Sulfide as a Chemical Stimulus for Deep-Sea Hydrothermal Vent Shrimp

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Organisms dependent on deep-sea hydrothermal vents for their existence face extinction when their vents expire, unless they can establish populations on neighboring vents or on new vent sites. Propagules, including larvae and motile adults, are readily dispersed broadly by seafloor currents, but how they recognize active hydrothermal sites is problematical. Compelling evidence that vent organisms can find and colonize hydrothermal sites has been provided by a series of observations on the East Pacific Rise (1). New hydrothermal vents created there following a volcanic eruption on the seafloor in March 1991 were colonized by sessile invertebrates in less than one year. On the Mid-Atlantic Ridge, shrimp that normally cluster on sulfide surfaces have been observed to swim directly back to the surfaces when displaced from them. How do vent animals locate new or existing vents? Passive transport by currents (2) or active swimming without guidance by some physical cue is not likely to result in success (3). Chemicals present in hydrothermal fluids have been proposed as attractants. We provide the first evidence of a chemosensory response in a vent invertebrate to sulfides, which are prevalent in vent fluids and provide the energy for chemosynthetic primary production at vents.

During recent field work at hydrothermal vents on the Mid-Atlantic Ridge where extremely motile shrimp (*Rimicaris exoculata* and a smaller, possibly new, *Rimi-*

caris sp.) dominate the surfaces of sulfide chimneys, we documented a strong orientation behavior of shrimp, perhaps guided by chemical cues, to a piece of sulfide removed from a chimney (Fig. 1). Specimens of *Rimicaris* sp. and *R. exoculata* collected by the deep submergence vehicle (DSV) *Alvin* survived sufficiently long aboard ship to allow us to examine physiological responses of antennal nerves to various chemical stimulants, as well as to preserve antennal filaments for later electron microscopic examination. The shrimp possess first and second antennae (Fig. 2A) similar to those of other decapod crustaceans whose antennae are known to respond to chemical as well as to tactile stimuli (4). The first and second antennae both bear sensilla (Fig. 2B; 1 sensillum/66 μm of filament circumference on average for *Rimicaris* sp. and 1/48 μm for *R. exoculata*) with an open pore at their tips (Fig. 2C, D). Transmission electron microscopy (TEM) reveals that each sensillum is innervated by 10 to 14 sensory dendrites (Fig. 2E, F). Light microscopic and TEM studies indicate that the channel containing the sensory dendritic segments extends to the pore at the tip of the sensillum, and that at least some of the dendritic processes in the channel reach the pore.

Nerves in excised filaments of both antennae frequently showed spontaneous activity and responded to tactile stimuli. Antennal filaments also responded to a variety of chemical stimuli, including an homogenate of bacterial cells isolated from the vents and mixtures of amino acids. We tested 7 filaments of the second antenna, and 4 medial filaments and 5 lateral filaments of the first antenna. The largest and most robust response was evoked in filaments
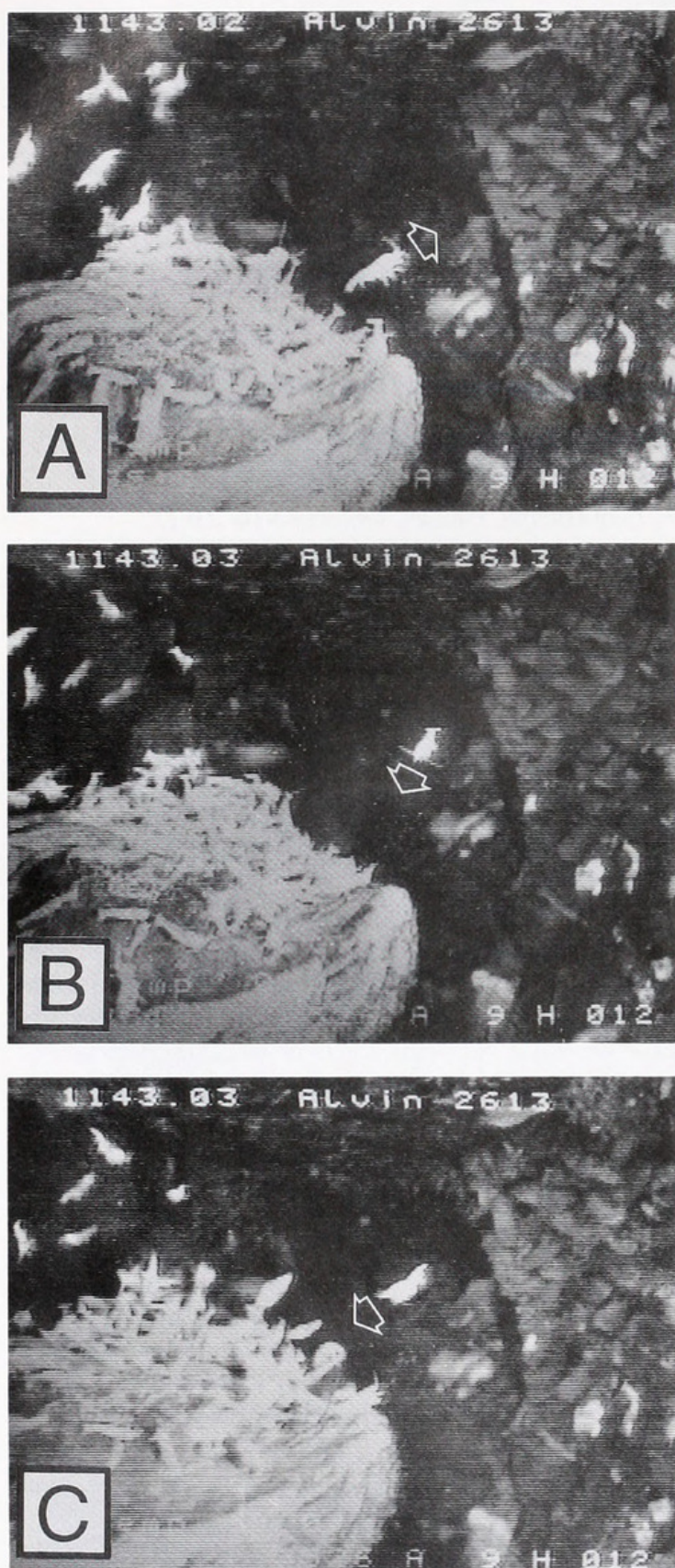
Figure 1. (A) A startled hydrothermal vent shrimp swims off a sulfide-bearing rock sample taken from a vent chimney, (B) reorients itself towards the rock, and then (C) swims back to the rock. Similar behavior by other shrimp occurred several times during collection of the rock sample by DSV *Alvin*. From videotape of DSV *Alvin* dive number 2613 on 14 June 1993 at the Snake Pit site on the Mid-Atlantic Ridge.

of the second antenna on exposure to solutions of Na_2S (Fig. 3A). All seven filaments tested responded to Na_2S; the response could be eliminated by removing the stimulant and could be evoked repeatedly. The steady-state response increased with the concentration of Na_2S. In the four filaments of the second antenna used to study the response to Na_2S at different concentrations, the relative responses (Fig. 3B) were (mean $\pm$ standard error of the mean): 0.113 ± 0.054 ($n = 3$) at $[Na_2S] = 1.3$ mM; 0.567 ± 0.137 ($n = 4$) at 13 mM; 0.654 ± 0.148 ($n = 4$) at 130 mM; and 1.014 ± 0.140 ($n = 4$) at 1300 mM. Two control solutions were used on two of these four filaments. The first was artificial seawater (ASW) with pH = 8, matching the pH of the lowest Na_2S concentration used, which gave a relative response of -0.078 ± 0.010. The second control solution (pH 13) was ASW with pH adjusted to 13, equal to that of the 1300 mM Na_2S solution, which gave a relative response of -0.030 ± 0.050. Three each of the medial and lateral filaments of the first antenna were tested for sensitivity to Na_2S. The medial filament of the first antenna responded less consistently than filaments of the second antenna in what appeared to be a transient manner. The lateral filament responded little, if at all, to Na_2S.

Of the three antennal filaments, only the responses of the second antenna showed a significant concentration dependence. A linear regression analysis of the mean values of the second antenna's responses as a function of $\log[Na_2S]$ gave a slope significantly different from zero ($P < 0.05$). Based on general experience with chemical senses, we expect that the actual dependence of the second antenna's response on sulfide concentration is not this linear one, but rather a sigmoidal dependence (10). The trend of the responses suggests that the threshold concentration for the sensory cells in the second antenna lies in the micromolar range of sulfide concentrations.

The responses of excised filaments of the second antenna of *Rimicaris* sp. and *R. exoculata* to stimulation by dissolved sulfide suggest that vent shrimp may be able

Figure 2. (A) Lateral view of *Rimicaris* sp., a small orange-colored hydrothermal vent shrimp collected at the Snake Pit site, together with dorsal view of cephalothorax, showing the two first antennae (each with a short medial and lateral filament close to the midline) and the two second antennae (each having one long filament shown deployed laterally); scale bar = 5 mm. (B) The second antenna of *Rimicaris* sp. showing the distribution of sensilla near the distal border of each segment. Bacterial populations encrust the surface, giving it a mottled appearance (scale = 25 μm). The second antenna of *Rimicaris exoculata* is similar in structure. For SEM examination, antennae were removed from animals which had been fixed in 5% paraformaldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.2) immediately after arrival at the ocean's surface. The tissue was dehydrated in a graded ethanol series, immersed in hexamethyldisilazane for 5–10 min and air dried (5). Pieces of dried antennae were mounted on stubs, coated with gold, and examined using an
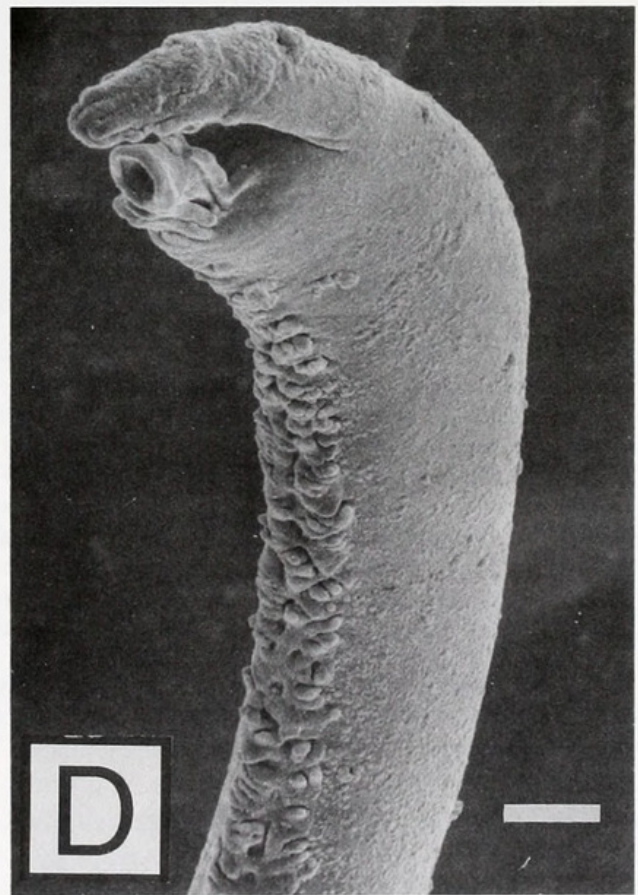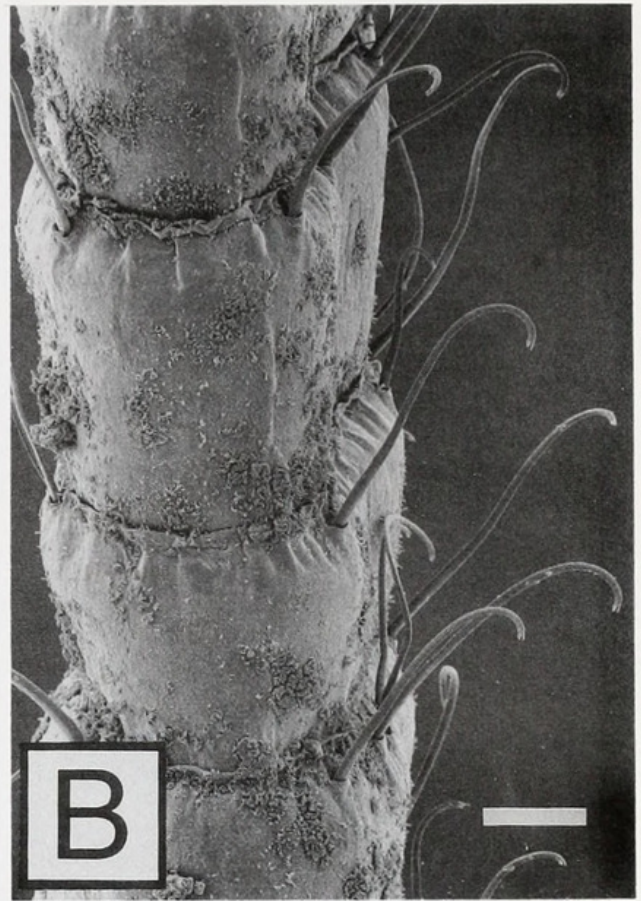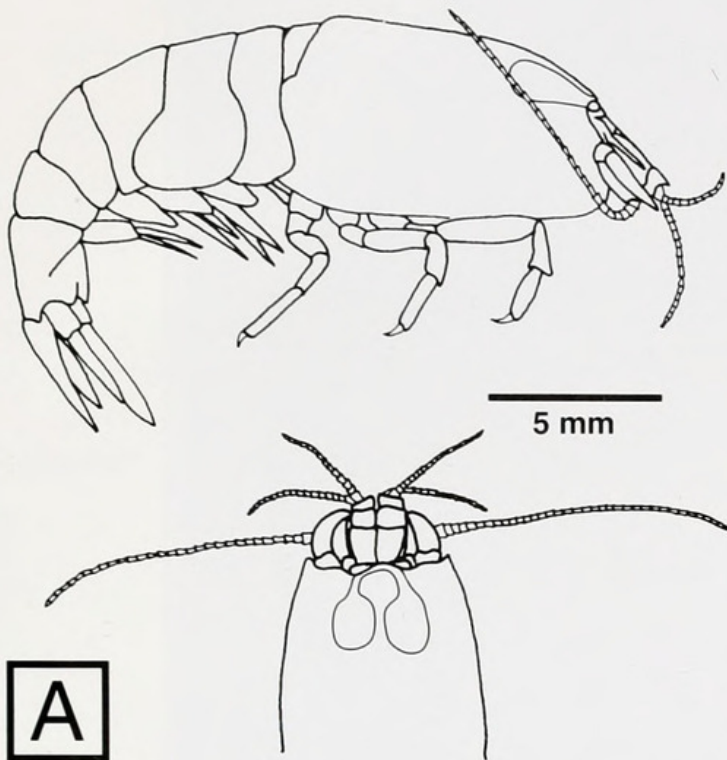
Hitachi 4000 scanning electron microscope at 6.0 kV. (C) A sensillum on the second antenna of *Rimicaris* sp. At the tip of the sensillum, fingerlike projections extend above the laterally facing terminal pore (arrowhead). Proximal to the pore are regularly arranged button-like microstructures (arrow) which are associated with the distal third of the sensillum (scale = 1 μm). (D) The sensilla on the second antenna of *R. exoculata* are similar in structure, with irregular crenulations replacing the button-like microstructures (scale = 1 μm).
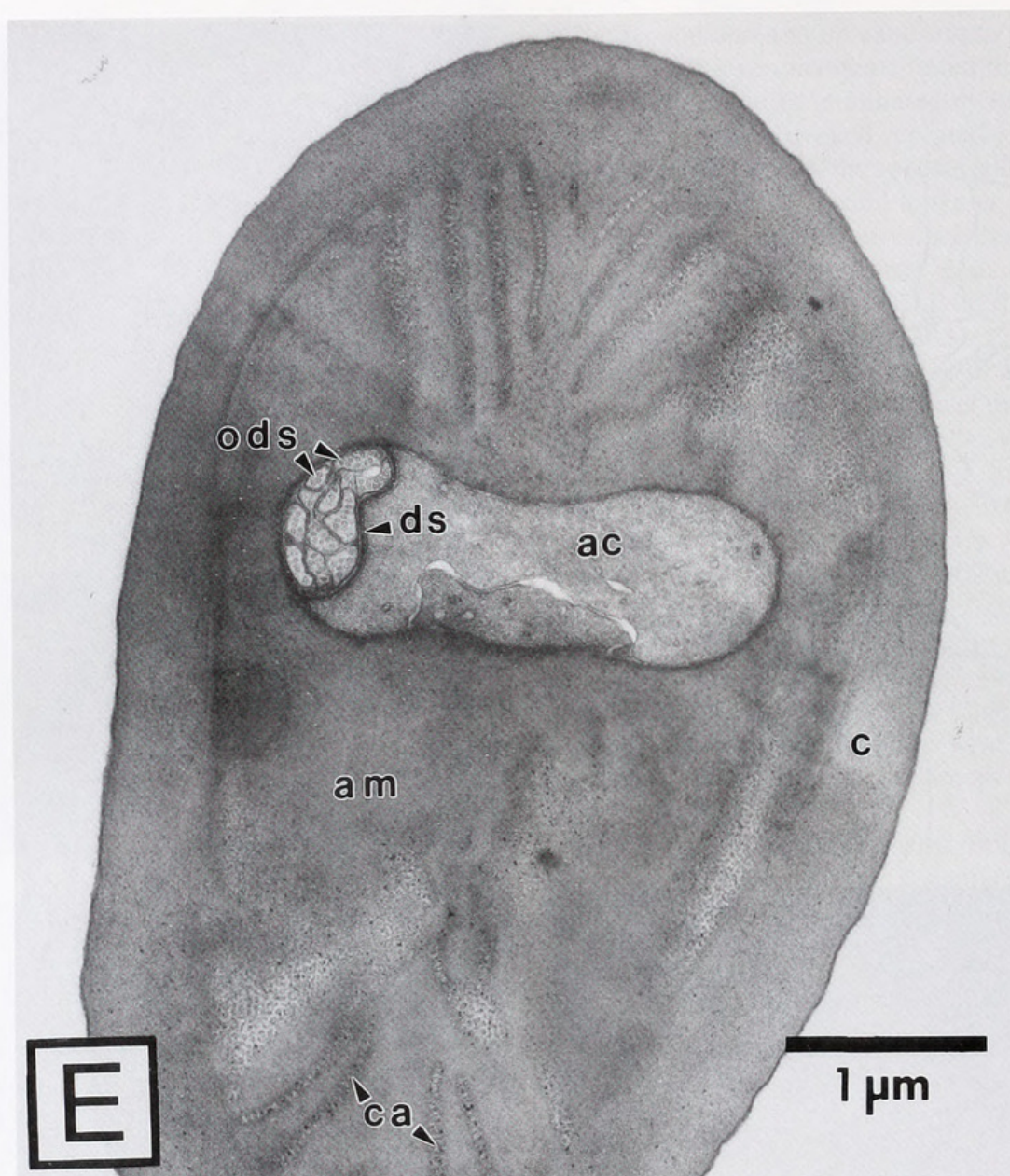
Figure 2. (E) Cross section in the proximal region of a sensillum from the second antenna of *R. exoculata*. The sensillum is composed of three major layers: a cuticle (*c*); an inner core of amorphous material (*am*) penetrated by canals (*ca*); and a central channel containing the processes of auxiliary cells (*ac*), together with the outer dendritic segments (*ods*) of sensory neurons. In this region the outer dendritic segments are surrounded by a well-developed dendritic sheath (*ds*). For TEM examination, antennae were removed from animals immediately after they were brought to the ocean's surface. The antennae were fixed overnight in 0.1 M Sorensen's phosphate buffer (PB; pH 7.2) containing 5% paraformaldehyde, 0.8% glutaraldehyde, 3% NaCl and 4.5% sucrose. Subsequently, antennae were (1) washed in PB containing 8% sucrose, 3 times for 5 min each; (2) post-fixed in PB containing 8% sucrose and 1% osmium tetroxide for 1 h; (3) washed in distilled water, 3 times for 5 min each; (4) dehydrated in a graded ethanol series followed by transfer to propylene oxide; and (5) embedded in Epon-araldite. Thin cross sections of the antennular sensilla were cut on an RMC MT-6000 XL ultramicrotome, stained with 5% aqueous uranyl acetate and lead citrate, and viewed on a Zeiss 10C transmission electron microscope.

to sense sulfides at concentrations occurring naturally in their environment, *e.g.*, 2–15 mM in vent fluids from the Menez Gwen and Lucky Strike segments, 0.01–1.0 μM in the vicinity of known venting sites 2–5 m above the bottom (7), 1–300 μM at diffuse, low temperature vents (8), and 6 mM at the orifice of a black smoker at the Snake Pit site (9). Sulfide may be useful principally as a short-range stimulus over distances of tens of meters from its source. Sulfide emitted from vents is oxidized in the seawater surrounding the vents, with a half-life of 380 h (11, 12). This half-life, however, is significantly shortened by the presence of sulfide-utilizing bacteria associated with
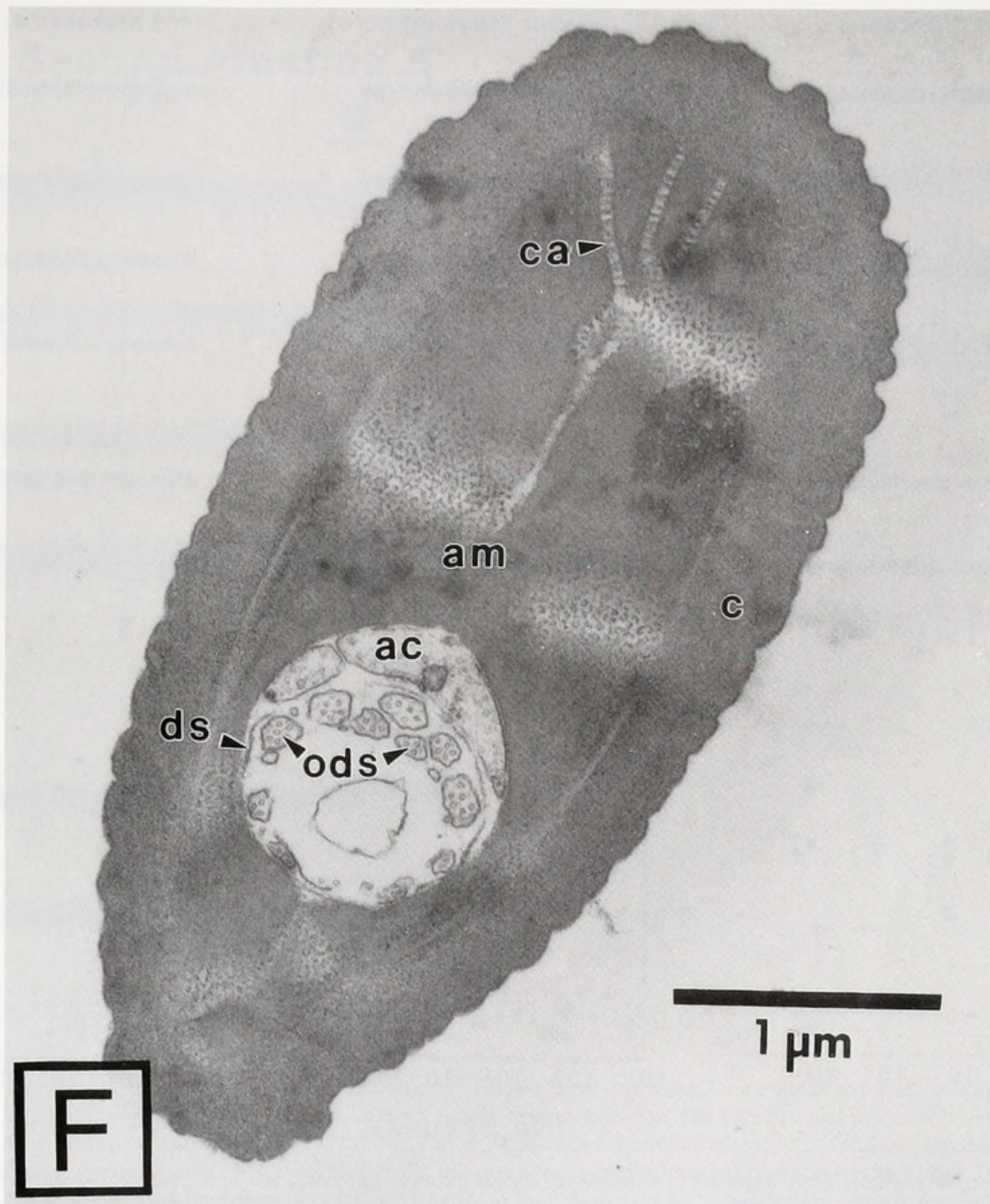
Figure 2. (F) Cross section in the distal portion of a sensillum from the second antenna of *R. exoculata.* In this region the outer dendritic segments are more dispersed and the dendritic sheath is fragmented.

the vents (13). Other compounds associated with hydrothermal plumes, such as methane which can be detected analytically tens of kilometers from the plume source (14), should be investigated for their ability to stimulate vent invertebrate chemoreceptors and thus possibly serve as long-distance cues.

Our observations that the antennae of vent shrimp are sensitive to sulfides led us to ask whether the antennae of other shrimp are sensitive to sulfides. The suction electrodes used on hydrothermal vent shrimp axons failed to reveal any response to sulfides or other chemical stimuli from excised antennal filaments of two species of shallow-water shrimp, *Penaeus aztecus* and *Palaemonetes pugio,* although the filaments responded to tactile stimuli and

were spontaneously active. We then used suction electrodes with finer tips especially developed to study chemosensory responses from antennal filaments of decapod crustaceans (15, 16) which allowed better discrimination between axon bundles in the second antenna of *P. aztecus.* We found that chemosensory axons which responded to broad-spectrum odorants also responded to sulfide stimulation in a concentration-dependent way (Fig. 4). The relative responses were 0.010 ± 0.012 ($n = 3$) for [Na_2S] $= 1.3$ mM; 0.083 ± 0.009 ($n = 3$) for 13 mM; 0.138 ± 0.019 ($n = 4$) for 130 mM; and 0.52 ($n = 1$) for 1300 mM. We used three control solutions in these experiments: ASW [-0.030 ± 0.020 ($n = 2$)]; pH 13 [0.26 ($n = 1$)]; and ASW with the pH adjusted to 10 to match
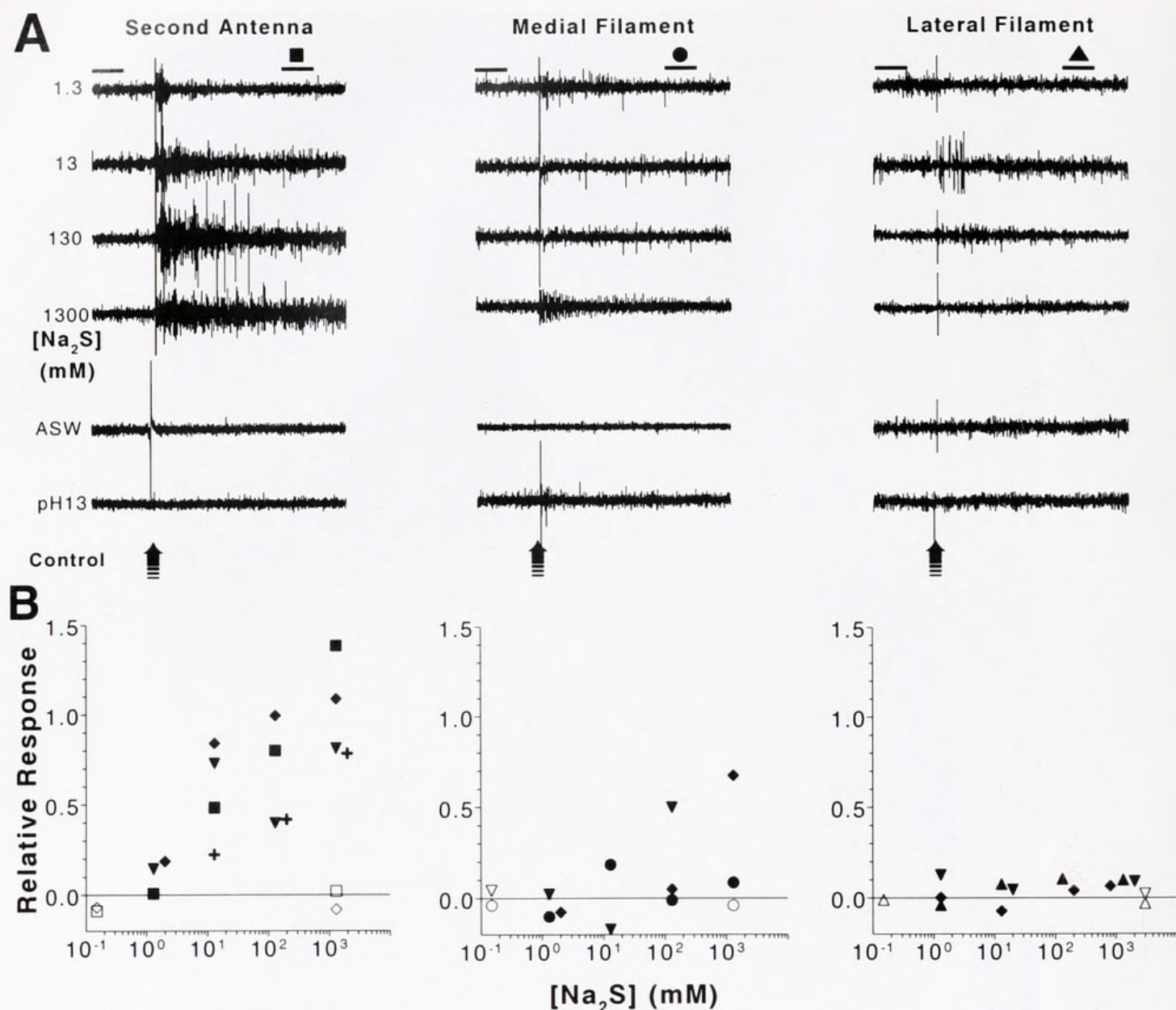
Figure 3. (A) Examples of multiunit nerve responses to solutions of Na₂S in the second antenna, and in the medial and lateral filaments of the first antenna excised from *Rimicaris* sp. The bold vertical arrow 0.5 s after the beginning of each 2 s record indicates when the stimulus, whether one of the Na₂S or control solutions, was applied. Transient responses following the exposure may have been due to tactile receptors activated by fluid motion. The activity following the transient response increased significantly with [Na₂S] in the second antenna. Exposure of filaments of either antenna to control solutions, either artificial seawater (ASW) or pH 13, evoked little or no response. These electrophysiological recordings from antennal filaments of hydrothermal vent shrimp collected from the Snake Pit site on the Mid-Atlantic Ridge (depth *ca.* 3600 m) were made aboard R/V *Atlantis II*. Filaments were excised from active shrimp and maintained in *Limulus* physiological solution (6). Several segments of the exoskeleton were removed to expose a short length of antennal nerve, which was either wholly or partially drawn into a suction electrode filled with the same solution. Filaments with spontaneous nerve activity and sensitivity to tactile stimuli were judged to be in good physiological condition and were chosen for further experimentation. A single drop of the test solution was gently merged with the fluid surface next to the antenna away from the tip of the suction electrode. Because of the differential behavior of the axonal responses from the various filaments, it is unlikely that the responses of the second antenna are due to some general injury response of the exposed ends of the axons to sulfide. Test solutions were as follows: Na₂S was dissolved in distilled water at a concentration of 1.3 M (pH 13), which was then diluted with ASW to produce lower concentrations (130 mM, pH 10.5; 13 mM, pH 9.5; 1.3 mM, pH 8). The stimulating solutions contained several chemical species of sulfide, namely, HS^-, H_2S, S_2^-, and NaHS, with concentrations dependent on the pH of the solution. In the pH range of the experiments, sulfide was present predominantly (> 90%) in the form HS^-. (B) Steady-state relative responses to concentrations of Na₂S and controls. Individual responses have been occasionally offset in the plot for the sake of clarity. The relative responses to Na₂S calculated for the sample recordings shown in (A) are represented here by the symbols shown in (A): ■ represents the second antennal responses in (A); ●, the medial filament of the first antenna; and ▲, the lateral filament. The other solid symbols represent responses to sulfides of the other filaments tested, whose responses are not shown in (A). Open symbols represent responses to the ASW control (shown near 0.1 on the abscissa) or to the pH 13 control (shown at or near 1300) for the filaments whose responses to sulfide are indicated by the corresponding solid symbols. Because individual nerve impulses could not be distinguished in these recordings, we determined the relative response for each filament in the following way: we calculated the root-mean-square (rms) deviation of the signal about its mean over an interval of duration 0.25 s beginning 0.5 s before exposure (B), together with the rms deviation over a similar 0.25 s interval beginning 1 s after exposure (R); then we took the relative response r to equal (R/B) − 1. These intervals are indicated by heavy horizontal bars in (A).
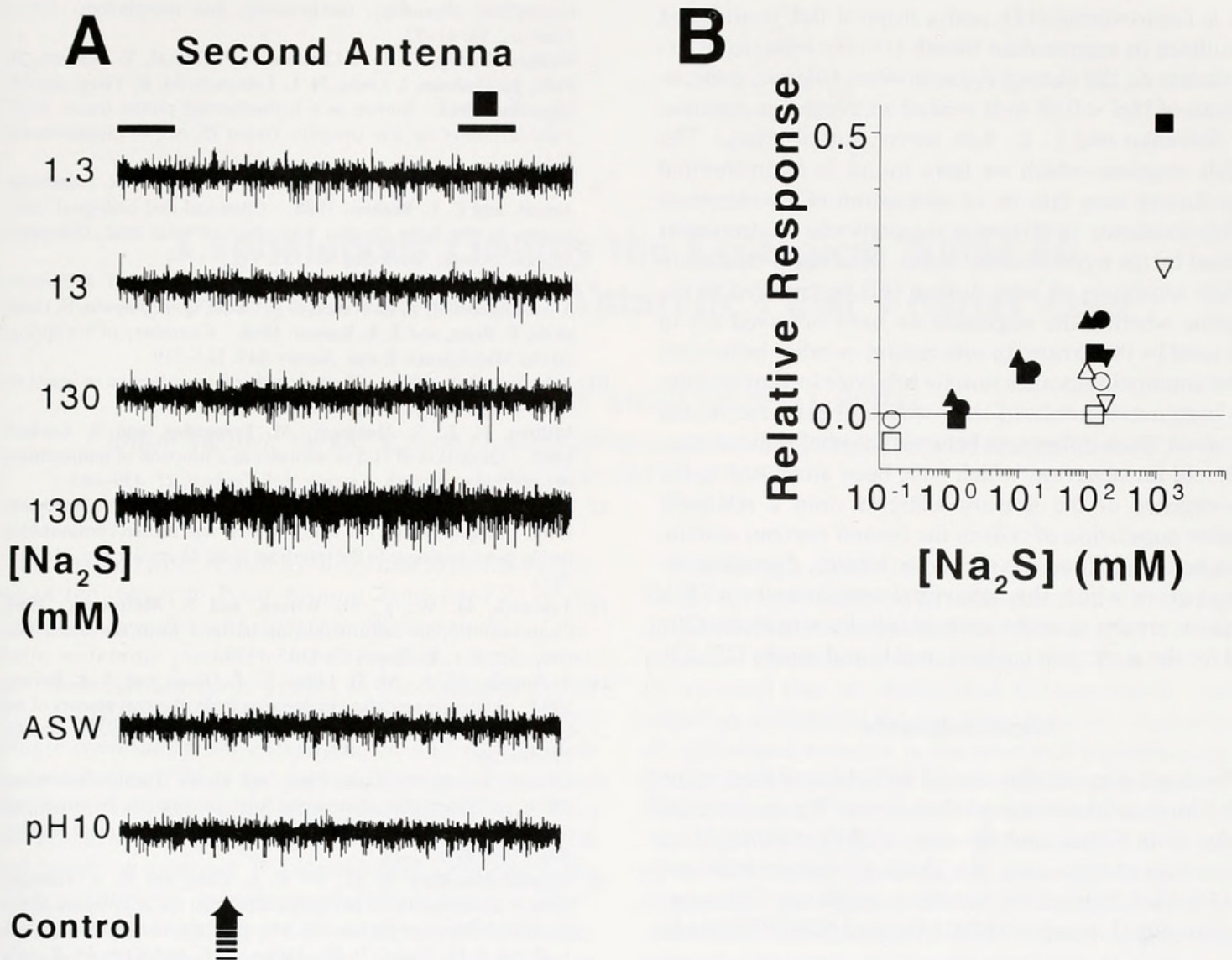
Figure 4. (A) Examples of multiunit nerve response from the second antenna of the shallow-water shrimp *Panaeus aztecus* to solutions of Na_2S and to control solutions. The experimental procedures used for *P. aztecus* are described in (15, 16). See Figure 3A for other details. (B) Steady-state relative responses to concentrations of Na_2S and controls. Note the expanded response scale in comparison with Figure 3B. The solid symbol ■ represents the relative responses to Na_2S shown in (A); the other solid symbols represent sulfide responses of the other second antennae tested. The open symbols represent responses to the three control solutions used on the antennal filaments whose sulfide responses are indicated by the corresponding solid symbols, namely, ASW (shown near 0.1), pH 10 (shown at or near 130), and pH 13 (shown at or near 1300). See Figure 3B for other details.

the pH of the 130 mM Na_2S solution [pH 10, giving a response = 0.040 ± 0.018 ($n = 4$)].

While the second antennae of both vent and shallow water shrimp responded to Na_2S, there are major differences between them. First, the axons of *P. aztecus* responded vigorously to the pH 13 control solution, unlike those of *Rimicaris* sp. or *R. exoculata.* Thus, the response to Na_2S in *P. aztecus* was confounded by a sensitivity to high pH. Second, the axons of *P. aztecus* responded only for about 40 min after excision, while axons of *Rimicaris* sp. and *R. exoculata* responded repeatedly to sulfide stimulation for several hours, and, in one experiment on *R. exoculata,* for 24 h. Furthermore, sensilla on the second

antenna of *Rimicaris* sp. and *R. exoculata* are more numerous than those in *P. aztecus* (1 sensillum/231 μm of filament circumference) and *P. pugio* (1 sensillum/110 μm). Although we do not know how many chemosensory neurons there are in each sensillum or what their individual sensitivities are, the larger density of sensilla in the vent species suggests that they may have an enhanced chemosensory capacity.

To our knowledge, this is the first physiological demonstration of a sensory response to a geochemical stimulus in a vent invertebrate. Sulfides have been reported to serve as physical cues for other invertebrates: a shallow-water polychaete may use sulfides as a settlement cue (17), but

this is controversial (18); and a tropical tick is attracted to sulfides in mammalian breath (19). In behavioral experiments on the shrimp *Palaemonetes vulgaris*, concentrations of $H_2S < 0.08$ mM evoked an avoidance response (N. Sofranko and C. L. Van Dover, unpub. data). The sulfide response which we have found in hydrothermal vent shrimp may thus be an adaptation of a widespread sulfide sensitivity in shrimp to the particular environment created by the hydrothermal vents. Behavioral studies on sulfide sensitivity of vent shrimp will be required to determine whether the responses we have observed are in fact used by the shrimp in orientation or other behaviors. If the antennal responses initiate behavior in vent shrimp, the behavioral threshold may well lie below the neural threshold. Such differences between thresholds for sensory cells and for behavior, which have been attributed to the convergence of the sensory afferents onto a relatively smaller population of cells in the central nervous system, have been discussed for the spiny lobster, *Panulirus interruptus,* in which the behavioral sensitivity to ATP is 30 times greater than the sensory cellular sensitivity (20), and for the American cockroach (21) and moths (22, 23).

Acknowledgments

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