comp. Neurol.* **441**, 23-43.

**Sullivan, J. M. and Beltz, B. S.** (2001b). Neural pathways connecting the deutocerebrum and lateral protocerebrum in the brains of decapod crustaceans. *J. Comp. Neurol.* **441**, 9-22.

**Wiens, T. J.** (1976). Electrical and structural properties of crayfish claw motoneurons in an isolated claw-ganglion preparation. *J. Comp. Physiol.* **112**, 213-233.

**Wolfrum, U.** (1990). Actin filaments: The main components of the scolopale in insect sensilla. *Cell Tissue Res.* **261**, 85-96.

**Wroblewska, J., Whalley, S. F. M. and Daniel, P. C.** (2002). Identification of chemosensory sensilla activating antennular grooming behavior in the Caribbean spiny lobster, *Panulirus argus*. *Chem. Senses* **27**, 769-778.

**Zimmer-Faust, R. K., Tyre, J. E., Michel, W. C. and Case, C. F.** (1984). Chemical mediation of appetitive feeding in a marine decapod crustacean: the importance of suppression and synergism. *Biol. Bull.* **167**, 339-353.