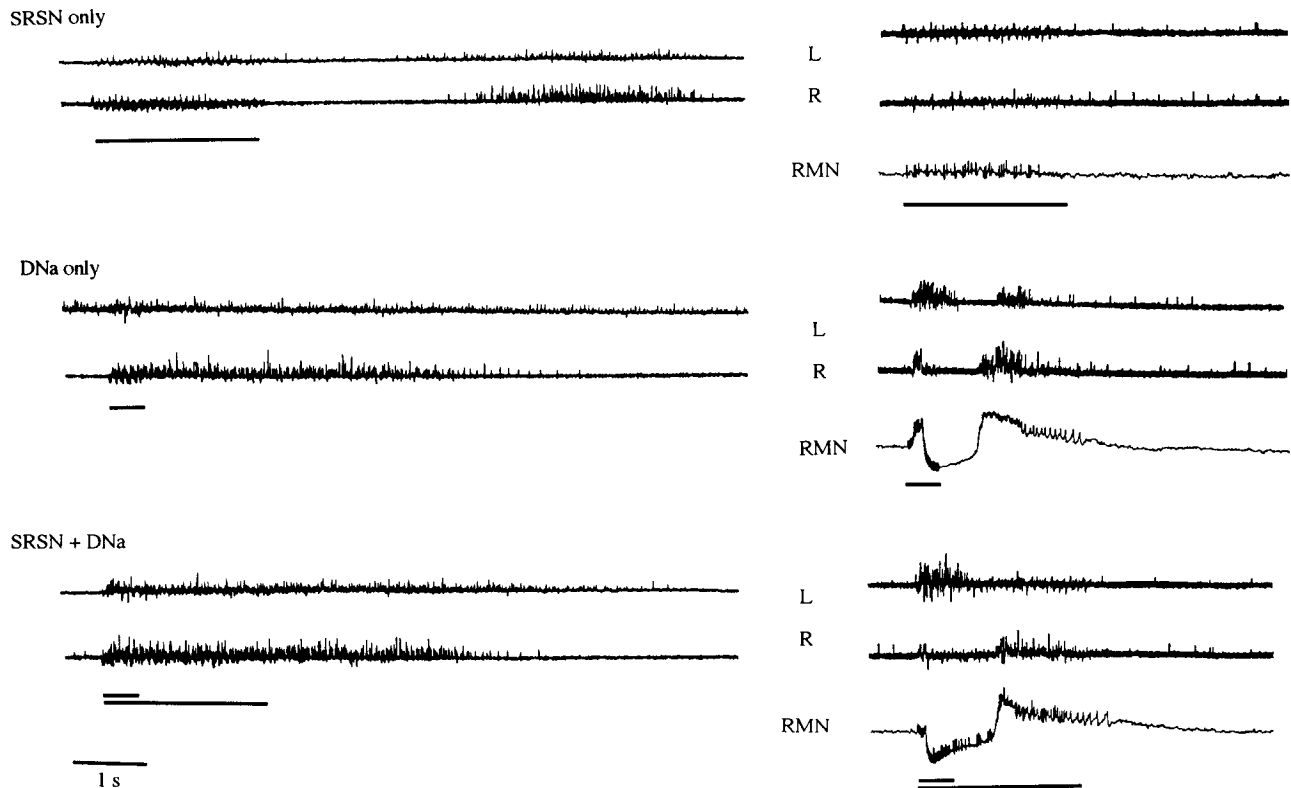


## LARVA

## PUPA



**Figure 2.** Records of bending-reflex motor activity in larval and pupal *Manduca sexta*. Larval extracellular records are responses of motor neurons in the left (L) and right (R) motor nerves innervating the intersegmental muscles (ISM). The responses are produced by electrical stimulation of the left stretch receptor sensory neuron (SRSN) alone (indicated by long bar); the right sensory nerve (DNa) carrying axons from mechanosensillae (indicated by short bar); or the stretch receptor and DNa together. When the SRSN was stimulated alone, the stimulus induced motor activity coincident with the stimulus and a long burst of motor activity that occurred after a period of inactivity (mean duration of inactivity = 3.7 s,  $n = 5$ ). Stimulation of the DNa only produced a typical, prolonged burst of activity on the stimulated side (mean duration of activity = 5.5 s,  $n = 5$ ) and weak, or no, activity on the opposite side. The response evoked by stimulation of the DNa and the SRSN was virtually the same as that evoked by DNa stimulation only, and the response evoked by SRSN stimulation alone was absent. Pupal responses to stimulation of the left DNa and the right SRSN are shown in recordings of left (L) and right (R) motor nerves and in an intracellular recording of a motoneuron innervating an intersegmental muscle on the right (RMN). Stimulation of the pupal SRSN only produced low frequency motor activity coincident with the stimulus. Stimulation of the DNa only produced a typical, triphasic patterned response in which two high frequency bursts of activity were separated by a period of inhibition. The response to simultaneous SRSN and DNa stimulation showed a weaker first burst of activity, a period of hyperpolarization during which action potentials occurred, and a high frequency second burst of activity ( $n = 4$ ).

Reference: *Biol. Bull.* 185: 316–317. (October, 1993)

### A Comparison of the Tuning Properties of Chemoreceptor Cells in the First and Fourth Walking Legs of Female American Lobsters

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The first and fourth pair of walking legs in the American lobster differ in morphology and behavioral function. Apart from their obvious use in walking, the first pair of walking legs are

chelated and serve in feeding behavior, *i.e.*, for grasping and transporting food to the maxillipeds and for grooming the anterior body (1). The fourth pair of walking legs is used for walking

and grooming of the posterior body (2). In the egg-bearing female, this prominently includes the grooming of the egg mass. Such behavioral differences may correlate with physiological differences in chemoreceptor function between the two leg pairs, e.g., lobsters may be able to differentiate between healthy and fouled eggs using fourth leg chemoreceptors. We therefore determined the spectral sensitivity of chemoreceptor cells in the first and fourth walking legs, focusing on chemicals present in food odor and products of bacterial degradation. Because of their egg-cleaning behavior, we used female lobsters for this initial study.

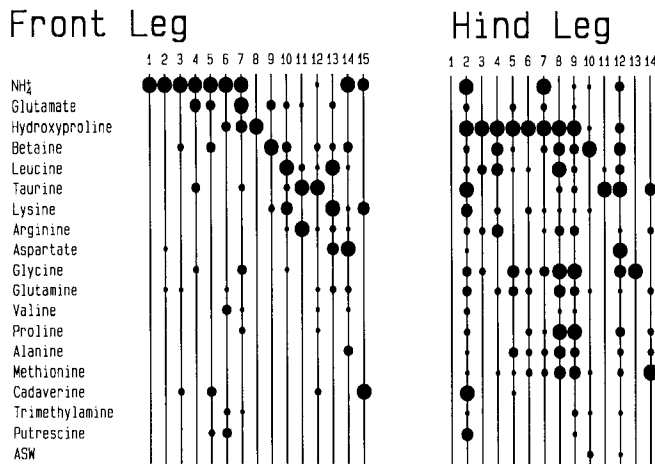
We assessed the tuning properties of chemoreceptor cells by measuring responses to single chemical stimuli; standard electrophysiological methods using suction electrodes were applied to record extracellular action potentials. From the first leg we excised the dactyl and from the fourth leg the dactyl and propus to ensure that both smooth and toothed sensilla would be present (3). The segment with the exposed nerve bundle was placed in the stimulating compartment of a two-compartment preparation chamber. The proximal end with the intact nerve bundle projecting into the recording compartment was immersed in lobster Ringer's solution. The stimulating and recording compartments were separated by a rubber plug (4). The segments were super-

fused with a carrier flow of artificial seawater (30 ml/min). Each single compound was injected as a 50  $\mu$ l aliquot into the carrier flow. The stimulation chamber was especially designed to provide thorough stimulus mixing for rapid stimulus onset and evacuation time; conductivity measurements (4) showed that the time course and dilution profiles were similar for both preparations. Single chemoreceptors were identified by their response to a mixture of 18 compounds (each at  $10^{-3}$  M; see Fig. 1). We then tested each compound separately at  $10^{-2}$  M. We evaluated cell viability with the mixture as every sixth stimulus, and we tested for mechanoreception with artificial seawater. Stimulus effectiveness was quantified as the total number of action potentials elicited in the first 5 s after stimulus injection. Action potentials from single chemoreceptors were differentiated on the basis of amplitude, wave form, and latency.

The first and fourth pairs of legs had different effective stimuli. Hind leg chemoreceptors responded best to hydroxy-L-proline, followed by glycine, methionine, glutamine, betaine, and leucine. Front leg chemoreceptors, on the other hand, responded best to ammonium chloride, followed by glutamate, lysine, leucine, betaine, and taurine. In contrast to Johnson *et al.* (4), we found no dominant population of glutamate-best cells in the front leg, but glutamate was the second-most effective stimulus. The tuning breadth of chemoreceptors in the two leg pairs was also different. Chemoreceptor cells in the hind legs were more broadly tuned than those in the front legs. Cells in the hind legs responded to between 1 and 18 stimuli, whereas cells in the front legs responded to between 1 and 10 stimuli. The H-metric, a measure of the tuning breadth, varies from 0 to 1, with 0 indicating narrow tuning and 1 indicating broad tuning (5). The H-metric mean was 0.55 for the hind legs and 0.42 for the front legs.

Our results indicate that the front and hind legs have different spectral sensitivities, which might be necessary for different behavioral functions. Whether this includes recognition of fouled eggs remains to be seen.

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**Figure 1.** Spectral tuning properties of chemoreceptor cells in the first and fourth walking legs of female American lobsters. A continuous line indicates no response, the smallest dots indicate less than 20% of the maximum response, next largest dots between 20 and 40%, third largest dots between 40 and 60%, fourth largest dots between 60 and 80%, and the largest dots between 80 and 100%. Cells are grouped by their best stimulus and, within each group, ordered by increasing tuning breadth based on their H-metric value (5). Numbers indicate individual cells.

**Literature Cited**

1. Derby, C. D., and J. Atema. 1982. *J. Exp. Biol.* 98: 317-327.
2. Bauer, R. T. 1989. In *Functional Morphology of Feeding and Grooming in Crustacea*, F. R. Schram, ed. A. A. Balkema, Rotterdam.
3. Derby, C. D. 1982. *J. Crust. Biol.* 2: 1-21.
4. Johnson, B. R., R. Voigt, P. F. Borroni, and J. Atema. 1984. *J. Comp. Phys. A.* 155: 593-604.
5. Smith, D. V., and J. B. Travers. 1979. *Chem. Senses Flav.* 4: 215-229.