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CHEMOSENSITIVITY OF WALKING LEGS OF THE LOBSTER *HOMARUS AMERICANUS*: NEUROPHYSIOLOGICAL RESPONSE SPECTRUM AND THRESHOLDS

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SUMMARY

Responses of chemoreceptors in the walking legs of the lobster *Homarus americanus* to 35 individual compounds and 3 mixtures (prey odours and extracts) were studied using extracellular recording techniques. Compared against a standard mussel (*Mytilus edulis*) extract, these receptors were most sensitive to the amino acids L-glutamate, hydroxy-L-proline, L-aspartate, L-arginine, glycine, taurine, and L-alanine, as well as such other compounds as ammonium chloride, betaine, and the tripeptide glutathione. Most of these excitants are among those compounds most prevalent in the prey of lobsters. Some proteins and odours from live prey were also effective stimuli. In general, carbohydrates, alcohols, nucleosides, and nucleotides were only slightly excitatory. The lowest thresholds for 10 compounds ranged from $3{\cdot}5\times10^{-6}$ to $3{\cdot}5\times10^{-14}$ M; these thresholds are lower than previously reported for crustacean taste receptors.

INTRODUCTION

Neurophysiological studies have demonstrated that chemoreceptors are located on many appendages of decapod crustaceans, including the antennules (Ache & Case, 1969; Ache, 1972; Shepheard, 1974; Derby, 1982), antennae (Tazaki & Shigenaga, 1974; Fuzessery & Childress, 1975; Derby, 1982), mouthparts (Shelton & Laverack, 1968, 1970; Fuzessery & Childress, 1975; Derby, 1982), and legs (Case, 1964; Shelton & Laverack, 1968, 1970; Fuzessery & Childress, 1975; Hatt & Bauer, 1980; Derby, 1982). The antennular chemoreceptors represent what in vertebrates and terrestrial animals is called the sense of smell (olfaction), while the legs and mouthparts contain the sensory structures involved in the sense of taste (gustation) (Atema, 1977, 1980).

The majority of neurophysiological studies analysing the spectrum of stimulatory chemicals for crustaceans has found that compounds of small molecular weight are most effective (Case, 1964; Ache, Fuzessery & Carr, 1976; Johnson & Ache, 1978;

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Heinen, 1980). The most stimulatory molecules are organic nitrogenous compounds; e.g. amino acids and amines (Case & Gwilliam, 1961; Case, 1964; Crisp, 1967; Shepheard, 1974; Allison & Dorsett, 1977; Johnson & Ache, 1978; Bauer & Hatt, 1980). L-Glutamate, glycine, betaine, and taurine are among the most stimulatory nitrogenous compounds for most of these crustaceans with the exception of the crayfish *Orconectes limosus* (Bauer & Hatt, 1980; Bauer, Dudel & Hatt, 1981). Other compounds, including carbohydrates, proteins, and nucleosides and nucleotides, have been found to occasionally show some activity but generally less than those mentioned above. However, behavioural experiments have shown that carbohydrates, which are commonly found in high concentrations in algae and diatoms, can be excitatory for certain crustaceans, including a herbivore (kelp crab *Pugettia producta*: Zimmer, Cook & Case, 1979), a filter-feeder (porcelain crab *Petrolisthes cinctipes*: Hartman & Hartman, 1977), a deposit-feeder (fiddler crab *Uca pugilator*: Robertson, Fudge & Vermeer, 1981), and an omnivore (crayfish *Procambarus simulans*: Ashby & Larimer, 1965). Peptides and proteins are also known to activate behavioural responses in some crustaceans (Carr & Gurin, 1975; Birch & Kagi, in Hindley, 1977; Zimmer-Faust & Michel, 1980; Robertson *et al.* 1981). The response spectra of many different species therefore seem to be tuned, within phylogenetically-imposed limitations, to extract chemical information most relevant to their particular habitat and lifestyle.

It is generally believed that there are differences in the chemosensory sensitivities or thresholds of the taste and smell organs of crustaceans; i.e. legs have high thresholds, serving contact chemoreception, and antennules have low thresholds, serving distance chemoreception (Shelton & Laverack, 1970; Shepheard, 1974). However, more experimental data are necessary to evaluate this conclusion because, as Thompson & Ache (1980) have pointed out, much of the data on receptor thresholds from the earlier neurophysiological studies may not be reliable due to lack of control of the health of the preparation or of the experimental protocol.

The present study characterizes the chemoreceptors in walking legs of the lobster *Homarus americanus* both in terms of response spectrum and threshold, and the results are interpreted in the context of the lobster's behavioural ecology.

MATERIALS AND METHODS

Neurophysiological experiments were performed on excised legs of locally caught lobsters; all four pairs of legs were examined. After a leg was removed, the articulations at the leg joints were cut and the exoskeletal coverings were slipped off, exposing the intact leg nerve. The dactylus and propodus of the leg remained intact and were inserted through a rubber dam of a stimulating-recording chamber (Fig. 1). This isolated the distal end of the leg, which was continuously rinsed in a constant 10 ml/min flow of M.B.L. formula artificial sea water (ASW) (423·0 mM-NaCl, 9·0 mM-KCl, 9·3 mM-$CaCl_2.2H_2O$, 22·9 mM-$MgCl_2.6H_2O$, 25·5 mM-$MgSO_4.7H_2O$, buffered to pH 7·4 with $NaHCO_3$), from the leg nerve bundle, which was bathed in *Homarus* Ringer's solution (472·2 mM-NaCl, 9·4 mM-KCl, 16·3 mM-$CaCl_2.2H_2O$, 6·9 mM-$MgCl_2.6H_2O$, 11·1 mM dextrose, buffered to pH 7·4 with $NaHCO_3$). Nerve bundles were carefully separated with minuten insect pins and fine forceps. A platinum

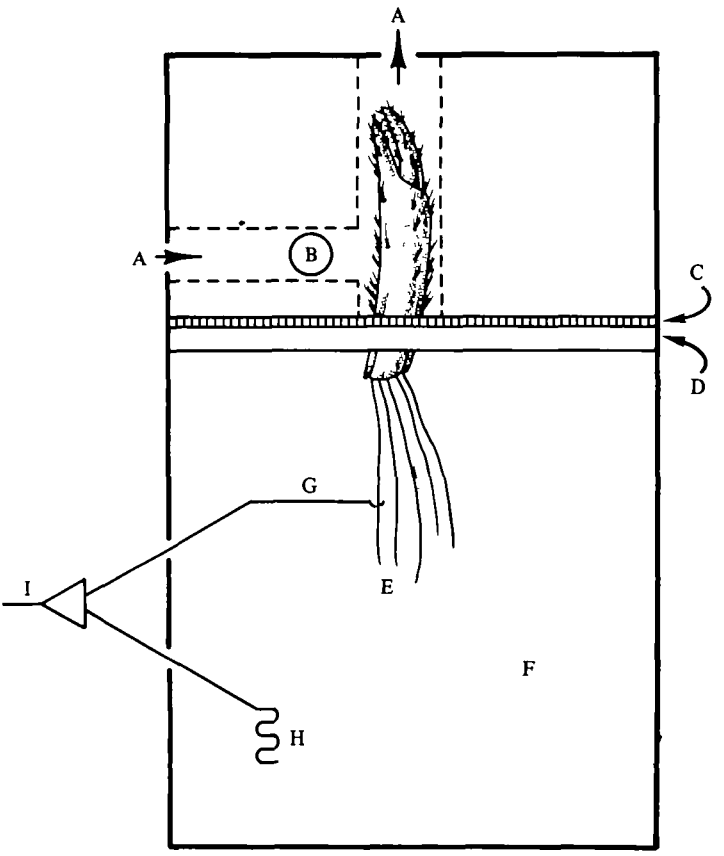

Fig. 1. Diagram of stimulating-recording chamber. Artificial sea water (A) flows over the distal segments of the leg. Chemical stimuli are introduced at (B). A rubber dam (C) and plexiglass plate (D) prevent the stimuli from reaching the exposed nerves (E), which are bathed in *Homarus* Ringer's solution (F). A platinum hook electrode (G) is used to pick up one of the nerve bundles, and a Ag–AgCl wire (H) serves as a reference electrode. The responses are amplified (I) and displayed on standard electrophysiological equipment and stored on magnetic tape for subsequent analysis.

hook electrode and standard electrophysiological instrumentation were used to record neural activity extracellularly.

Stock solutions of chemical compounds were mixed in ASW and the pH of each was adjusted, if necessary, to 7·6 with either HCl or NaOH. The L-isomers of amino acids were used in these experiments since they have generally been found to be more effective than the corresponding D-isomers in both neurophysiological (Case, 1964; Bauer *et al.* 1981) and behavioural (Mackie, 1973) experiments. Standard mussel (*Mytilus edulis*) extract was prepared by homogenizing 20 g wet weight of soft tissue in one litre ASW, centrifuging at 25000 *g* for 15 min, removing the pellet, and diluting the supernatant 10-fold. This extract was kept frozen until used each day. Rinses from live intact prey species (= prey odours) were prepared by placing five 5 cm long mussels (*Mytilus edulis* or *Modiolus modiolus*) in one litre ASW for 6 h,

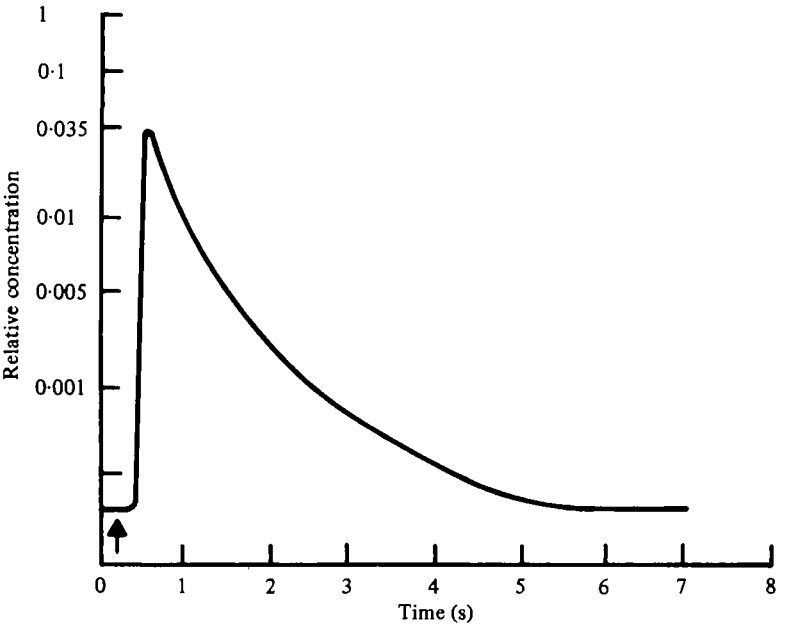

Fig. 2. Temporal profile of stimulus introduced into chamber as it flows over the leg hairs. A 50 μl aliquot of salt solution was introduced at the time indicated by the arrow. As the stimulus flowed through the chamber, electrodes connected to a Wheatstone bridge measured the change in conductance. Relative concentration was determined by measuring the conductance of known dilutions of the stock salt solution.

followed by filtration of the water. These prey odours were only used fresh, within a few hours of preparation.

Chemical stimuli in 50 μl aliquots were introduced into the ASW flow of the chamber. The stimulus became more dilute as it reached the sensory hairs of the leg. The time course and dilution factor of the stimulus were determined by measuring with a platinum wire connected to a Wheatstone bridge the change in conductance following introduction of a salt solution into flowing distilled water (Meredith & Moulton, 1978). The stimulus reached its peak concentration 0·5 s after introduction and was eliminated from the chamber 4–10 s later, depending on the stimulus concentration introduced; the stimulus achieved a maximum concentration of 0·035 times that introduced (Fig. 2). This dilution factor is included in the reported concentrations of chemical stimuli. This dilution factor is probably a conservative measure of the stimulus concentration near some of the receptor cells, such as those situated in the centre of a tuft of setae, since access of the stimulus to these receptor sites is most likely physically impeded.

Active chemoreceptors were identified as such with a broadly stimulating search stimulus, mussel extract. Responses to a series of compounds were then monitored. The compounds tested included many known and potential feeding attractants of marine animals, including amino acids, amines, alcohols, nucleosides and nucleotides, peptides, proteins, carbohydrates, and prey odours. Following the termination of each

test response, the leg was rinsed by using suction to remove the ASW surrounding the hairs and by increasing the ASW flow rate several times. Threshold responses for a given compound, defined as the lowest concentration at which an increase in neural activity was recorded, were determined by presenting an ascending concentration series. Action potentials elicited following stimulation were counted either manually or with a spike counter containing a window discriminator. Response magnitudes were determined by taking the difference in the total number of spikes during equal time periods before and after stimulus introduction; mechanoreceptor responses were corrected for by also subtracting the responses to an ASW control stimulus. Activity ratios, defined following Johnson & Ache (1978) as the ratio of the response to each compound and the response to the mussel extract, were computed for the responses of each nerve to every compound tested. Mean activity ratios and standard errors ($\bar{X} \pm$ S.E.) were then determined for each compound. During this study, both multi-unit and single-unit recordings were obtained. The response spectrum for the population of leg chemoreceptors was determined by means of multi-unit recordings; both multi- and single-unit recordings were used in the threshold determinations.

RESULTS

The population of walking leg chemoreceptors of *H. americanus* generally responded to the same compounds that are effective stimuli for other crustacean chemoreceptors; i.e. certain amino acids and amines, but not sugars, alcohols, or nucleosides and nucleotides (Table 1). The amino acids that were most stimulatory to this population of cells were L-glutamate, hydroxy-L-proline, L-aspartate, L-arginine, glycine, taurine, and L-alanine. Among the other compounds as excitatory as these amino acids were ammonium chloride, glutathione, and betaine. Although proteins and prey odours are difficult to compare with these other compounds due to uncertainty of molarity in the case of the proteins and the composition in the case of prey odours, some of the stimuli were quite effective, most notably haemoglobin and *Mytilus* prey odour.

Thresholds were determined for 10 compounds using single- and multi-unit recordings (Table 2). Individual thresholds ranged from $3{\cdot}5 \times 10^{-4}$ M to $3{\cdot}5 \times 10^{-14}$ M. Many cells responded to compounds at concentrations as low as $3{\cdot}5 \times 10^{-9}$ M, and some cells were responsive to ammonium chloride at $3{\cdot}5 \times 10^{-10}$ M. In addition, one cell was excited by a much lower ammonium chloride concentration – $3{\cdot}5 \times 10^{-14}$ M; the responses of this cell are shown in Fig. 3. The responses of a single cell to a dilution series of L-glutamate are illustrated in Fig. 4. The mean response threshold of 22 single cells sensitive to L-glutamate, as shown in Fig. 5, is between 10^{-7} and 10^{-8} M.

DISCUSSION

Chemoreceptors in walking legs of *H. americanus* have a chemical response spectrum generally similar to many of the other crustaceans that have been studied, responding best to L-glutamate, hydroxy-L-proline, ammonium chloride, L-aspartate, L-arginine, glycine, glutathione, betaine, taurine, and L-alanine (Table 1). All of these compounds,

Table 1. *Response spectrum of* H. americanus *walking leg chemoreceptors*

Stimulus*	Activity ratio ($\bar{X}$)	S.E.	N
Mussel extract (*Mytilus*)†	100	—	100
L-Glutamate	66·5	14·3	62
Hydroxy-L-proline	61·6	18·9	31
Ammonium chloride	57·8	21·8	9
L-Aspartate	52·6	26·2	26
L-Arginine	43·3	14·9	51
Glycine	42·0	11·4	35
Glutathione (reduced)	41·6	10·4	15
Betaine	39·1	14·0	21
Taurine	34·6	10·4	36
L-Alanine	32·4	17·6	27
L-Phenylalanine	27·4	7·6	56
L-Isoleucine	25·7	15·9	49
Inosine 5′ monophosphate	24·6	17·8	10
L-Histidine	18·1	13·5	16
L-Methionine	16·3	9·3	16
L-Leucine	16·0	11·7	17
L-Valine	13·4	5·8	29
L-Serine	9·9	8·6	15
β-Alanine	7·0	4·2	13
Sucrose	6·7	4·5	12
L-Tryptophan	4·5	4·0	14
L-Cysteine	4·0	3·2	16
Ethanol	2·8	1·7	6
Adenosine 2′ & 3′ monophosphate	2·5	1·9	5
L-Proline	2·3	1·7	9
D-Ribose	1·0	1·0	10
L-Asparagine	0·8	0·8	5
Urea	0·4	0·4	10
Inosine	0	0	5
Glucosamine	0	0	5
L-Lysine	0	0	6
Haemoglobin‡	41·0	20·1	26
Mytilus prey odour§	34·4	20·8	6
Modiolus prey odour§	18·8	17·8	5
Egg albumin‡	13·9	6·6	25
Glycogen (from oyster)‡	6·2	4·2	13
Bovine serum albumin‡	5·5	2·4	25

* All compounds tested at $3{\cdot}5 \times 10^{-4}$ M unless noted.
† 70 mg wet weight soft tissue/litre ASW.
‡ 0·125 mg/ml; purified by 10000 mol. wt. molecular filtration.
§ One mussel in one litre ASW for 1 h.

with the exception of hydroxy-L-proline, are among the most common free amino acids and amines in the tissues and excretory products of many invertebrates upon which lobsters feed (e.g. Awapara, 1962; Kittredge *et al.* 1962; Florkin & Schoffeniels, 1969; Takagi *et al.* 1970; Konosu, 1971; D'Aniello, 1980; Suyama & Kobayashi, 1980). The essential amino acids for lobsters (Gallagher & Brown, 1975; Conklin, 1980) – leucine, isoleucine, lysine, threonine, valine, methionine, phenylalanine, histidine, and arginine – are generally not the most prevalent free amino acids in the tissues of prey species. The chemoreceptors of *H. americanus* therefore appear to be tuned to those amino acids that are most abundant in their prey rather than

Table 2. *Threshold responses of* H. americanus *walking leg chemoreceptors as determined from combination of single- and multi-unit recordings*

Stimulus	Lowest threshold (M)	Median threshold (M)	N*
Ammonium chloride	$3{\cdot}5 \times 10^{-14}$	$3{\cdot}5 \times 10^{-8}$	14
L-Glutamate	$3{\cdot}5 \times 10^{-9}$	$7{\cdot}0 \times 10^{-8}$	38
Taurine	$3{\cdot}5 \times 10^{-9}$	$3{\cdot}5 \times 10^{-7}$	12
Betaine	$3{\cdot}5 \times 10^{-9}$	$3{\cdot}5 \times 10^{-7}$	7
L-Isoleucine	$3{\cdot}5 \times 10^{-9}$	$3{\cdot}5 \times 10^{-6}$	3
L-Arginine	$3{\cdot}5 \times 10^{-8}$	$3{\cdot}5 \times 10^{-7}$	10
L-Glutamine	$3{\cdot}5 \times 10^{-8}$	$3{\cdot}5 \times 10^{-7}$	4
L-Phenylalanine	$3{\cdot}5 \times 10^{-7}$	$3{\cdot}5 \times 10^{-5}$	7
L-Aspartate	$3{\cdot}5 \times 10^{-8}$	$3{\cdot}5 \times 10^{-5}$	5
L-Valine	$3{\cdot}5 \times 10^{-6}$	$3{\cdot}5 \times 10^{-5}$	5

* Number of recordings for each stimulus.

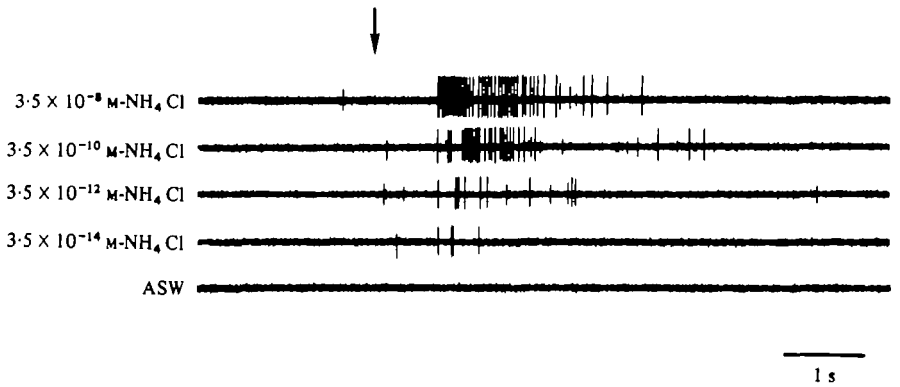

Fig. 3. Threshold determination for chemoreceptor responsive to ammonium chloride. The order of traces in this figure is inverse of the order of stimulus presentation in the experiment, since thresholds were determined by presenting an ascending concentration series of stimuli. Arrow indicates stimulus introduction.

those that are nutritionally essential. The amino acid hydroxy-L-proline is interesting in that while it is not an abundant constituent of prey species, it is a highly effective stimulus for both legs (Table 1) and antennules (Shepheard, 1974) of *H. americanus*. Proline, on the other hand, is a common free amino acid in invertebrates, but it is comparatively non-stimulatory to leg and antennular chemoreceptors of *H. americanus*. Ammonium chloride, which is a major metabolic waste product excreted by common prey to lobsters (Campbell & Bishop, 1970; Bayne & Scullard, 1977) and is a major bacterial degradation product of rotting flesh (Frobisher *et al.* 1974), is a highly effective stimulus. In contrast, urea, which is not a common excretory product of these prey species, is non-stimulatory. A dilution of prey odour from live *Mytilus* is also stimulatory, indicating that leg chemoreception is important in the detection of live undamaged prey (Hirtle & Mann, 1978; Derby & Atema, 1981, 1982*a*). Larger molecules also excite the leg chemoreceptors of *H. americanus*. One such compound

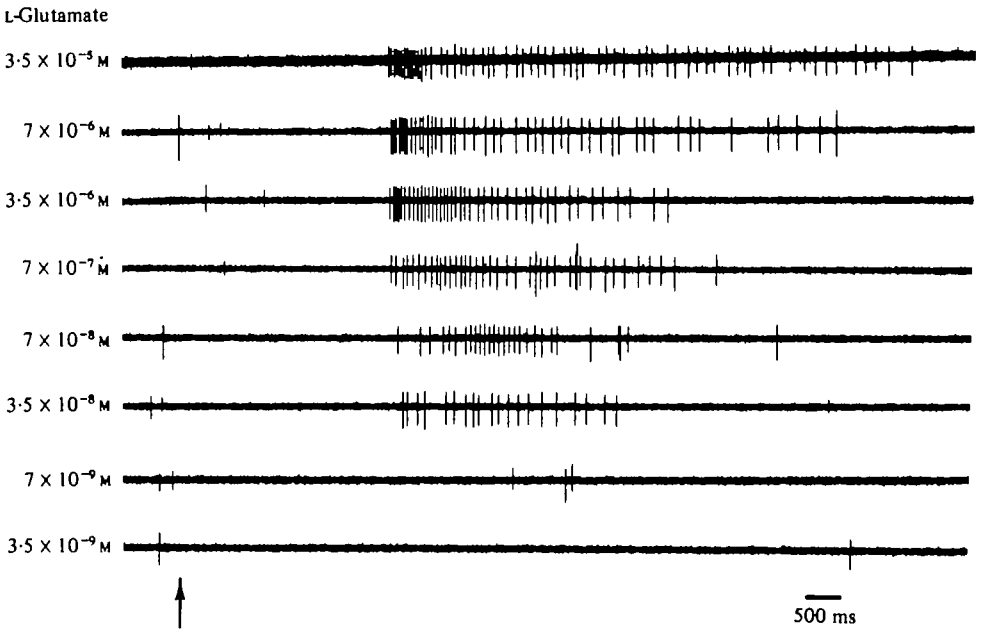

Fig. 4. Threshold determination for chemoreceptor responsive to L-glutamate. The order of traces in this figure is inverse of the order of stimulus presentation in the experiment, since thresholds were determined by presenting an ascending concentration series of stimuli. Arrow indicates stimulus introduction.

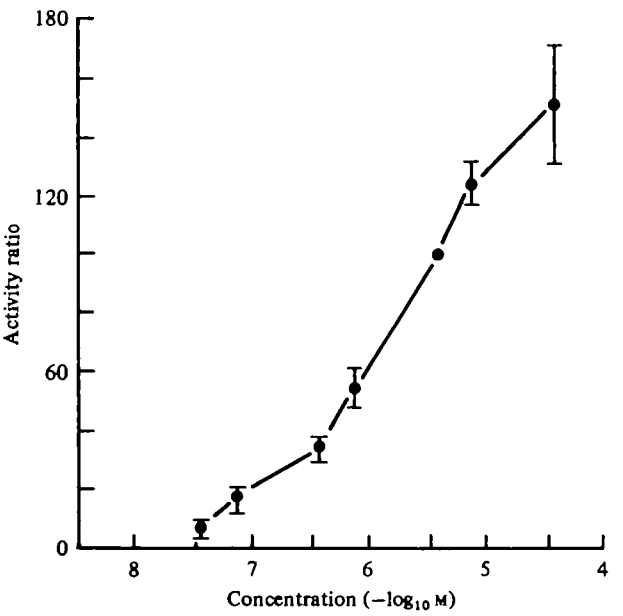

Fig. 5. Response thresholds of 22 single cells sensitive to L-glutamate. Values are means ± standard errors. Mean activity ratio of responses to $3{\cdot}5 \times 10^{-6}$ M was arbitrarily set at 100; activity ratios of responses to other concentrations were set relative to this value as described in Materials and Methods section.

glutathione, a tripeptide (glutamylcysteinylglycine) important in the feeding responses of many invertebrates, especially coelenterates (Lenhoff & Lindstedt, 1974). Haemoglobin (0·125 mg/ml) is another highly stimulatory compound, although comparisons of effectiveness are tenuous since the precise molarity of the protein solution is uncertain. Although neurophysiological experiments generally find proteins to have no (Case, 1964) to moderate (Ache, Johnson & Clark, 1978) activity, behavioural experiments indicate that peptides and proteins can be important for crustaceans in finding hosts or food (Davenport, 1966; Carr & Gurin, 1975; Birch & Kagi, in Hindley, 1977; Zimmer-Faust & Michel, 1980; Robertson *et al.* 1981). Thus, protein-sensitive cells such as those reported here may be involved in feeding or social behaviours.

The response spectrum described above represents that of the entire population of leg chemoreceptors. When the activity of single neurones could be clearly monitored, it was found that the response spectra of different neurones fell into discrete groups, as is discussed in more detail elsewhere (Derby & Atema, 1982*b*).

Neurophysiological analyses of antennular chemoreceptors of *H. americanus* (Ache, 1972; Shepheard, 1974) provide a basis for comparing the response characteristics of taste (leg) and smell (antennular) chemoreceptors. Shepheard found that glutamate, hydroxy-L-proline, glycine, taurine, and aspartate were five of the seven most effective compounds that excited antennular chemoreceptors, and that sugars, alcohols, and organic acids were less effective. Although Shepheard's data are biased for L-glutamate, since this single compound was used as the search stimulus, the reported responses of antennular chemoreceptors are very similar to responses of chemoreceptors in the legs. Obviously, a complete characterization of the response spectra of neither the antennular nor the leg chemoreceptors has been carried out. More thorough descriptions of responses to both food and social chemical stimuli will be necessary before it can be stated unequivocally that antennular and leg response spectra of *H. americanus* are the same. Yet all the neurophysiological data presently available indicate that the smell and taste receptors are similar in this respect. There is behavioural evidence that leg and antennular chemoreceptors of some marine crustaceans may be differentially sensitive to some chemicals, such as sex pheromones (e.g. Gleeson, 1980) and host odours (Ache, 1975; Derby & Atema, 1980); however, it is not certain in these studies whether pheromone receptors only exist on antennules or whether pheromone receptors of other appendages do not mediate the particular behavioural actions monitored in these studies.

Observations of the chemosensory behavior of *H. americanus* are in general agreement with the responses of leg and antennular chemoreceptors. Of the 25 amino acids tested at concentrations estimated to be 0·7 ppm (approximately 10^{-5} M), L-proline, glutamate, and alanine elicited walking and feeding movements, whereas leucine, tryptophan, lysine, methionine, cysteine, phenylalanine, histidine, serine, and valine did not. In contrast to the neurophysiological experiments, hydroxy-L-proline, aspartate, L-arginine, and glycine were not effective behavioural excitants (McLeese, 1970). Whole prey extracts and complex mixtures were more effective than single compounds (McLeese, 1970; Mackie, 1973) and no single class of compounds was as attractive as a complex mixture of several of these classes (Mackie

& Shelton, 1972; Mackie, 1973; Zimmer-Faust & Michel, 1980), suggesting that stimulation of a variety of different types of chemoreceptors (Fuzessery, Carr & Ache, 1978; Derby & Atema, 1982*b*) is most effective in initiating food searching behaviour of several lobster species. Experiments with *H. americanus* indicate that the small molecule fraction (< 1000 mol. wt.) is responsible for eliciting grasping responses and ingestion (C. D. Derby, unpublished data).

The lowest thresholds of chemoreceptors in the legs of *H. americanus* for the 10 compounds tested (Table 2) ranged from $3{\cdot}5 \times 10^{-6}$ to $3{\cdot}5 \times 10^{-14}$ M. Responses to $3{\cdot}5 \times 10^{-9}$ M concentrations of many compounds were common, and one cell was excited by $3{\cdot}5 \times 10^{-14}$ M ammonium chloride. In their natural environment, the lobster's amino acid and ammonia receptors (Derby & Atema, 1982*b*) would become adapted to the background levels present in sea water and would function only at concentrations above these levels. The background levels are variable but are often in the nanomolar range for many amino acids and in the micromolar range for ammonia (Dawson & Pritchard, 1978; Mopper & Lindroth, 1982). Cells sensitive to compounds that are not present at such high concentrations in sea water could function over a greater range of concentrations; this would include taurine cells of lobsters (Fuzessery *et al.* 1978; Thompson & Ache, 1980; Derby & Atema, 1982*b*).

The wide variation between cells in response thresholds observed here could have been due to real characteristics of the cells (e.g. range fractionation). Fractionation of response range has been described for chemoreceptors in the antennules of the lobster *Panulirus argus* (Thompson & Ache, 1980). The observed variations in thresholds of leg chemoreceptors of *H. americanus* may also have been due to experimental artifacts, such as differential access of the stimulants into the tufts of chemosensory sensilla due to the indirect method of stimulus delivery. Another possible experimental artifact is differential adaptation of the receptors, although we attempted to control for this effect by maintaining a strict protocol of stimulus introduction.

Traditionally, leg chemoreceptors have been assumed to be less sensitive than antennular chemoreceptors (Shelton & Laverack, 1970; Shepheard, 1974). The thresholds reported here are lower than most previous reports for leg chemoreceptors (Case & Gwilliam, 1961; Laverack, 1963; Case, 1964; Levandowsky & Hodgson, 1965). They are also lower than thresholds reported for antennular chemoreceptors of *H. americanus* (Shepheard, 1974). Unfortunately, many of the earlier reports on thresholds of crustacean chemoreceptors are of limited comparative value because of inconsistencies in methodology. As Dethier (1976) and Thompson & Ache (1980) have pointed out, methodology can have an important effect on threshold measurements. Important methodological considerations in threshold studies include: the use of relatively low concentrations of stimulants in the search for active chemoreceptors in order to avoid adaptation of the receptors; strict adherence to the experimental protocol (e.g. order of stimulus presentation); and maintenance of the health of the preparation (e.g. perfusion of the antennular preparation seems to be essential for maintaining its viability) (Thompson & Ache, 1980; Ache & Macmillan, 1980). Therefore, comparisons of thresholds of leg and antennular thresholds should be based only on the few studies in which methodology was regulated carefully. Using methods similar to those performed in these experiments, Fuzessery *et*

1978) found that taurine-sensitive cells in the antennules of the spiny lobster *Panulirus argus* have thresholds from 10^{-8} to 10^{-10} M, which is within the range of thresholds of many of the leg chemoreceptors of *H. americanus*. In even more rigorously controlled experiments, however, Thompson & Ache (1980) found that these same taurine-sensitive cells of *P. argus* were excited by concentrations of taurine near 10^{-13} M and presumably have thresholds several orders of magnitude lower than this. These results, taken together, suggest that the distinction between leg and antennular chemoreceptors of crustaceans, and therefore also between taste and smell, on the basis of threshold sensitivity must await a comparative study using the same techniques on the chemoreceptors in different sensory appendages of the same species.

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