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Title: Chemosensory Physiology and Behavior of the Desert Sand Scorpion, *Paruroctonus mesaensis*.

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Philip H. Brownell

This is a neuroethological study of two major chemosensory systems found in all scorpions - the large, ventral appendages, called pectines, found uniquely in this taxon, and setaform chemoreceptors of the tarsal leg segments. These sensory organs are closely associated with the substrate and their microstructure suggests specialized function in gustation or near-field olfaction of chemical substances on dry surfaces. In this study I present behavioral and electrophysiological evidence that the numerous peg sensilla on the pectines are important chemosensory channels in scorpions and probably fill similar functional roles to antennal sensilla of mandibulate arthropods. The subject of these investigations was the desert sand scorpion *Paruroctonus mesaensis*.

Sand scorpions displayed vigorous, stereotyped behavior in response to substrates treated with water and chemicals derived from conspecific

scorpions. Ablation studies showed the pectines are important in the detection of substrate-borne pheromonal signals while the tarsal chemosensory hairs are important detectors of substrate water. Electrophysiological investigation of individual peg sensilla on the pectines showed these structures are sensitive to chemostimulants applied directly to the sensillar tip or blown across its pore. Neurons within each peg gave characteristic patterns of response to organic stimuli of various classification (alkanes, alcohols, aldehydes, ketones, esters) that were generally independent of carbon chain length (C2 to C12). Tarsal hair sensilla were responsive to water applied directly to the hair tips. Mechanoreceptive units were observed in both types of sensilla indicating dual sensitivity to both chemical and mechanical stimulation.

A novel finding of these studies was the identification of synaptic interactions between chemosensory units in peg sensilla. Electrophysiological evidence now confirms morphological evidence of a dense plexus of axo-axonic synapses between peg sensory neurons. The local circuitry formed by these synapses appears to modify primary sensory information prior to its relay to the central nervous system. The accessibility of the pectines to detailed electrophysiological analysis suggests this will be a useful model for investigations of sensory detection and coding of substrate-associated chemical stimuli.

Chemosensory Physiology and Behavior of the Desert Sand Scorpion, *Paruroctonus mesaensis*

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CHAPTER 1

INTRODUCTION

To varying degrees, the behaviors of all animals are affected by chemical sensory information. Sensitivity to the host of substances that identify potential food or mate, shelter or potential predator, is essential for initiation and guidance of appropriate behavior. It is paradoxical, therefore, that of all the senses used by animals the chemical senses remain the least understood at the physiological and biochemical levels. This is likely due to the enormous structural diversity of natural chemical stimuli and the lack of tractable experimental models for analyzing nervous systems and behaviors affected by these substances. The majority of work on chemosensory physiology in invertebrates has been done on the relatively specialized organs of arthropods, particularly chemosensory hairs of insects and crustaceans (Dethier 1976, Kaissling 1987, Derby and Atema 1988). The chelicerate arthropods have been largely ignored even though experimental preparations of these animals have many features comparable to those of the mandibulate subphylum. In this thesis we present scorpions as a new model for experimental work. In particular, these chelicerate arthropods have special advantages for study of chemosensory systems involved in detection of signals on the substrate. The desert sand scorpion, *Paruroctonus*

mesaensis, has been our model of choice for laboratory and field studies because of the accumulation of information about its biology (Bowerman and Burrows 1980; Brownell 1977; Hadley 1974; Polis 1979, 1980; Stahnke 1966).

For several reasons scorpions are excellent models for study of chemosensory physiology and behavior. From a practical stand point, scorpions are abundant animals and kept easily in captivity. The primary organs mediating chemosensitivity in scorpions are paired, mid-ventral appendages of large size that are accessible structures for electrophysiological analysis. These "pectines" contain numerous sensilla called pegs, which are similar to other arthropod setaform chemoreceptors. However, the pectines differ from insect antennae in a fundamental way: they are specialized for survey of the substrate surface rather than for monitoring volatile chemostimulants in air.

The pectines have long been recognized as unique organs among the Arthropoda. The first descriptions of pectine morphology and behavior were accompanied by speculations about their functions which ranged from respiratory organs to external genitalia (see Cloudsley-Thompson for summary of early literature). Blanchard (1853) was first to recognize that each organ was richly supplied with nerve fibers, and Gaubert (1889) showed this innervation extended into the pectine "teeth". Schröder (1908) described the peg-shaped sensilla (peg sensilla) borne on these teeth and

determined that the nerve fibers terminated within these cuticular structures. He concluded that the pectines "were chemotactic organs and had a double function as receptors for taste and smell and were perhaps used during mating as a stimulatory organ in the recognition of the sexes." After many intervening years of misguided study, this early hypothesis would be shown to be largely correct.

In the late 1950s Alexander (1957,1959) produced behavioral evidence of the use of male pectines as substrate texture discriminators. During the "promenade aux deux", the mating dance of scorpions, the male typically grasps the female with his pedipalps and walks backwards, leading the female, until a suitable site is located for deposition of his spermatophore. In almost all accounts of this behavior (6 families studied to date) the male's pectines are highly active organs, tapping or sweeping the substrate periodically (see Polis and Sissom 1990 for a review of this literature). Alexander found in her studies of *Parabuthus planicauda* (Buthidae) and *Opisthophthalmus latimus* (Scorpionidae) that each species chose particular surface textures on which to deposit spermatophores. Courting males which had their pectines amputated or, alternatively, fixed to the overlying leg coxae conducted otherwise normal promenades, but did not deposit spermatophores, regardless of surface texture. Normal courtship and spermatophore deposition behavior recovered when the glued pectines were released.

In the 1960s a series of papers provided additional support for a mechanosensory role for the pectines. In a variety of behavioral choice tests Abushama (1964) assayed taxic responses of scorpions (*Leiurus quinquestriatus*) to various stimulus modalities. Animals with pectines amputated or covered with varnish responded as normal animals in tests of sensitivity to temperature, chemicals (naphthalene), and ground vibrations, but choice of substrate texture based on particle size was different between the groups. Hoffmann (1964), using metal electrodes to impale the base of individual pegs, obtained the first electrophysiological recordings from these sensilla. Distinct responses were shown for mechanical deflection of the peg tip, but not to application of various chemicals. Carthy (1966), using transmission electron microscopy, described the fine structure of the peg sensilla as lacking external pores required for chemoreceptive sensilla and concluded the peg sensilla were exclusively mechanosensory structures. He conjectured from the arrangement of the sensilla that the pectines could be used to distinguish substrates of particular grain textures. The results of Hoffmann and Carthy discouraged further investigation of a chemosensory function for pectines for nearly two decades.

Anatomical (Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983) and behavioral studies (Krapf 1986) of scorpions renewed interest in the possibility of a chemosensory function for the pectines. Behavioral observations provide the most compelling evidence for this hypothesis and

further suggested they are used primarily for mate recognition and food-locating behaviors. During normal movements, the pectines are tapped intermittently on the substrate or dragged lightly over the surface as the animal walks. This tapping behavior is seen in *Hadrurus arizonensis* where the frequency of pectine tapping of an adult male increased dramatically when it was moved from its home container into a container previously occupied by a mature conspecific female (Fig 1.2). Movement of the animal into the container of a conspecific male resulted in a much lower tapping frequency. Moreover, it was commonly noted that pectines of males show a remarkable increase in activity during the "promenade aux deux" (Baerg 1954; Alexander 1959; Rosin and Shulov 1963; McAlister 1965; Auber-Thomay 1974; Polis and Farley 1979a), the mating dance of the scorpion, suggesting some potential role in mechano- or chemosensory orientation.

The only behavioral study to date demonstrating general chemosensitivity of the pectines was conducted by Krapf (1986). Stereotyped behavioral patterns were released in buthid scorpions (*Androctonus australis* and *Buthus occitanus*) in response to freshly killed prey items or paper dummies soaked with solvent extracts of prey, but not from control dummies soaked with pure solvent. Similar behavior was observed when only the pectines contacted the test object, indicating they were sufficient input for releasing the consummatory behavior. Furthermore, scorpions located and consumed dead insects buried 5 mm

beneath the sand surface after the pectines contacted sand particles besmeared with prey extract on the surface just above the insects. It was also reported that crickets which were stung and managed to escape (e.g. by leg autotomy) or alternatively, removed from the scorpion's grasp and placed approximately 10 cm away were all relocated and consumed by their predator. Krapf argued that such chemically mediated searching behavior was important in relocating prey freshly killed by envenomation and that the pectines may be important in such a role.

The unique importance of the pectines for mate recognition and localization is supported by the high degree of sexual dimorphism they display anatomically and biochemically. In most species of scorpions the pectines of males are longer and contain more teeth and peg sensilla than those of females (Table 1.1; Swoveland 1978; Brownell 1988, 1989). Even in species where the number of teeth is the same for males and females, there are substantially more peg sensilla per tooth in males (e.g. *Superstitionia donensis*). Backfilling of the pectinal nerve in males and females reveals striking differences in the terminal projections of the afferent fibers in the subesophageal ganglia of the brain (Brownell 1988, 1989). In the male the afferent terminal fields are conspicuously larger and distinctly layered while the female projection is smaller and contains fiber tracts that extend anteriorly into the supraesophageal ganglia and laterally to the leg motor centers (Brownell 1988, 1989). Finally, the pectines of male scorpions

contain a family of low molecular weight (13-18 kd) proteins that are sex and species-specific in 5 species examined so far (Bulseco and Brownell 1989). These sensilla-specific proteins are turned over at an unusually high rate and otherwise show several properties that characterize pheromone binding proteins of lepidopteran insects (Kaissling 1986; Kaissling and Thorson 1980; Vogt and Riddiford 1981). Thus, by the same morphological and biochemical criteria that distinguished female and male antennae in insects, the pectines of scorpions also appear to be functionally dimorphic in the sexes.

In the late 1960's Carthy (1966, 1968) published ultrastructural reports stating that the peg sensilla lacked cuticular pores and were therefore probably tactile organs specialized for the detection of substrate texture. Ivanov and Balashov (1979) reexamined the fine structure of the peg sensilla of a buthid scorpion (*Mesobuthus eupeus*) using scanning and transmission electron microscopy and improved fixation techniques to clearly show the presence of a slit-like pore at the terminal end of each peg sensillum. These and other features of sensory innervation indicated the pegs were like gustatory sensilla found in other arthropods (Slifer 1970). A subsequent investigation of two North African species (*Euscorpius italicus* (Chactidae) and *Androctonus australis* (Buthidae)) showed that the peg sensilla are innervated by approximately 12 sensory neurons with 11 of the unbranched dendrites terminating in a receptor lymph near the pore opening (Foelix and

Müller-Vorholt 1983). The remaining neuron was shown to terminate near the base of the sensillum in a structure typical of arthropod mechanoreceptors (McIver 1975). Thus, by microanatomy each peg sensillum appears to be capable of both mechanical and chemical sensitivity.

In spite of their unique external appearance, the peg sensilla of scorpions have many structural features in common with chemosensory sensilla of insects. The shape and size of individual pegs varies between species, with sand dwelling species having shorter, flatter pegs than tropical species (Brownell unpubl.). Generally, pegs are cylindrical structures approximately 1 μm in diameter and 2-5 μm in length. These double-walled shafts extend from circular sockets and gradually flatten at the peg tip where a slit-shaped pore connects to a fluid-filled chamber inside the sensillum (Fig. 1.3). The reported number of dendrites per peg varies from 12 to 18, but averages about 15 where it has been carefully measured (Brownell unpubl.). Most of the bipolar sensory neurons extend into the sensillar chamber within a few microns of the slit opening. Foelix and Müller-Vorholt (1983) described one dendrite as terminating near the peg base and possessing the tubular body characteristic of arthropod mechanosensory cells. The sensillar chamber is lined by an inner enveloping cell that isolates the outer segments of the dendrites from hemolymph and probably secretes substances unique to fluids of this chamber (Slifer 1970). Proximal to the basal body of each dendrite (approx. 30-50 μm from the tip of the peg) the

inner segments form a fixed arrangement, with five neurons in an array opposite the microvillar projections of the inner enveloping cell. This gives polarity to the arrangement of dendrites within the peg and aids in correlating morphology with physiological data. The cell bodies of sensory cells are large and rounded (ca. 10 - 30 μm diam.) and form a ganglia layer approximately 100 μm below the cuticular surface.

Chemosensory sensilla are also abundant on other cuticular surfaces of scorpions. In particular, dense assemblages of setaform sensilla are located on ventral surfaces of tarsal leg segments (Foelix and Schabronath 1983). These pore-containing hairs have been described morphologically, but have not been investigated as to their behavioral or functional relevance. As of yet, no side-walled pored sensilla, characteristic of olfactory receptors of insect antennae, have been found in scorpions. As such, it appears that scorpion chemosensory structures are designed specifically to "taste" and utilize chemical signals associated with the substrate.

In this thesis I have developed a behavioral assay to describe and quantify the response of desert sand scorpions, *P. mesaensis*, to two biologically important chemostimulants: pheromones (Chapter 2) and water (Chapter 3). I have adapted specific electrophysiological techniques to these preparations in order to characterize the sensory responses of pectinal (Chapters 4, 5) and tarsal (Chapter 3) chemosensitive sensilla -- sensory structures that mediate sensitivity to these substances. Finally, in Chapter 5,

I present physiological evidence of a unique neural plexus between first-order chemosensory neurons in pectinal peg sensilla. I outline the computational approach I used to interpret the connectivity and functionality of this specific neural network and discuss the importance of these results and this type of analysis to the larger problem of understanding sensory coding of gustatory information.

Table 1.1 Sexual dimorphism of scorpion pectines.

Family	Species	Sex	No. teeth/ 2 pectines	No. pegs/ tooth	Total no. pegs	No. pegs (♂)/ no. pegs (♀)	Ref.*
Vaejovidae	*Anuroctonus phaiodactylus*	♂	19	201	3819	2.55	1
		♀	13	115	1495		
	Nullibrotheas allenii	♂	24	160	3840	2.75	1
		♀	17	82	1394		
	Paruroctonus mesaensis	♂	75	1600	120000	13.89	1
		♀	48	180	8640		
	Uroctonus mordax	♂	27	1100	29700	9.71	1
		♀	20	153	3060		
	Vaejovis confusus	♂	34	1000	34000	4.31	1
		♀	27	292	7884		
	V. spinigerus	♂	46	1000	46000	4.01	1
		♀	37	310	11470		
Buthidae	*Centruroides sculpturatus*	♂	50	800	40000	1.86	1
		♀	44	490	21560		
	Parabuthus pallidus	♂	72	1650	118800	1.82	2
		♀	62	1050	65100		
Chactidae	*Superstitionia donensis*	♂	12	800	9600	1.63	1
		♀	12	490	5880		
Diplocentridae	*Didymocentrus comondae*	♂	17	1050	17850	7.83	1
		♀	15	152	2280		
Scorpionidae	*Pandinus gregoryi*	♂	36	1400	50400	1.65	2
		♀	34	900	30600		

*References: 1 Swoveland (1978); 2 Brownell (unpubl.)

Figure 1.1 External morphology of the pectines. **A** Ventral view of *P. mesaensis* showing larger size of pectines (p) in males (**A1**), and greater numbers of sensilla-bearing teeth (t) than in female (**A2**). **B** Scanning electron micrograph of distal face of female tooth showing dense arrays of peg-shaped sensilla (Pe). **C** Peg fields of adjacent teeth overlap in the horizontal axis; only peg sensilla and tactile hairs contact substrate when the pectines are swept forward during locomotion. **D** Epi-fluorescent illumination of peg sensilla in a live animal. (h, tactile hairs; ps, pectine spine) (from Brownell 1988)

Figure 1.1

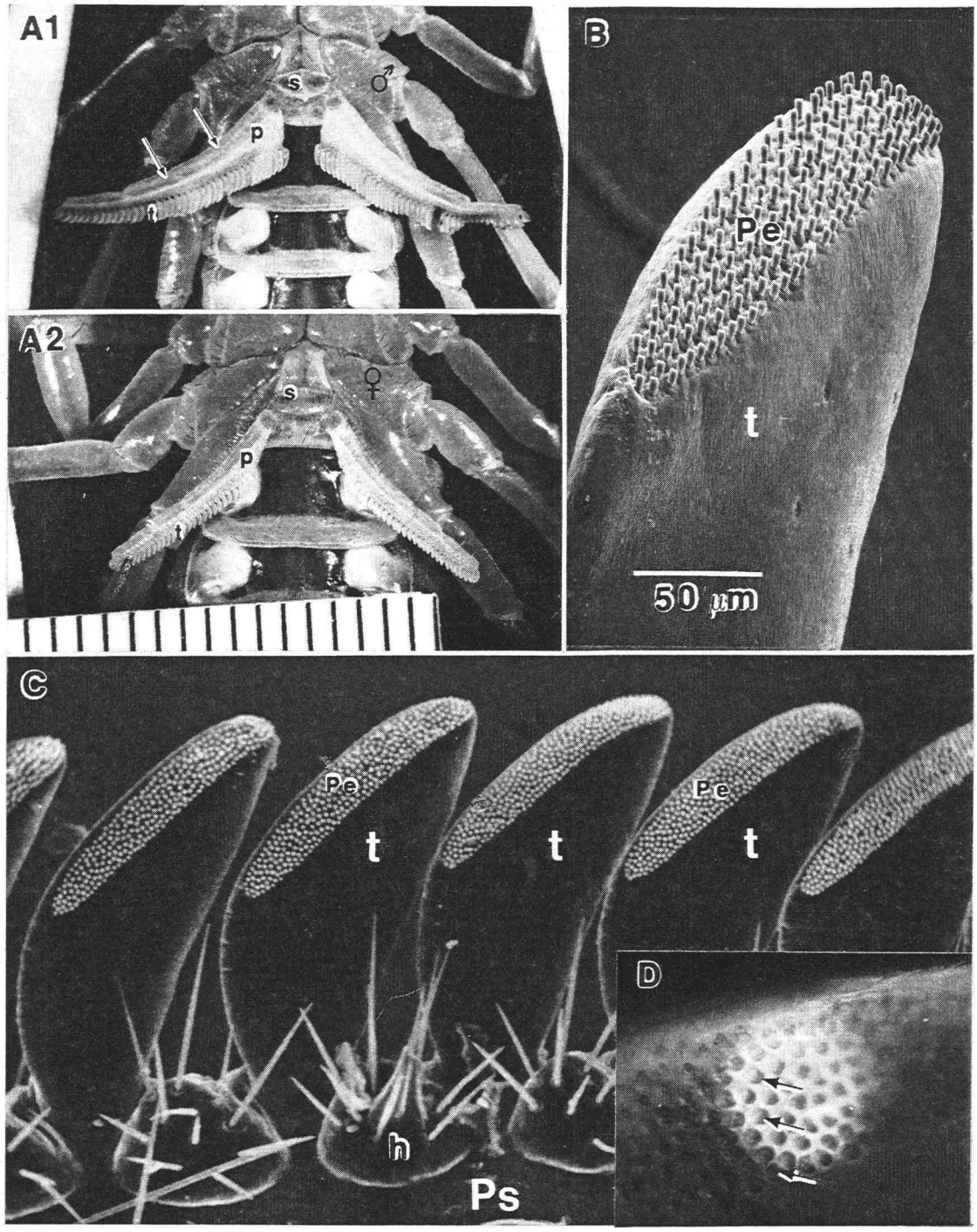
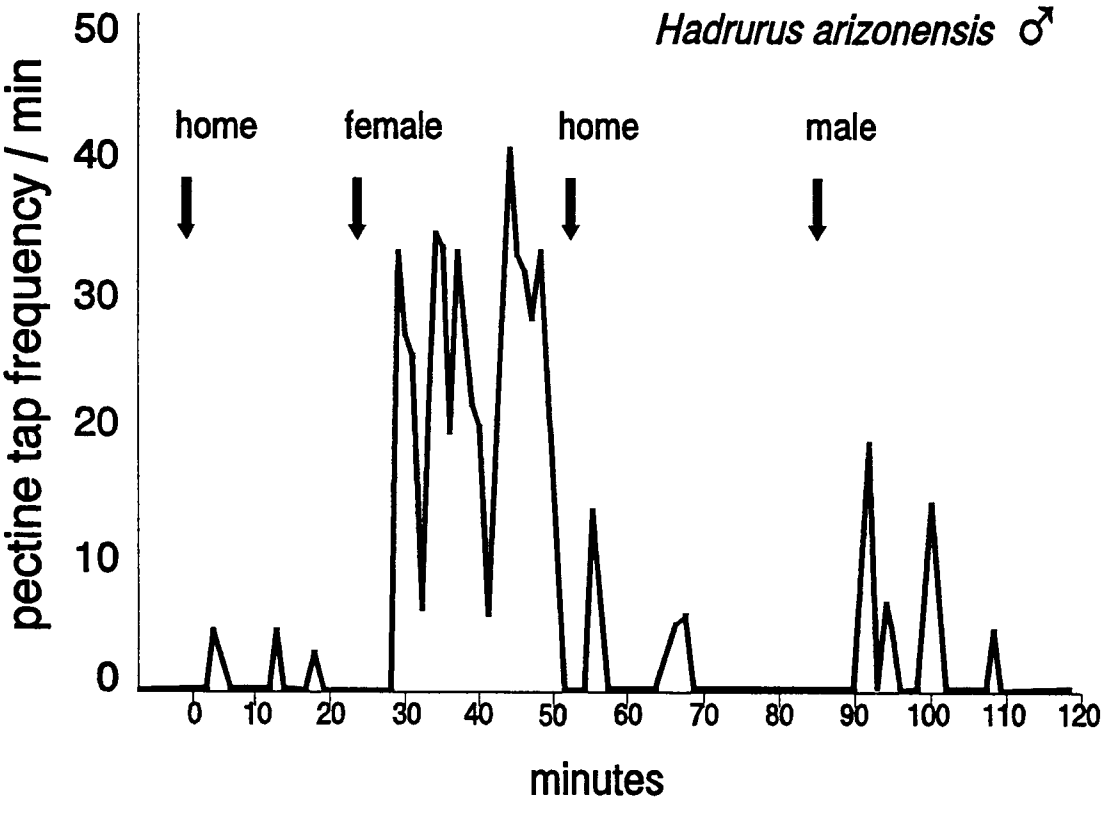


Figure 1.2 Behavioral indication of chemical signaling in scorpions. The activity of the pectines of a male *Hadrurus arizonensis* was assayed as the animal was moved from home container to container previously occupied by conspecific female, back to home container, then to container of conspecific male. Number of contacts the pectines made with substrate is expressed as frequency of taps per minute. (from Brownell 1988)

Figure 1.2


Hadrurus arizonensis ♂
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20
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0
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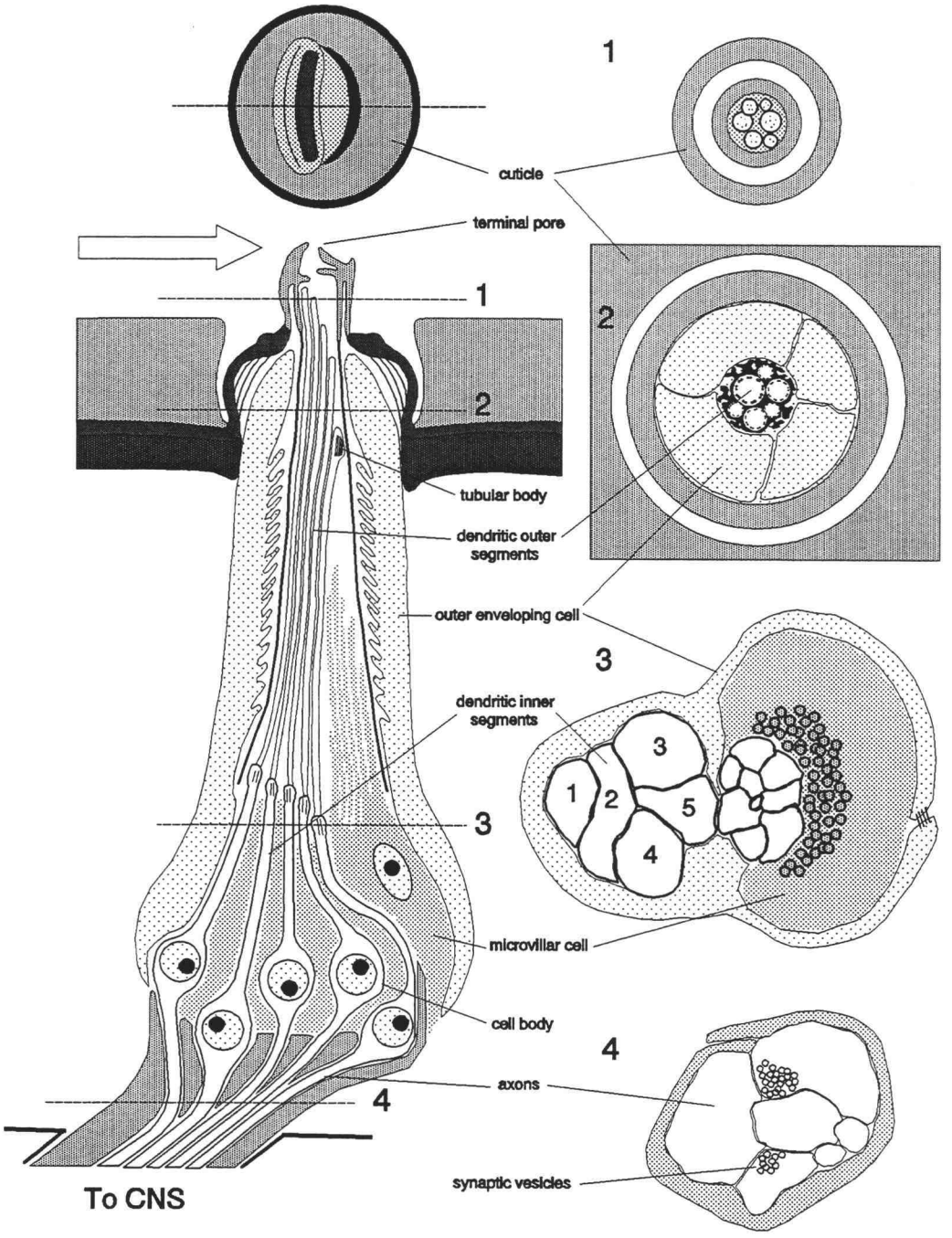
120
minutes

Figure 1.3 Morphology of a typical peg sensillum. **Left:** Longitudinal section through sensillum showing arrangement of dendrites, cell bodies, microvillar cell and structural elements (compiled from studies by Ivanov and Balashov 1979, Foelix and Müller-Vorholt 1983, Foelix 1985, and Brownell unpubl.). **Right:** Cross-sections taken from levels 1-4 (left) showing (1) double-walled shaft containing several dendritic outer segments inside receptor lymph; (2) cuticular base of sensilla, extensions of outer enveloping cells, inner sheath, and dendrites with microtubules arranged at periphery; (3) regular arrangement of five dendritic inner segments relative to microvillar and other enveloping cells; (4) axo-axonic synaptic profiles just below sensory cell body layer.

Figure 1.3


1
cuticle
terminal pore
1
2
2
tubular body
dendritic outer segments
outer enveloping cell
3
dendritic inner segments
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microvillar cell
cell body
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axons
4
synaptic vesicles
To CNS

CHAPTER 2

CHEMICAL COMMUNICATION IN THE DESERT SAND SCORPION

ABSTRACT

This study presents evidence of intraspecific chemical communication in scorpions. The subject of our investigation was the desert sand scorpion, *Paruroctonus mesaensis*. During the mating season, mature males show a sex-specific wandering behavior ostensibly directed at locating conspecific females that remain in the vicinity of their home burrows. Searching behavior was stimulated in the laboratory by releasing males onto substrates that had previously been occupied by females. Receptive males exhibited changes in locomotory behavior that favored occupation of the female-exposed area. Males occasionally displayed a precourtship behavior, called juddering, indicating the presence of a pheromone on the substrate. Juddering, and two newly described behavior patterns, tail-wagging and pedipalp-reaching, were also induced by solvent extracts of female cuticle. Most behavioral responses began vigorously within the first few seconds of stimulus contact and gradually adapted within 10 min. Males and females responded to deposits from conspecifics; males showed greater sensitivity to female deposits than male deposits. Strong behavioral responses were evoked in males by polar and non-polar extracts of female cuticle. The response of pectineless males to female extracts was significantly lower than

that of intact males, suggesting that these mid-ventral sensory appendages are important in mediating the response to specific chemical signals. These results are consistent with the hypothesis that mate identification and localization in sand scorpions are mediated in part by a contact sex pheromone.

INTRODUCTION

Given the importance of pheromonal signaling in stimulating and directing behavior of mandibulate arthropods (Tumlinson and Teal 1987; Kaissling 1987), it is surprising that so little is known about intraspecific chemical communication in the other great division of the phylum, the chelicerates. Arachnids in particular are a diverse and ancient group of terrestrial arthropods with several large forms that can be easily studied, yet the whole of our understanding of arachnid pheromonal communication is based on investigations of ticks (Sonenshine 1985), phytophagous mites (Sonenshine 1985) and spiders (Tietjen and Rovner 1982; Pollard et al. 1987). Notably lacking are studies of scorpions, among the earliest of terrestrial animals and the order believed by some workers to be ancestral to other arachnid groups (Störmer 1977). Kjellesvig-Waering (1986) notes that the general body form of scorpions and the structure of a putative chemosensory organ, the pectines (ventral antenna-like appendages), changed very little as Devonian aquatic forms began to invade land more than 300 million years ago. From this perspective a study of scorpionids may give insights into the evolution of chemical communication and related behaviors in terrestrial chelicerates.

Apart from their evolutionary significance, scorpions offer special advantages for behavioral analysis and experimentation owing to their longevity, large size, fluorescent cuticle, and ease of maintenance in

captivity. The desert sand scorpion, *Paruroctonus mesaensis*, has become a particularly important species for ethological study due to the simplicity of its dune environment and the substantial body of physiological, ecological and natural history literature that has accumulated on this species (Brownell 1977; Brownell and Farley 1979a,b,c; Hadley and Williams 1968; Polis 1979, 1980; Polis and Farley 1979a,b, 1980; Root 1985; Stahnke 1966). As part of an integrative investigation of chemical communication in arachnids, we have conducted morphological (Brownell 1989), neurophysiological (Gaffin and Brownell 1990), and biochemical (Bulseco and Brownell 1989) studies of the sand scorpion chemosensory systems and behavior. The present study demonstrates the existence of chemical signaling in this species and describes some aspects of the chemical signals involved and the behaviors they release.

The natural reproductive behavior and environment of *P. mesaensis* suggest that it uses some form of chemical signaling to increase the probability of a reproductive encounter. This is a solitary, cannibalistic species that seldom wanders farther than 1 m from its home burrow (Polis 1980). Most between-individual interactions occur during the reproductive season when, for a few weeks each year, mature males abandon their burrows and wander across the dune at night apparently in search of potential mates (Polis and Farley 1980). Since at least some, and perhaps all, matings occur on the surface of the dune, the chance of a successful

encounter is dependent on the synchrony of male and female surface activities. Furthermore, as a heavily predated species, *P. mesaensis* is a "time-minimizer," emerging from its burrow on less than 40% of nights and then for only a few hours (Hadley and Williams 1968; Polis and Farley 1979b; Polis and McCormick 1987). Thus, most matings occur during times when nightly surface densities of mature males and females are about 35 and 40 individuals per hectare, respectively (Polis 1980). At these densities random movements by the males are not likely to account for the number of successful matings observed. Another obstacle to successful mating is the tendency of larger females to cannibalize the smaller males (Polis 1980); successful encounters occur only after an elaborate courtship dance (Polis and Farley 1979a) when males presumably signal their status as potential mates and suppress the predatory responses of females. One behavior that may signal courtship intention is "juddering", an abrupt lurching of the body that produces substrate vibrations (Alexander 1959; see Polis and Farley 1979a for review). Juddering may itself be released by chemical stimuli since Krapf (1986) observed that adult males of the African species *Pandinus imperator* judder following contact with objects transferred from the container of female conspecifics.

The study presented here supports the hypothesis that substrate-borne chemical signals direct the navigation of male sand scorpions to prospective mates and trigger behavior patterns important to a successful mating.

The sensory organs involved in pheromone detection in scorpions are unknown. Chemosensory sensilla are abundant on the cuticular surfaces of *P. mesaensis* and other scorpions, but are most concentrated on the pedipalps, the tarsal leg segments and pectinal appendages (Hoffmann 1967; Foelix and Müller-Vorholt 1983; Foelix and Schabronath 1983; Krapf 1986). In this regard the sexually dimorphic pectines are of particular interest. These ventromedial sensory appendages support dense arrays of minute peg-shaped sensilla which are structurally similar to contact chemoreceptors found in other arthropods (Cloudsley-Thompson 1955; Boeckh et al. 1965; Hoffmann 1964, 1967; Carthy 1966, 1968; Ivanov and Balashov 1979; Ivanov 1981; Foelix and Müller-Vorholt 1983). Krapf (1986) noted that the pectines may be involved in chemosensory responses to prey. Anatomical and physiological studies of the pectines (Brownell 1988, 1989; Gaffin and Brownell 1990) are consistent with the hypothesis that these are the primary chemosensory organs of scorpions and the ones most likely to be involved in detection of a substrate-borne pheromone.

In this paper we: 1) characterize the response of male *P. mesaensis* to chemical signals derived from female conspecifics, 2) evaluate the sensitivity of males and females to deposits from either sex, 3) assay the behavioral activity of polar and non-polar cuticular lipids, and 4) compare the responses of intact and pectineless males to cuticular extracts of

conspecific females. Parts of this study have appeared in the literature (Gaffin and Brownell 1992a,b).

MATERIALS AND METHODS

I. Response of male *P. mesaensis* to female deposits and cuticular extracts

Field observations and collection of animals for laboratory studies were conducted in sandy regions of the Mojave Desert near Indio, California (San Bernardino Co.) using portable ultraviolet lamps to observe surface activities at a distance of several meters. Collected animals were measured and sexed according to the procedures of Stahnke (1970), and only animals judged to be reproductively active adults (stage 5 instars or older) were used in behavioral experiments. Individuals were kept in an environmental chamber (27° C; 15L/9D) in separate, clear plastic dishes containing natural sand. They were maintained at capture weight by regular feedings of wax worms (Northern Bait Co., Chetek, WI).

The test chambers were cylindrical, clear plexiglass arenas (13.5 cm diam., 8 cm height) placed over natural desert sand (80 ml). Sand was cleaned of organic substances by overnight washing in chloroform:methanol (2:1, 100 ml per 80 ml of sand) in a reflux apparatus or baked at 250°C for 4 h. Opaque-plexiglass partitions were used to isolate test animals in specific quadrants within the arenas.

Trials were monitored by a video camera (Sony V9, 8 mm format) positioned above the test arenas (Fig. 2.1). Tapings occurred at night under ultraviolet illumination (single fluorescent bulb: F8T5/BLB Sylvania, 8 watt;

50 cm distance). The brightly fluorescent scorpion cuticle gave clear and detailed images for behavioral analysis under this illumination.

We have witnessed successful matings of *P. mesaensis* in the arena environment under UV illumination. All component behaviors described by Polis and Farley (1979a) for matings of this species in the field were observed in laboratory situations including initial female aggression, male juddering, "promenade aux deux", and spermatophore release. This indicates that the reproductive behavior of *P. mesaensis* is not overtly affected by the arena environment.

Two experiments were conducted to test the ability of adult male *P. mesaensis* to detect (1) natural sand substrates previously exposed to live female conspecifics from the same population, and (2) sand substrates labeled with chloroform:methanol (2:1) washes of conspecific female cuticle. In the first experiment the stimulus female (n=24, no repeats) was confined to one quadrant of the test arena for at least 24 h where it was constantly in contact with the substrate. The stimulus animal was removed from the arena just prior to release of the test male (n=24, no repeats). In this experiment, 15 of the 24 test animals crossed the exposed quadrant at least once constituting 15 legitimate trials. In the second experiment, females (n=29) were anesthetized by chloroform and cuticular extracts were prepared by submerging tail segments (telson and 5th metasomal segment) of the stimulus female in 1.5 ml of chloroform:methanol (2:1) for 2 h. This

extract was subsequently dried onto 1.0 g of sand under a stream of nitrogen gas and divided into 0.25 g portions which were placed in test arenas at known location just prior to release of the test animal (n=43 different males, 19 males repeated after at least 2 days rest for total of 62 trials). Fifty-five legitimate trials (at least one crossing of treated quadrant) were recorded for this series. Trials with pure solvent dried onto sand (n=16 males, no repeats; none used in female treated trials) were conducted as a control (14 legitimate trials).

For each trial the test animal was initially fenced in a quadrant of the test arena opposite ("O") the area where the stimulus was to be applied ("E", Fig 2.1). The positions of test animals and stimuli were randomized spatially within the room. A trial was initiated by removing the plexiglass fence that separated the test animal from the stimulus field. All trials were begun between 1900 h and 2400 h and were recorded for 2 h thereafter. Ultraviolet illumination began 15 min before each trial.

Analysis. Video records of each trial were analyzed and scored for the occurrence of several characteristic behaviors that took place during single "excursions" within the arena. An excursion was defined as an ambulatory movement lasting at least 10 s and containing no pauses greater than 30 s. For each excursion the beginning and ending times, the number of crossings of the treated substrate, and the position of final rest were recorded.

Scores were assigned the initial 5 entries into the experimental quadrant as follows: 1 = no alteration of behavior; 2 = slight but noticeable alteration of behavior; 3 = distinct alteration of behavior including "back-ups" (cessation of forward locomotion followed by quick backward movement of 1 to 2 steps) and/or increased turning tendency (animal moves away from container walls); 4 = prolonged alteration of behavior including "push-ups" (animal's body pushes forward and downward while tarsal segments remain stationary, pectines brush forward and laterally over substrate), "creeping" (distinct change of normal forward stepping movements to shorter forward movements, usually accompanied by increased turning movements), "tail-wags" (described in results section), and/or "pedipalp-reaches" (also described in results section); 5 = presence of juddering. The highest score received constituted the overall score for the trial.

The binomial distribution (Snedecor and Cochran 1967) was used to test the statistical significance of a behavioral pattern (BP) initiated in the treated quadrant (E) compared to a hypothetical random quadrant of initiation. In a random situation, the probability of initiation in quadrant E (p_E) would be 1/4 while the initiation in any of the other three quadrants would be 3/4. Thus, we test for H_0: $p_E \leq 1/4$ and H_a: $p_E > 1/4$ where:

$$p_E = \sum_{i=x_E}^{N} \left(\frac{N!}{i!\,(N-i)!}\right)\left(\frac{1}{4}\right)^{i}\left(\frac{3}{4}\right)^{N-i}$$

x_E is the number of scorpions which first exhibited the BP in quadrant E; N is the total number of scorpions exhibiting the BP. Mann-Whitney analysis was used to test for significant differences between treatments with female cuticular extracts and solvent controls.

II. Factors affecting response to intra-specific chemical signals

Sex-specificity of behavioral response

This set of experiments was conducted at the Zzyzx field station near Baker, CA during a period of new moon (September 9-16, 1991). Animals used in these experiments were adult *P. mesaensis* obtained nightly from the Crescent dune system located 3 mi south of the station. Only animals judged to be instar stage 6 or older were used; wandering males were selectively used when found.

Two sets of trials were run: one with males as responders, the other with females as responders. Testing arenas were the same as previously described. Test stimuli were sand substrates exposed to conspecific males or females; trials with untreated sand were run as controls. Stimulus animals were allowed 2 h exposure time in quadrant E and were removed just prior to placement of test animals in quadrant O (See Fig. 2.1); positions of O and E were randomized with each trial. Fifteen minutes acclimation time was allowed between placement of responding animals and lifting of partitions to initiate trials. Ultraviolet illumination was begun at the time of

placement of the test animals. All trials were conducted between the hours of 2100 and 0200, the normal active period for *P. mesaensis*; filming duration was limited to 1 h. In all cases, each animal was used only once.

Behavioral activity of polar and non-polar cuticular lipids

These experiments were performed between September 26 and October 14, 1991 in our laboratory at Oregon State University using adult animals recently returned from the Crescent dune system. Animals were maintained as described in Part I above.

Extractions were made of recently caught animals (mid-September) during early night of Sept. 25. Cuticular lipids of anesthetized (CO_2) adult males and adult females were obtained by sequential extraction of 10 animals (2 min dip / animal) in 30 ml of hexane or methylene chloride solvent. Total weights of animals extracted in each solvent were as follows: hexane ♀♀ = 11.14 g, hexane ♂♂ = 8.79 g, methylene chloride ♀♀ = 10.86 g, methylene chloride ♂♂ = 7.53 g. After evaporation under nitrogen of each extract to a final volume of 20 ml, final lipid concentrations were as follows: hexane ♀♀ = 13 μg / 200 μl, hexane ♂♂ = 23 μg / 200 μl, methylene chloride ♀♀ = 13 μg / 200 μl, methylene chloride ♂♂ = 13 μg / 200 μl. Extracts were stored at -80°C until time of use.

For behavioral trials, 2.0 ml of extract was added to 2.5 g of untreated desert sand and dried under nitrogen; 0.5 g of extract treated

sand, representing 0.2 animal equivalents ([10 animals / 20 ml extract] * [2.0 ml extract / 2.5 g treated sand] * [0.5 g / trial]), were used per trial. Solvent controls were made in the same manner using 2.0 ml pure hexane or methylene chloride in place of extract.

Treated sand was added to quadrant E of test arenas just prior to placement of test animals in quadrant O; positions of O and E were randomized with each trial. Ultraviolet illumination was begun prior to placement of test animals; in these trials, partitions were lifted immediately after placement of test animals. All trials were conducted between the hours of 2100 and 0200; filming duration was 1 h. In all cases, each animal was used only once.

Pectineless males were used as test animals in some of the female extraction trials described above. Pectines were surgically removed from animals (anesthetized by cooling) at least two weeks prior to testing; locomotory behavior and activity level of pectineless animals were judged to be normal at time of testing.

Analysis. Behavioral scores for all trials in Part II represent the averaged scores of two independent reviewers unaware of the particular test crosses. Scores were assigned to movements in the experimental quadrant (E) based on the scoring system described in Part I; the highest score obtained constituted the overall score for the trial. Data were tested for significance using Mann-Whitney analysis.

For experiments in Part II, legitimate trials were defined as trials with responding animals spending 2 min of non-riled locomotory behavior (tail down, normal walking) within the experimental quadrant (E) or 3 non-riled entries into the experimental quadrant, whichever came first. Rests were defined as more than 30 continuous seconds of non-movement and were not included in calculation of cumulative time of quadrant occupancy. Table 2.1 shows total number of trials and number of legitimate trials for the experiments in Part II.

RESULTS

I. Response of male *P. mesaensis* to female deposits and cuticular extracts

When placed in a foreign environment, such as the test arena of these experiments, *P. mesaensis* were quiescent for several minutes ($\bar{x}$ = 18.5 ± 16.8 min). Thereafter, most animals began to explore the new environment, generally by walking along the walls of the circular arena. These exploratory movements were characterized by quick forward steps interrupted by brief pauses. If no stimulus was encountered during this time the animal eventually began other behaviors in the vicinity of the container walls such as "wall-climbing" (animal attempts to climb container walls) or "rototiller-digging" (first three leg pairs quickly rotate and scrape surface sand away). A strikingly different behavior was observed for male scorpions that encountered substrates that had been exposed to female conspecifics: their exploratory and escape behavior abruptly changed to shorter, creeping movements, with a tendency to turn and move toward areas away from the container walls. As a consequence, stimulated males tended to linger in the quadrant labeled by exposure to the female; a representative example is shown in the tracing of movement over time for one individual in Fig. 2.2A. Some stimulated males also displayed juddering behavior (Fig. 2.3A) as they traversed substrates previously exposed to a female. Figure 2.2B shows the locations where juddering behavior was performed during the trial depicted in Fig. 2.2A. When the animal moved out of the exposed quadrant, creeping

and juddering behavior ended abruptly as turning behavior continued, serving to bring the animal back into the exposed quadrant.

These changes in locomotory behavior of males were likely stimulated by substances deposited on the sand by the stimulus female or, alternatively, by mechanical disturbances of the substrate (e.g. tarsal-print patterns) resulting from her movements. To eliminate the latter as a potential cue and to minimize potential variability in the distribution and intensity of the stimulus signal we tested the male's response to organic extracts of conspecific females. When male scorpions encountered undisturbed substrates with sand grains labeled with these dried extracts they showed distinct changes in locomotory activity and movements of their appendages. Behavioral changes (score of 2 or greater, see Materials and Methods for criteria) were seen in 62% of the males as they first moved across the extract-treated region (34 of 55 trials). Juddering behavior was elicited in eight of these trials.

In addition to juddering behavior, female tail extracts also evoked two other behaviors referred to here as "tail-wagging" and "pedipalp-reaching" (Fig. 2.3). These behaviors have not been previously described for *P. mesaensis* or other scorpions. In tail-wagging (Fig. 2.3B), the animal's first four metasomal segments are held low to the substrate and in line with the body axis. The terminal (fifth and sixth) segments of the metasoma are held perpendicular to this axis and rotated approximately 15 degrees side to side at about 1 Hz. The rest of the body remains stationary except for the

pectines, which brush forward and backward against the substrate at a frequency similar to that of the tail-wag. In pedipalp-reaching (Fig. 2.3C), the pedipalps appear to grasp for an illusory object immediately in front of the chelicera. This behavior was accompanied by a short backing or turning movement of the body, which places the pedipalps in an area where the pectines had been moments before. Pedipalp-reaching and tail-wagging occurred in 6 and 15 of the 55 trials, respectively.

Figure 2.4 shows a particularly vigorous response of a male to a cuticular extract of a conspecific female. Figure 2.4A shows position per second for 8 min of movement during the animal's second excursion (39 min post-release); the animal did not move into quadrant E during its first excursion. All three of the behaviors described in Fig. 2.3 were observed in this response (Fig 2.4B), but only after the animal's initial movement into the treated quadrant. Also depicted in Fig. 2.4A is the frequency of pectine contacts with the substrate during the male's initial movement into the treated quadrant. As the male moved through quadrants O and L, pectine-tapping frequency was 18 and 13 taps per min, respectively. Upon initial contact with the treated substrate in quadrant E, pectine-tapping frequency increased to 84 taps per min.

For all trials taken together, the sites of initiation of juddering, pedipalp-reaching, and tail-wagging behaviors were significantly associated with the quadrant containing sand treated with extracts of female cuticle.

All pedipalp-reaching (n=6) and juddering behaviors (n=8), and all except one of the tail-wagging behaviors (n=15), began within the quadrant containing the extract (binomial distribution $p<0.001$ for each).

The results of all trials of male response to female deposits, female cuticular extracts, or solvent controls as categorized by behavioral score (see Materials and Methods for details) are summarized in Fig. 2.5. Mann-Whitney analysis of trials with female cuticular extract compared with solvent controls was significant at the $p<0.001$ level. Mean behavioral responses to deposits and extracts were 2.1 and 2.9, respectively, reflecting the large number of non-responders (behavioral score = 1) in each group.

The response of males to female cuticular extracts was generally immediate and intense but adapted quickly with continued exposure. The dynamics of adaptation are shown in Fig. 2.6 as a record of the initial 6 min of occupancy of the experimental quadrant (E) treated with either female cuticular extracts or pure solvent. Most responses decayed to spontaneous levels within 3-5 min.

II. Factors affecting response to intra-specific chemical signals

Sex-specificity of behavioral response

During the mating season, the surface density of adult male *P. mesaensis* is often twice that of adult females (Polis 1980). If wandering males use substrate-borne chemical signals to detect and locate isolated

females, they may also detect and selectively avoid males or show some form of sex-recognition of chemical signals. As such, we were interested in whether males showed differential responsiveness to deposits from females and males. Using animals caught nightly during the mating season (mid-September), we assayed the behavioral response of male and female *P. mesaensis* to substrates exposed (2 h) to conspecifics of either sex. The results of these trials are shown in Fig. 2.7. Males responded most strongly to female-exposed substrates ($\bar{x}$ = 2.4 $\pm$ 0.3). Males showed some response to male-exposed substrates, but not significantly different from control trials. Females also responded to female-exposed substrates at a level significantly different from control trials. By these observations it appears that both males and females respond to intraspecific chemical cues.

Behavioral activity of polar and non-polar cuticular lipids

In this set of experiments we tested the behavioral activity released by superficial lipids of polar (methylene chloride) and non-polar (hexane) classification. The response of male *P. mesaensis* to polar and non-polar lipids from either sex was tested using pooled (n = 10) extracts of animals freshly returned from the field (see methods). Males responded strongly to female extracts of either polarity (Fig. 2.8; $\bar{x}$ = 4.1 $\pm$ 0.2, polar; $\bar{x}$ = 3.6 $\pm$ 0.4, non-polar). Males also responded to polar male extracts ($\bar{x}$ = 3.0 $\pm$ 0.4), but not to non-polar male extracts ($\bar{x}$ = 1.5 $\pm$ 0.3). Juddering occurred

in 4 of 12 responses to polar female extract and in 1 of 12 response to non-polar female extract. No juddering was observed in response to male extract (polar or non-polar) or pure solvent controls. Tail-wagging was observed in 3 responses to polar female extract and in 4 responses to non-polar female extract. Tail-wagging also occurred in 2 responses to polar male extract, but in none of the responses to non-polar male extract or pure solvent controls. Thus, it appears that pheromonal activity exists in both polar and non-polar female extracts and that juddering may be selectively released in males by female cuticular lipids.

In part I of this study we often observed increased activity of the pectines as males moved across surfaces treated with female-derived substances. As such, we were interested if removal of these organs was sufficient to effect a change in male response to female cuticular extracts. The results of this treatment are also shown in Fig. 2.8. The response of pectineless males to polar ($\bar{x} = 1.8 \pm 0.5$) and non-polar ($\bar{x} = 1.4 \pm 0.4$) female extracts was significantly reduced from the response of intact males to female extracts of either polarity. No juddering or tail-wagging behavior was observed in trials with pectineless males ($n = 15$). These results suggest that the ventral-medial pectines are the likely sense organs involved in detection of chemical signals in these animals.

DISCUSSION

This study shows that male scorpions (*P. mesaensis*) markedly alter their locomotory behavior when they encounter substrates previously exposed to female conspecifics. Sand substrates exposed to live females or treated with organic extracts of female cuticle suppressed non-directed locomotory behavior and released mate-searching behavior that was of greatest intensity in the vicinity of stimulus application. The response of males was significantly stronger to female deposits and extracts than to male deposits and extracts. The simplest explanation of these observations is that male scorpions are responding differentially to a chemical signal deposited on the substrate from the cuticle of conspecific females. In their natural sand dune environment such responses would have the effect of congregating male scorpions in areas frequented by females, that is, in the vicinity of a female's burrow. In most instances we only observed changes in the pattern of locomotion although some trials also stimulated behavior related to courtship -- juddering, and two previously undescribed behaviors, tail-wagging and pedipalp-reaching. These behavioral observations are consistent with the hypothesis that scorpions, like many other terrestrial arthropods, use pheromonal signals to trigger and direct orientational behaviors related to mating.

The natural history of *P. mesaensis* suggests the need for some means of species-specific chemical communication during reproduction. For most of

their 5-7 yr life span, males and females of this species live in solitary burrows; these are generally located among dunegrass and creosote vegetation where thermal and water stresses of the diurnal dune environment are minimized. At night their surface activities are confined to areas near their home burrows, where they lie motionless on the surface in ambush of arthropod prey, some of which are conspecific scorpions of smaller size. During the summer mating season mature *P. mesaensis* males become increasingly mobile and are often seen walking with extended pectines in regions between patches of vegetation. During this time males are heavily predated by larger *P. mesaensis* and the sympatric scorpion, *Hadrurus arizonensis*, indicating that wandering behavior involves substantial risk. Wandering appears to diminish in the vicinity of a female's burrow since clusters of 2 or more immobile males are often seen there (Polis pers. comm. 1990). Clustering of males is particularly evident in two other species, *Paruroctonus luteolus* and *Paruroctonus borregoensis*, that are rarely seen on the surface.

Such observations lead us to hypothesize that some form of chemical signal attracts male scorpions to female burrows. Once within detectable range, surface vibrations generated by male juddering may function to suppress the female's predatory attack response. *Paruroctonus mesaensis* is known to be highly sensitive to disturbances of the substrate (Brownell 1977; Brownell and Farley 1979a,b,c). Near-range behaviors may also be governed

by specific qualities of the stimulus. Pedipalp-reaching behavior (Figs. 2.3 and 2.4), which appears to be a male's attempt to make direct contact with the female, only occurs at the central region of the stimulus field.

Nature of chemical cues

Our studies indicate that chemical signaling in *P. mesaensis* may involve more than one pheromonal moiety since strong behavioral responses (including juddering and tail-wagging) were evoked in males by polar and non-polar washes of female cuticle. Male extracts also evoked behavioral changes in male responders, but did not release juddering (0 of 20 trials). Male signals appear to partition selectively into polar solvent; tail-wagging was evoked in 2 of 8 trials with polar solvent and 0 of 12 trials with non-polar solvent.

Our observations also suggest the chemical signals that trigger reproductive behaviors are substances of low volatility and high potency. Males responded strongly to sand that had been exposed to females for a brief period, and extracts of female cuticle dried onto sand and allowed to stand for 3 d were still capable of stimulating changes in locomotory behavior. Furthermore, extracts that produced vigorous behavioral responses (e.g. juddering) on contact during locomotion did not affect test animals from a distance. For instance, the response depicted in Fig. 2.4 occurred on the animal's second excursion, 39 min after release into the test

arena. During the first excursion (26 min post-release) the animal remained in regions away from the treated quadrant, with no indication of behavioral change. These observations are consistent with those of Abushama (1964) who found no tendency for *Leiurus quinquestriatus* to be attracted to regions of a test chamber in which conspecifics were confined behind a wire gauze barrier. Thus, stimulus detection appears to require direct contact with a substrate bearing a non-volatile signal.

Seasonal fluctuations may exist in the receptivity of males or in the production and release of signals by females. A pilot study of male response to female deposits (n = 8) was conducted in August 1991 at Zzyzx Station using the same protocol as for deposit experiments described here with the exception that depositing females were given only 1 h exposure time (as opposed to 2 h exposure time in September trials conducted at Zzyzx). The behavioral score for August trials was 3.8 $\pm$ 0.1 (mean $\pm$ SE) as compared to 2.4 $\pm$ 0.3 for the same test cross in conducted in September.

Our observations suggest that females may also use chemical cues to detect and recognize the sex of conspecific scorpions. Since females tend to remain throughout the year in areas near their home burrows (Polis 1980), it seems unlikely that females would often encounter other females in the field. The possibility exists that sex-recognition is a primitive ability and that spatial isolation of male and female *P. mesaensis* is a more recent development. As such, ability to recognize sex would be particularly

exploited by males as they encounter female-labeled sand during their annual wanderings.

Sensory detection

The sexual dimorphism of the pectine sensory organs suggest they may function differentially in males and females. In most species of scorpions the pectines of males are longer and contain more teeth and peg sensilla than those of females. While the number of teeth per pectine is determined as early as the first instar stage and does not change with subsequent moults, the rate of pectine elongation in sexually mature *P. mesaensis* males is greater than that of either immature males or females of any age (Polis and Farley 1979b). A dimorphism also exists in the number of chemosensory peg sensilla on the pectines with males of most species having substantially more than females (Swoveland 1978, Brownell unpublished).

The activity of the pectines of male scorpions as they move across sand chemically labeled by conspecifics supports the idea that these organs are involved in mediating pheromone-induced behavior. During normal ambulatory movements the pectines are swept lightly over or brushed intermittently against the substrate as the animal pauses between forward movements. We observed changes in substrate-sweeping activity of the pectines as males walked across female-treated substrates in laboratory test

arenas. When adult males contacted a female-exposed surface, pectine tapping increased in frequency and changed to longer sweeping movements over the substrate.

Comparison of responses of intact males and pectineless males to organic washes of female cuticle further supports their role in the detection of chemical signals. The response of pectineless males to polar and non-polar female cuticular extracts was significantly reduced from that of males with intact pectines and similar to the response of intact males to sand treated with solvent alone (Fig. 2.8).

Male pectines appear to serve a tactile role in the discrimination of surface texture for spermatophore deposition during the promenade aux deux (Alexander 1959). This mechanosensory function is supported by morphological (Ivanov and Balashov 1979, Foelix and Müller-Vorholt 1983) and physiological (Hoffmann 1964, 1967; see also Chapter 4) studies showing the presence at least one mechanoreceptor in each peg sensillum.

Recent physiological studies, however, show the pectines are sensitive to a variety of chemostimulants (Chapter 4). Morphological studies showing a population of from 10 to 20 chemosensory cells per peg sensillum (Foelix and Müller-Vorholt 1983, Brownell unpublished) support this finding. Taken together with the results presented here, it appears that the pectines are important organs for detection of ground-based chemical stimuli and, in

addition to texture discrimination, serve a primary role in males in the detection of pheromonal molecules.

Table 2.1 Number of legitimate and total trials in assays of sex-specificity and polarity of chemical signals.

Sex specificity:			
Responding sex	Depositing sex	# Legitimate trials	Total # trials
♂	♀	11	11
♂	♂	11	18
♂	ns	5	6
♀	♀	9	10
♀	♂	9	14
♀	ns	5	5
Totals:		50	64

Polar vs. non-polar extracts:			
Responding animal	Extract/sex	# Legitimate trials	Total # trials
Intact ♂	hex/♀	12	12
Intact ♂	hex/♂	12	12
Intact ♂	hex	8	8
Pectineless ♂	hex/♀	7	8
Intact ♂	mc/♀	12	12
Intact ♂	mc/♂	12	12
Intact ♂	mc	7	8
Pectineless ♂	mc/♀	8	8
Totals		75	79

ns = no-stimulus controls, hex = hexane, mc = methylene chloride

Figure 2.1 Arena for monitoring response of scorpions to test substances. Responding animal is acclimated behind fence in quadrant opposite test stimuli. A trial is initiated by lifting the partition, allowing responder access to remainder of arena. Overhead mounted camera monitors trial for subsequent analysis; illumination is via fluorescent "black light" bulb. E refers to quadrant containing the female-exposed sand; L, R, and O refer, respectively, to quadrants on the left, right and opposite the exposed quadrant.

Figure 2.1

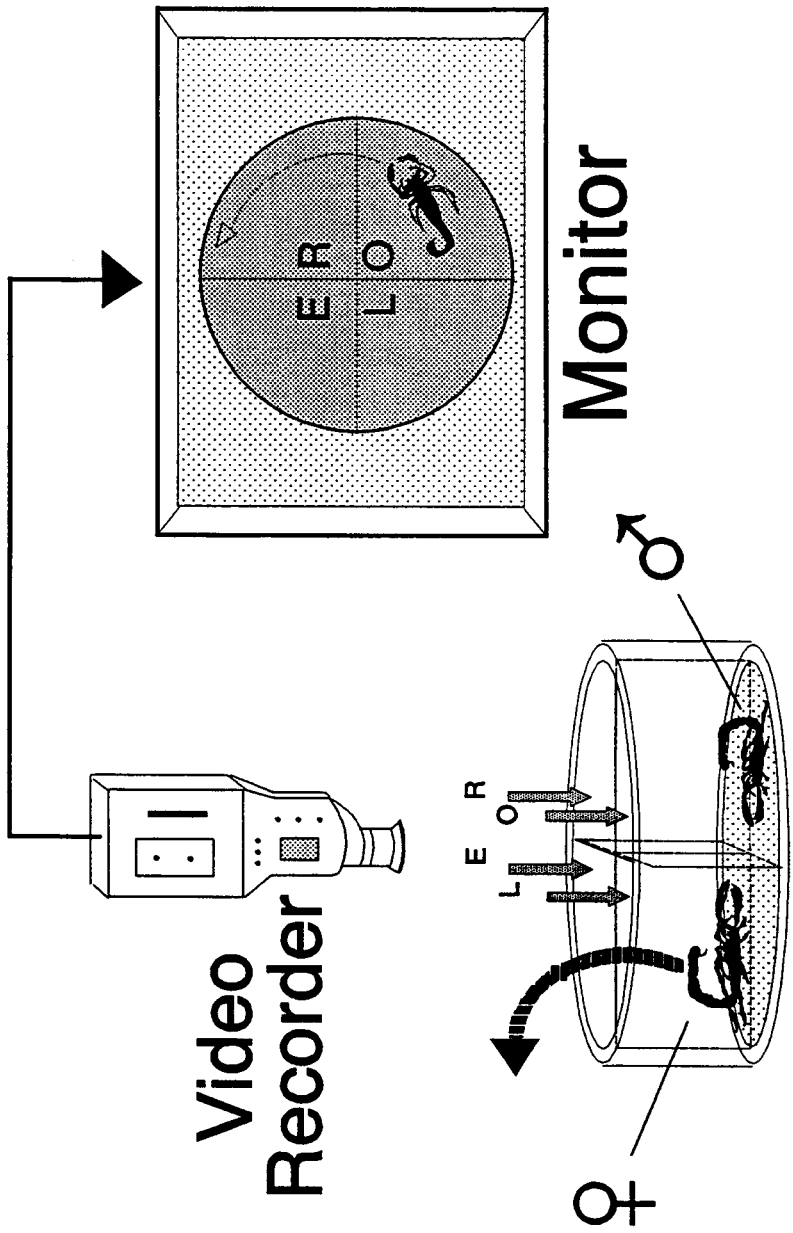


Figure 2.2 Behavioral response of a male *P. mesaensis* to sand previously exposed to a conspecific female. **A** Initial 5 min of locomotory movements plotted as position at 1 s intervals. Quadrant E was exposed to a live conspecific female for 24 h prior to testing. **B** Location of juddering behavior during first 10 min of male movement.

Figure 2.2

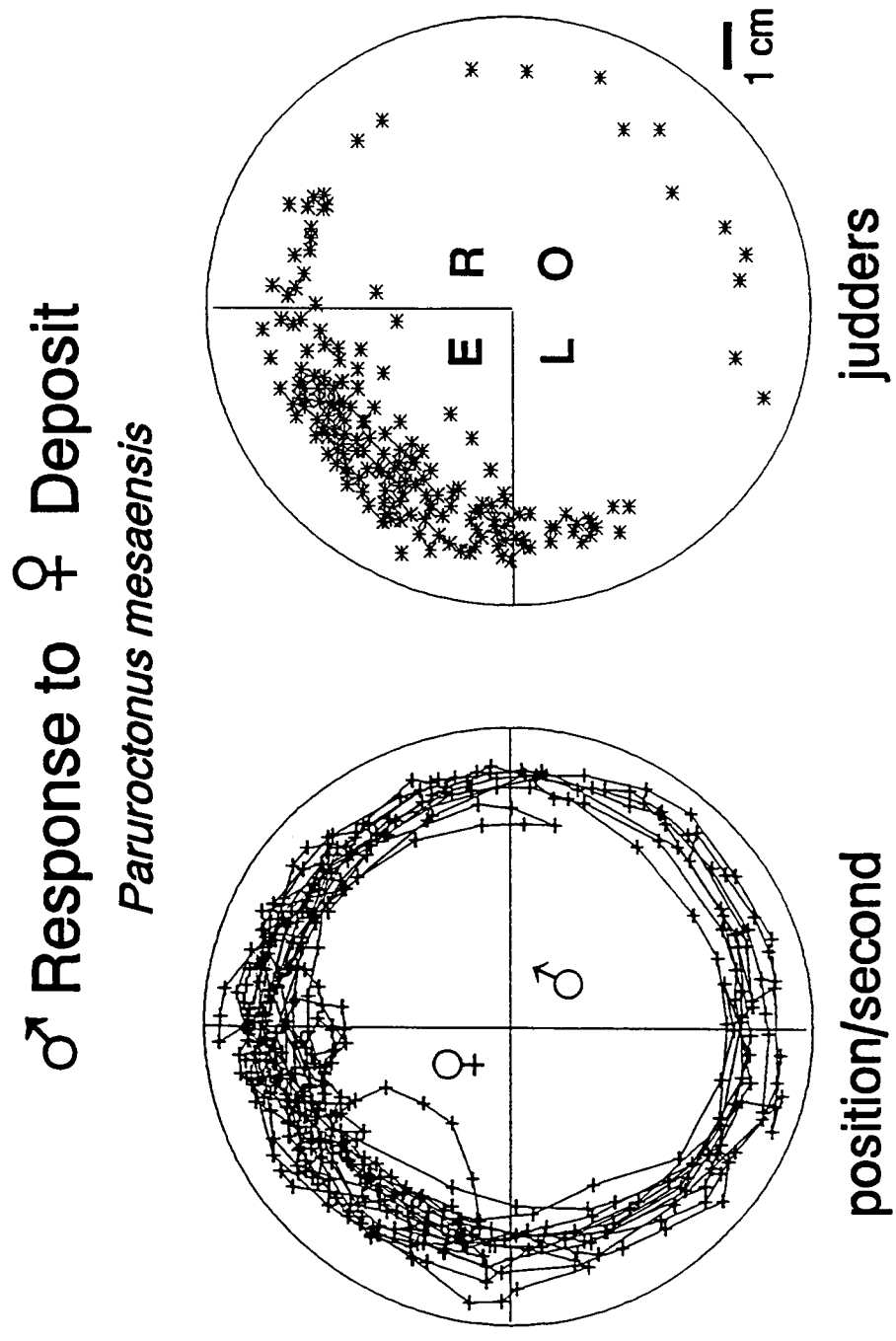

♂ Response to ♀ Deposit
Paruroctonus mesaensis
1 cm
judders
position/second
♀
♂

Figure 2.3 Three discrete behaviors observed when male scorpions contact substrates previously exposed to female conspecifics or extracts of females. **A** Juddering, **B** Tail-wagging, **C** Pedipalp-reaching. See text for descriptions. The black spot in C indicates a point on the sand touched initially by the right pectine then subsequently by the pedipalp tips at the conclusion of the behavior.

Figure 2.3

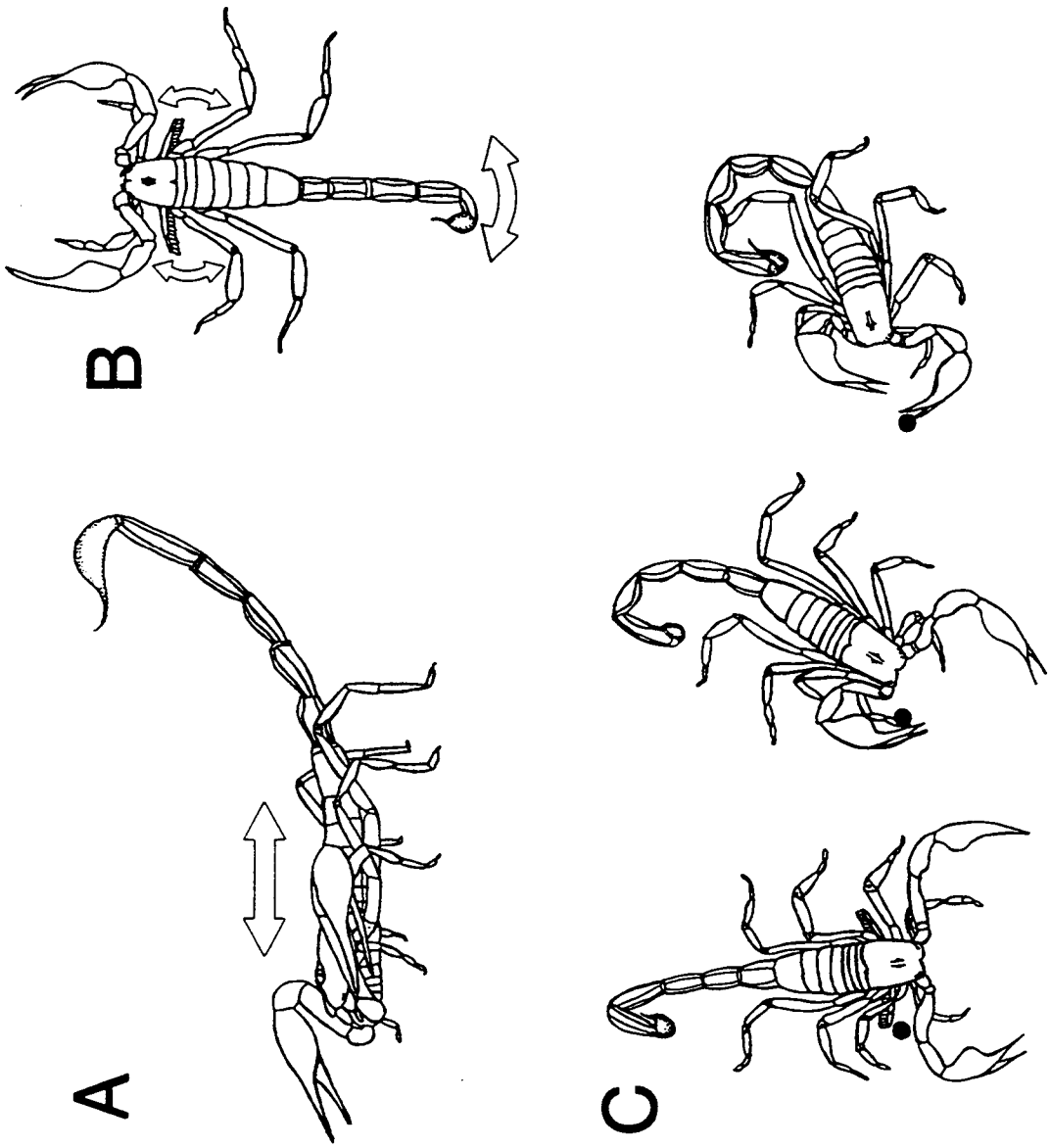


Figure 2.4 Response of a male *P. mesaensis* to sand treated with chloroform/methanol extract of a female's metasomal segments. **A** Scatter plot of male location at each second during 8 min observation of animal's first excursion into treated quadrant (second excursion of trial). Numbers in parentheses indicate the frequency of pectine-tapping per min by quadrant during the animal's first movement into the treated quadrant. **B** Location of juddering (*), pedipalp-reaching (□), and tail-wagging (T) behaviors for the same 8-min period. The quadrants were labeled according to the scheme shown in Fig. 2.1. Extract-treated sand was placed in quadrant E.

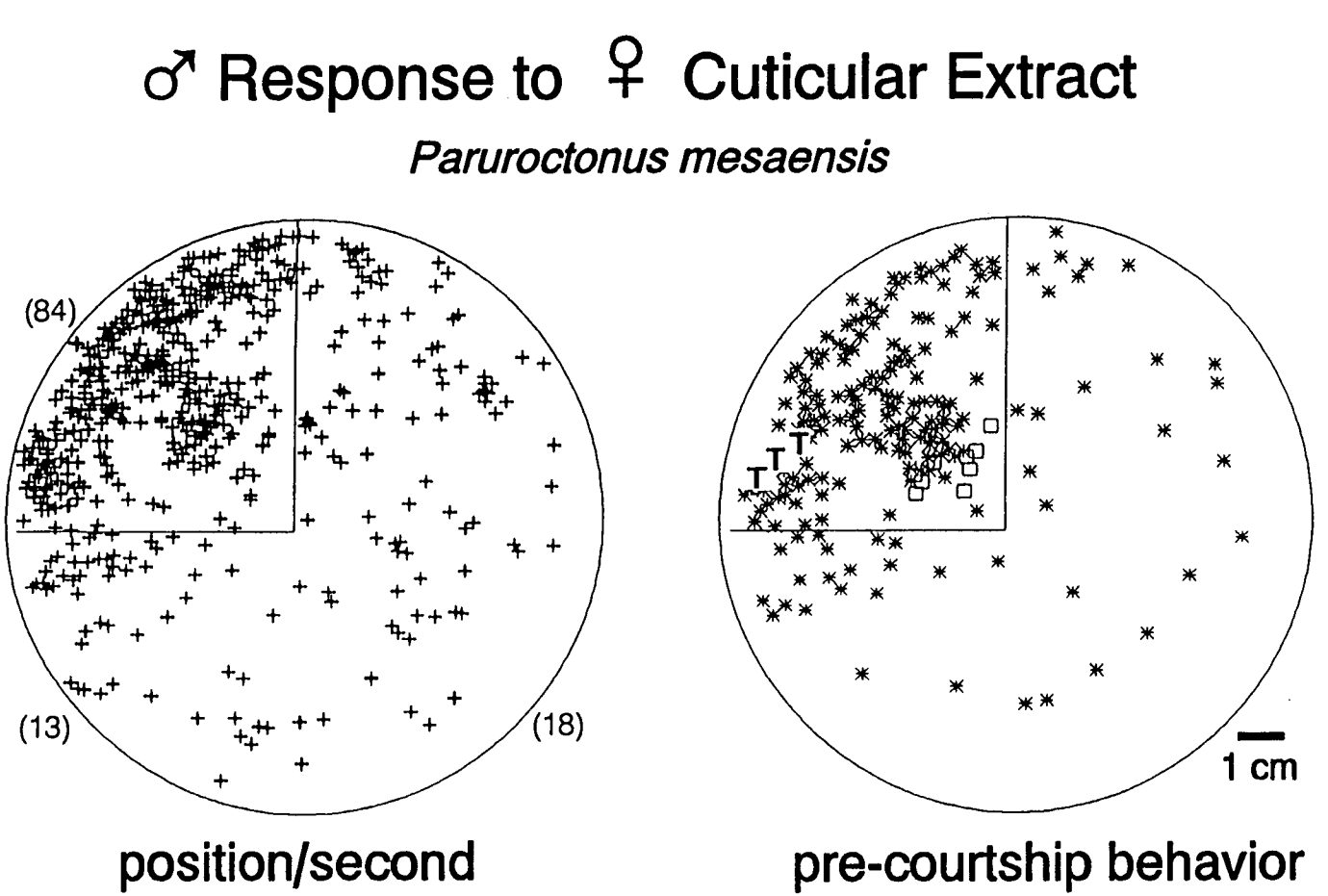

♂ Response to ♀ Cuticular Extract
Paruroctonus mesaensis
(84)
(13)
(18)
T
T
T
1 cm
position/second
pre-courtship behavior

Figure 2.4

Figure 2.5 Summary of male behavioral responses to female-derived chemical stimuli. Animals in all trials were grouped by behavioral score to obtain frequency of response. Mean values: female deposit $\bar{x}$ = 2.01 (n=15), female cuticular extract trials $\bar{x}$ = 2.93 (n=55), solvent control $\bar{x}$ = 1.14 (n=14). Responses to solvent control differ from female cuticular extract responses at $p < 0.001$ level (Mann-Whitney analysis).

Figure 2.5

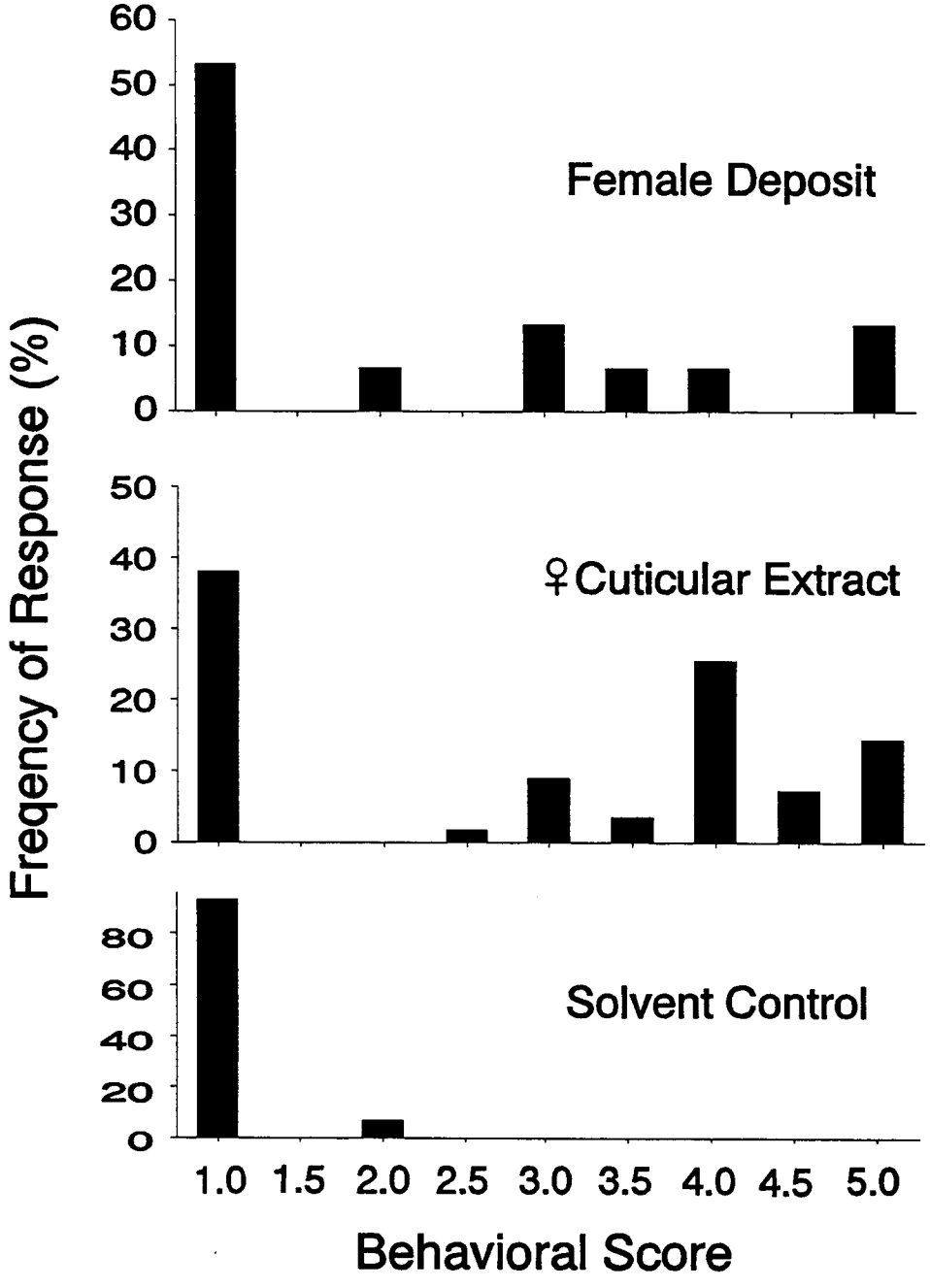

Freqency of Response (%)
60
50
40
30
20
10
0
Female Deposit
50
40
30
20
10
0
♀Cuticular Extract
80
60
40
20
0
Solvent Control
1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
Behavioral Score

Figure 2.6 Adaptation of male's response to extract-treated sand. The percent time of occupancy of quadrant treated with either female cuticular extract or pure solvent (n=12) during first 6 min of movement is shown ($\bar{x}$ ± SE). Trials with cuticular extract are grouped as responders (behavioral score ≥ 2; n=21) or non-responders (behavioral score = 1; n=11). Only trials with at least 10 min of total movement were compiled.

Figure 2.6

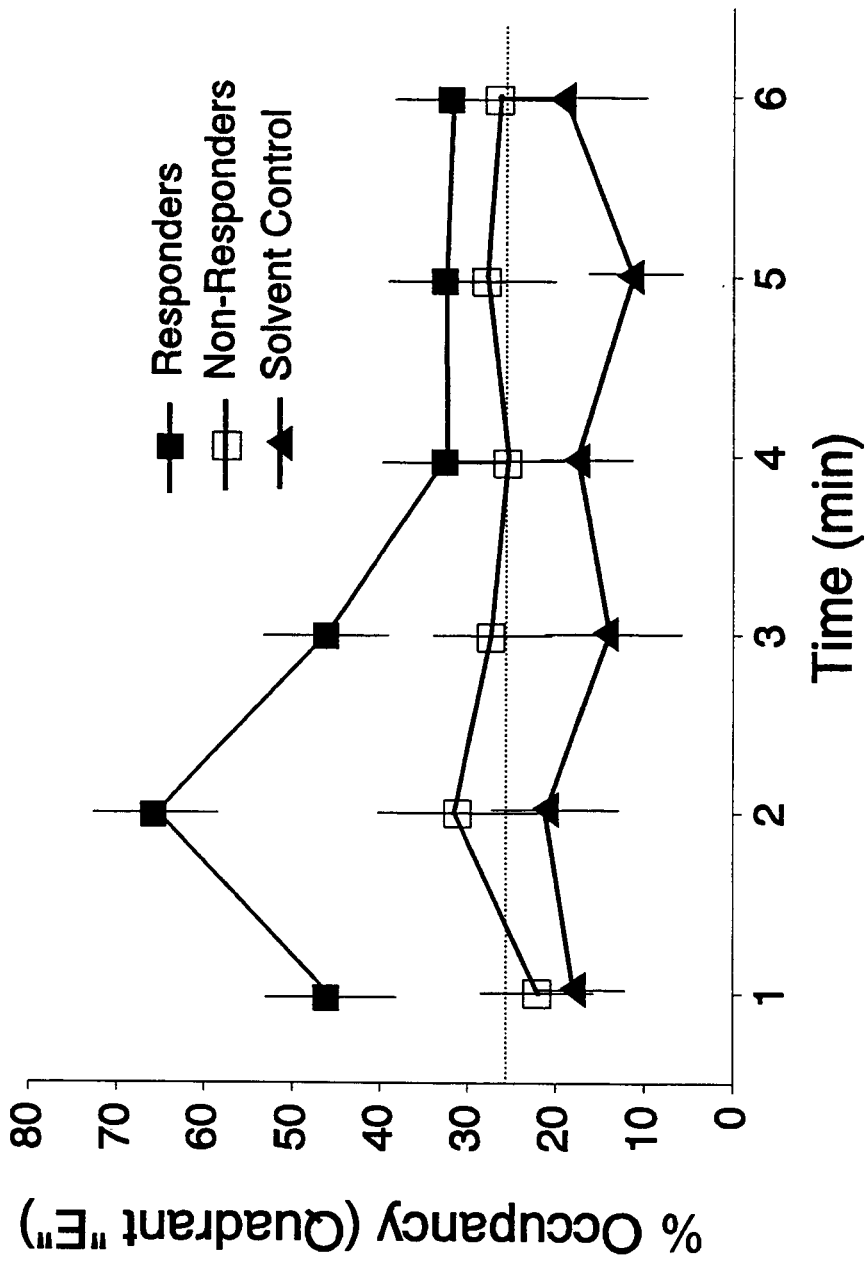

Responders
Non-Responders
Solvent Control
% Occupancy (Quadrant "E")
Time (min)
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6

Figure 2.7 Sex-specificity of pre-courtship behavior in *P. mesaensis*. Histograms show mean behavioral scores (±SE) of male and female *P. mesaensis* to conspecific female and male deposits and no-stimulus controls. Values represent averaged scores assigned by two independent observers unaware of test crosses. Within each responder sex, treatments with same letter are not significantly different at $p < 0.05$ (Mann-Whitney analysis); n indicates the number of legitimate trials for each treatment.

Figure 2.7

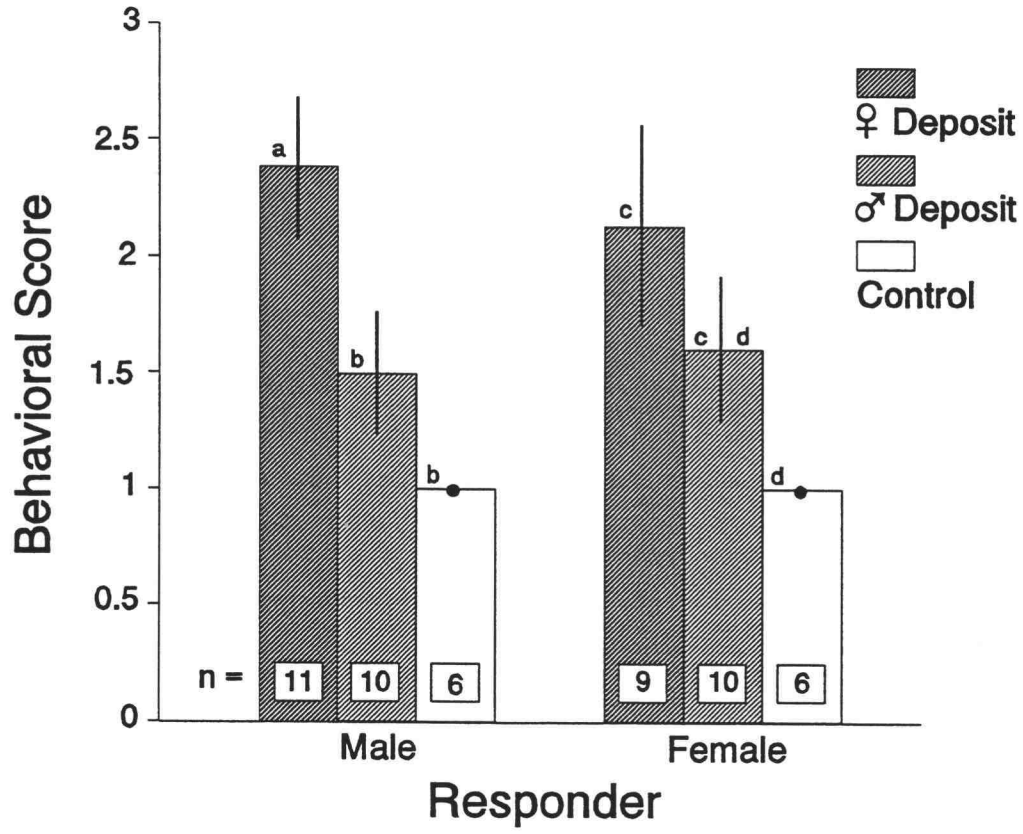
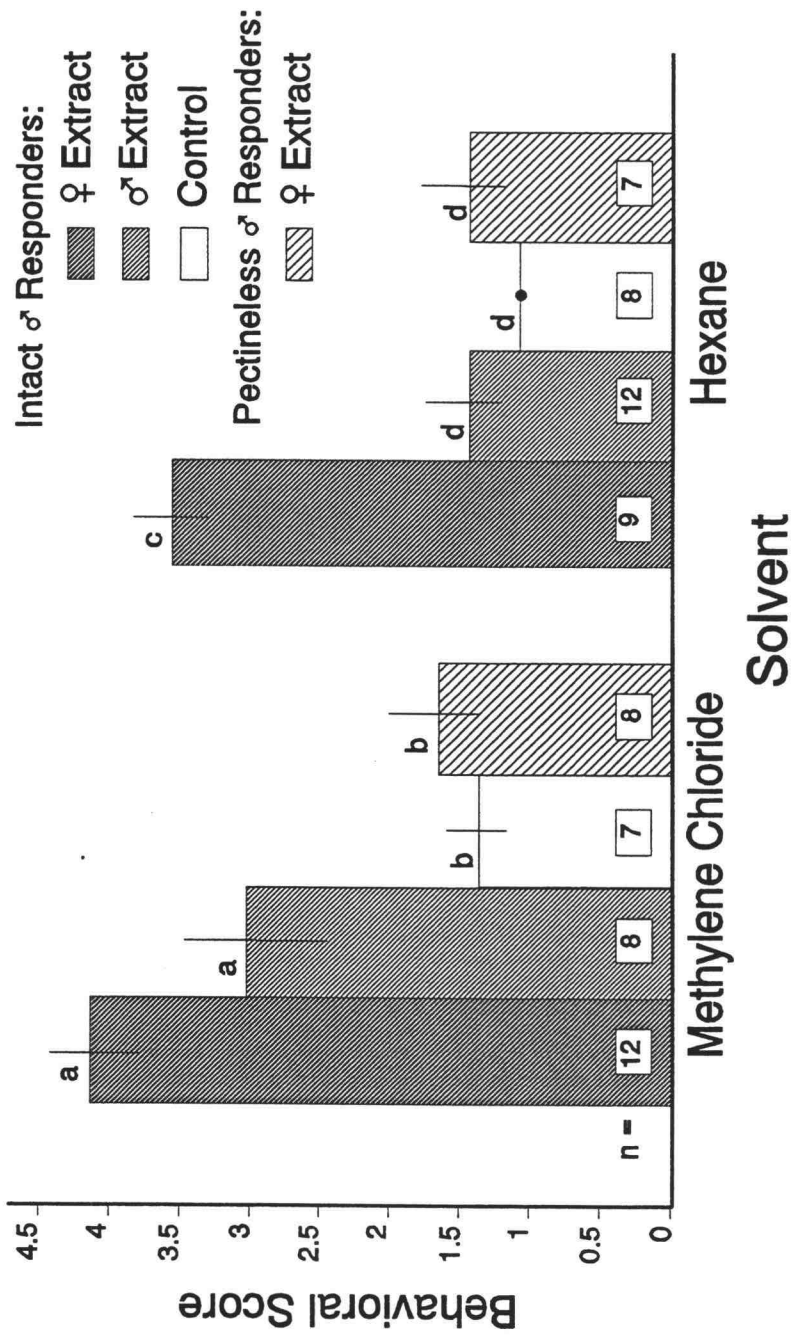

Behavioral Score
3
2.5
2
1.5
1
0.5
0
a
b
b
c
c
d
d
n =
11
10
6
9
10
6
Male
Female
Responder
♀ Deposit
♂ Deposit
Control

Figure 2.8 Courtship assay for pheromonal activity in cuticular lipids, and the effect of pectine ablation on male pre-courtship response. Histograms show mean behavioral scores (±SE) of intact male *P. mesaensis* to polar and non-polar cuticular extracts from male and female conspecifics and response of pectineless males to female cuticular extracts. Cuticular extracts were pooled from 10 animals (2 min dip each in 20 ml solvent, dried onto small volume of sand; approx 0.2 animal equivalents used per trial). Pectines were surgically removed from animals at least two weeks prior to testing to allow full recovery; locomotory behavior and activity level of pectineless animals were otherwise normal at time of testing. Values represent averaged scores assigned by two independent reviewers unaware of the test stimulus and condition of the test animal. Within each solvent group, treatments with same letter are not significantly different at $p < 0.05$ (Mann-Whitney analysis); n indicates the number of legitimate trials for each treatment.

Figure 2.8


Intact ♂ Responders:
♀ Extract
♂ Extract
Control
Pectineless ♂ Responders:
♀ Extract
Behavioral Score
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
n =
12
8
7
8
9
12
8
7
a
a
b
b
c
d
d
d
Methylene Chloride
Hexane
Solvent

CHAPTER 3

WATER DETECTION IN THE DESERT SAND SCORPION

ABSTRACT

For the sand scorpion, *Paruroctonus mesaensis*, substrate moisture is a powerful and fast-acting stimulus of discrete behaviors related to localization and imbibitory uptake of water. These behaviors are readily observed in the field and quantified in the laboratory when free-roaming animals encounter sand substrates dampened by small amounts of water. Of ten behaviors we monitored in laboratory tests, five (pedipalp-pull, rototiller-digging, prolonged stops, headstand, and backing-up) occurred after contact with a moistened substrate. These water-stimulated behaviors were selectively blocked when all eight tarsal leg segments were coated with wax; coverings of the chemosensory pectine appendages had little to no effect. Electrophysiological recordings from chemoreceptor organs on the tarsi showed that neurons innervating the dorsal tarsal organ, were highly sensitive to humid air stimuli while the numerous, pore-tipped hairs on the ventral surface were responsive to aqueous solutions applied directly to their tips. Selective blocking of the eight tarsal organs had no effect on water sensitive behavior indicating that the chemosensory hairs mediate detection of substrate moisture. Such localized, sensory triggering of a robust and

directed behavior presents a useful model for further neuroethological studies.

INTRODUCTION

Water is of obvious importance to all living things; its gain and loss are powerfully regulated, especially in species living under chronic water stress. Desert arthropods have provided several interesting examples of physiological adaptations that conserve total body water by minimizing its rate of loss (Edney et al. 1974; Hadley 1970; Hadley 1974), but less is known about sensory mechanisms for detection and orientation to substrate water or humid air (Altner et al. 1983; Steinbrecht 1984). Although sensory detection is a prerequisite for orientation to moist microenvironments, the mechanisms involved in the detection of water are likely to be diverse and difficult to localize experimentally.

In this regard, psammophillic scorpions, which live in the driest of desert environments, may be excellent subjects for sensory and behavioral analysis. Under normal circumstances these animals show a simple repertoire of locomotory behaviors which can be used in the field or laboratory to assess responsiveness to a variety of stimuli. Anecdotal field observations show that simulated rain or localized application of water to the substrate evokes unusual behavior from *Paruroctonus*, including *en masse* emergence from burrows and cheliceral-chewing of the dampened sand. Physiologically, water is a potent stimulus for chemosensory neurons in the pectines, two antenna-like, ventral appendages that intermittently touch the substrate as the animal walks (Brownell 1988; Gaffin & Brownell 1990).

Additionally, on the tarsi of each leg there are numerous chemosensory hairs on the ventral surface and a single "tarsal organ" approximately mid-length along the dorsal surface (Foelix and Schabronath 1983). These organs are similar to those found in spiders where more extensive structural and physiological studies indicate they are chemoreceptors that may be sensitive to water (Foelix and Chu-Wang 1973; Dumpert 1978). Abushama (1964) demonstrated that sensitivity to humid air in the scorpion *Leiurus quinquestriatus* was destroyed by cutting off the terminal (tarsal) leg segments. This would suggest that sensory structures on the tarsus are necessary in water detection and that the pectines are not sufficient for of triggering this mode of water-orientating behavior.

In this study we have examined the behavioral responses of *P. mesaensis* as they encountered localized areas of water-dampened sand in a test arena. We describe several stereotyped behaviors stimulated by substrate water and use electrophysiological procedures to determine which of the tarsal sensory organs are responsive to water and humid air.

MATERIALS AND METHODS

The animals used in this study were adult male and female *Paruroctonus mesaensis* collected from the Mojave Desert near Indio, California in San Bernardino County. They were housed in an environmental chamber (27°C; 15:9 L/D) in individual containers of clear plastic containing sand from the site of capture and fed biweekly with wax worm larvae (Northern Bait Co., Chetek, WI).

All behavioral observations were recorded by a video camera (Sony V9, 8 mm format) using ultraviolet light (15 W fluorescent "black lights") to illuminate the test site; scorpion cuticle fluoresces bright yellow-green under UV illumination giving sharp video images of their movements in the dark without noticeably disrupting their behavior. All trials were conducted in cylindrical arenas of clear plexiglas (13.5 cm in diameter, 8 cm in height) placed over natural desert sand that had been cleaned by baking (220°C, 2 h) after each use. For 15 min prior to the beginning of each trial each test animal was confined to one quadrant (randomly determined) of an arena by an opaque partition. At the end of this adjustment period a stimulus substance (0.5 ml water or paraffin oil) was placed in the quadrant opposite the scorpion and the partition removed. All trials were carried out in a darkened room and the camera and arenas were enclosed in a black felt tent to reduce visual cues for orientation. Trials were recorded four at a time, and the tapes were subsequently reviewed and scored by an observer

unaware of the experimental treatments for each trial. A trial ended after 2 h or after an animal had occupied the quadrant for 3 min (cumulative time).

Important contact chemoreceptor organs for *P. mesaensis* appear to be the pectines and a dispersed group of chemosensory hairs on the ventral and lateral surfaces of each tarsus. The numbers of ventro-lateral chemosensory hairs vary between 23 (1st leg tarsi) to 14 (4th leg tarsi) in accordance with anterior-posterior gradient first observed by Foelix and Schabronath (1983) for two species of buthid scorpions. Additionally, each tarsus contains a single "tarsal organ" on its dorsal surface, which does not contact the substrate directly, but may be sensitive to water vapor (Foelix and Chu-Wang 1973; Foelix and Schabronath 1983). Using various combinations of shrink tubing, low-melting temperature wax and organic adhesives it was possible to selectively block chemosensory function of the pectines, the entire tarsus or the tarsal organs, but the tarsal hairs were too numerous and small to be selectively ablated in this fashion.

For ablation experiments, behaviors of experimental (sense organs covered) and control (unaffected) animals were scored each time they crossed the site of water application. Scores were assigned as follows: 0, no behavior change; 1, altered behavior (non-specific); 2, altered behavior (water-specific). A trial ended after 2 h or 10 crossings of the stimulus application site unless a score of "2" was achieved in which case the trial was terminated immediately. A minimum of 2 crossings were required for a

legitimate trial. For each trial the cumulative score for an animal was divided by the number of crossings it made of the treated site. This "response index" for individuals was rank-ordered and differences between experimental and control groups were tested for significance by Mann-Whitney analysis.

For electrophysiological investigations, scorpions of either sex were paralyzed by cooling and immobilized in a recording chamber using wax. Electrolytically-sharpened tungsten electrodes were inserted at the tip of the tarsus (indifferent electrode) and through the cuticle of the tarsal organ (Fig. 3.5) or at the base of single chemosensory hairs on the tarsus (recording electrodes). Alternatively, saline filled (10-150 mM NaCl) glass electrodes slipped over the tips of individual hairs (Fig. 3.4) were used to detect the presence of water and/or salt sensitive units within these structures. Electrical signals were amplified and stored on audio cassette tapes for subsequent playback and computer analysis (TSG software, Gaffin and Jubran 1991).

Scanning electron microscopy was of tarsal cuticle (unfixed) lightly coated with gold and viewed at 7 kV in an AmRay 1000A SEM. In most instances the cuticle surface was cleaned by applying a thin layer of polyethylene film (Hobby Technik, D-7850 Lörrach, Germany) and stripping-off the hardened shell once it dried.

RESULTS

Behavioral responses to water

In its natural environment and in captivity, *P. mesaensis* is a nocturnal animal showing peak locomotory activity from 1 to 5 h after sunset (Polis 1980). In the course of these studies we found that animals tested in the late afternoon to early evening (1700 h to 2100 h, PST) were less responsive to water stimulation than animals tested after 2100 (27.8% vs 63.6% of total normal control animals tested during these times, respectively). Nevertheless, significant changes in behavior could be observed at all times.

For the initial description of water stimulated behavior, ten discrete activities were monitored as each test animal was released from confinement in the test arena. Animals were observed to walk-through (W) a test quadrant without showing overt changes in behavior, or to pause (P) locomotion for less than 2 min or stop (S) for longer time in an alert, standing posture; stops with the body resting on the substrate, usually with the post-abdominal segments and legs retracted, were scored as rests (R). Turns (T) to the left or right, backing-up (B) and wall-climbing (C) were other locomotory behaviors monitored during the test period. When responsive animals encountered dampened sand they displayed several stationary behaviors: headstands (H), where the pre- and post-abdomen were elevated as the chelicera and pre-oral cavity were pressed to the substrate (Fig. 3.2A); pedipalp-pull (PP), where sand is pulled toward the mouth by

the pedipalps (Fig. 3.2B); and rototiller-digging (RD), where rotary movements of the first three leg pairs scrape surface sand away.

As shown in Fig. 3.1, five of the ten behaviors we scored during these experiments were only observed when animals encountered water-dampened sand (n=18); these behaviors (PP,H,RD,S and B) were not observed in control trials (n=6) or trials where non-aqueous material (paraffin oil) was used to wet the sand (n=9). Three of these behaviors (PP,H,S) appear to be strictly related to water stimulation while two behaviors (B,RD) have also been observed when animals encounter pheromonal stimuli on the substrate (Brownell 1988; Gaffin and Brownell 1992a,b).

Water responsive animals showed a stereotypical sequence of behaviors when they encountered a small spot of dampened sand. This was characterized by cessation of forward locomotion and initiation of Turning or Backing-up until the chelicerae were directly over the moist sand. At this point the animal would Stop, in some instances for more than 1 h, or immediately display Pedipalp-pulls and Headstand behaviors, often in succession. The PP/H sequence was sometimes interrupted by Backing-up or Rototiller-digging.

Localization of water receptors

Since the behavioral responses to water appear to require direct contact with dampened substrate, we conducted a series of behavioral tests on

animals with blocked chemosensory organs. As shown in Table 3.1, blocking of pectinal input had little to no effect on water-sensing behavior; these animals continued to orient to damp substrates and to display one or more of the water-specific behaviors (PP,H,S). Selective blocking of the tarsal organ also had no apparent effect on the threshold or expression of these behaviors. Selective ablation or blocking of tarsal chemosensory hairs was not practical, although covering all chemosensory structures on the tarsi significantly altered water sensitivity without interfering with normal ambulation and searching behavior. The unique importance of tarsi for water detection was confirmed by retrospective examination of video-taped records of responding animals; tarsal contact with the dampened substrate nearly always preceded initiation of water-specific behaviors in these trials.

Anatomy of tarsal chemoreceptors

To further localize and identify the tarsal receptors involved in water detection, we used scanning electron microscopy (SEM) to characterize their gross structure and standard electrophysiological procedures to record unitary sensory responses from chemosensory hairs and the tarsal organ. Individual hairs were approximately 100 μm long and 7 μm in diameter at their base and protruded from a cup-shaped socket approximately 25 μm in diameter; most possessed a distinct 30°-90° bend in their shaft near the tip

(Fig. 3.3A,B). SEM inspection clearly showed each of these hairs contained a single, terminal pore approximately 0.1 μm in diameter (Fig. 3.3C). Tarsal organs were readily identified as indentations (10 x 30 μm) of the dorsal tarsal cuticle with two morphologically distinct pores spaced approximately 20 μm apart (Fig. 3.3C,D). The larger of the pair (Fig. 3.3D) has a 4 μm diameter opening similar to wax-secreting ducts of other scorpions (Hadley, pers comm). The second pore consisted of two "D-shaped" openings, each approx 1 μm diameter, that were occluded internally (Fig. 3.3E). By appearance, these structures appear to be specialized cuticular sensilla and, thus, the source of the physiological recordings discussed below.

Electrophysiology

The small, curved hairs on the ventral surface of the tarsi contain both mechano- and chemosensory neurons. Extracellular recordings with metal electrodes inserted at the base of these hairs showed each contained at least one unit responsive to deflections of the hair shaft, but unit responses to aqueous solutions applied to the hair tip were not well resolved in this recording configuration. Chemosensory units were more clearly seen when glass electrodes containing dilute saline solutions were slipped over the hair tip (Fig. 3.4). Figure 3.4B shows the initial seconds of spiking activity after a recording electrode containing 150 mM NaCl made contact with a hair tip.

At least two distinguishable units were transiently excited within the first 0.5 s of contact (lower trace) while other units showed more delayed and sustained responses (upper trace, Fig. 3.4B). Mechanosensory units in recordings from the hair tip were easily triggered and distinguished from the chemosensory responses by deflecting the hair shaft side to side with the recording electrode. Tip recordings using saline of lower ionic strength (10, 50, and 100 mM NaCl) gave similar responses indicating that water as it occurs naturally on dampened sand would excite these units.

Extracellular recordings from the tarsal organ (Fig. 3.5) were obtained by impaling the cuticle near this depression with a bluntly-tapered metal electrode. Sensory neurons in the tarsal organ were notably silent in the absence of stimulation which made it difficult to position the recording electrode. When properly positioned, at least three units (Fig. 3.5B) were highly responsive to humid air stimuli presented either as moist breath blown over the organ or as a moist cotton swab brought to within 1 cm of the tarsus. Spike activity of these units adapted quickly to sustained stimulation (Fig. 3.5C, top trace) and recovered sensitivity rapidly between presentations (Fig. 3.5C, bottom trace) indicating this is a phasic receptor. These neurons were also strongly excited by direct application of water to the tarsal organ pit even though this mode of stimulation probably does not occur when the tarsus comes in contact with dampened sand substrates.

DISCUSSION

Our results indicate that point sources of dampened sand release a series of stereotyped behaviors in *P. mesaensis* directed toward localization and imbibition of substrate-associated water. Selective masking of candidate water receptors gave behavioral evidence that chemosensory hairs on tarsal leg segments are the most important sensory structures for detection of substrate moisture. Morphological and physiological analysis of these sensilla further support our conclusion that the robust behaviors associated with water localization and uptake are triggered by this relatively simple and localized sensory system.

Like many desert arthropods, *P. mesaensis* is under chronic water stress in its natural dune environment (Hadley 1970). Although standing surface water or dampness rarely occurs on sandy substrates, behaviors that increase the likelihood of finding and utilizing ingestible water may have adaptive value for animals that encounter infrequent rainstorms. Hadley's (1971) field observations of *Centruroides sculpturatus* show it is capable of imbibition of standing water and Polis and Seely (1990) have observed *Parabuthus villosus* drinking precipitated fog in the Namib Desert. In the current study the stereotyped Headstand and Pedipalp-pulling behaviors were clearly discernable attempts to bring dampened sand to the vicinity of the mouth.

In addition to detection of substrate water, our electrophysiological investigations of *P. mesaensis* indicated the tarsal organs are very sensitive to water vapor and may mediate orientation to high-humidity environments. In this regard, it is noteworthy that the burrows of *P. mesaensis* maintain a steep humidity gradient ranging from almost 0% near their entrances to near saturation 30-50 cm below the sand surface (Edney et al. 1974; Polis and Farley 1980). Previous studies by Abushama (1964) on *Leiurus quinquestriatus* show that these animals were capable of orienting to humidity gradients and that ablation of the tarsi on all legs destroys this sensitivity. Stahnke (1966) observed that ground scorpions living in desert washes follow the receding moisture line as summer progresses, indicating the preference for higher humidity microclimates is expressed behaviorally in the field. These results indicate the tarsal organs are likely to be essential for such detection.

The location of chemosensory hairs on the ventral surfaces of the tarsi is appropriate for detection of substrate moisture and close examination of the behavioral responses reported here confirm this view. In one instance we observed a responding animal as it contacted a small spot (50 μl) of water with its second right leg. This animal immediately turned to the right, found the precise location of the moisture, and began performing Pedipalp-pulls. The location and size of tarsal chemoreceptive sensilla in *P. mesaensis* were similar to that described by Foelix and Schabronath (1983) for two species of

buthid scorpions although *P. mesaensis* had fewer sensilla per tarsus and gradation in number between first and fourth leg pairs was not as steep as in the buthids. It remains to be determined, however, if functional and/or morphological differences exist among these hairs as described for chemosensory hairs of insect tarsi (Dethier 1976). The buthid chemosensory hairs are innervated by 22-23 bipolar neurons, four of which terminate at the hair base in a manner typical of mechanoreceptor cells (Foelix and Schabronath 1983). Our electrophysiological investigations are consistent with this arrangement since recordings made from the base of individual hairs showed units distinctly responsive to deflection of the hair shaft while tip recordings with saline-filled electrodes did not show large-amplitude mechanoreceptor responses. Analysis of extracellular action potential wave-forms for water responsive units revealed three or more distinguishable units in tip recordings, many fewer than the number believed to innervate individual hairs. Other neurons in these sensilla are not spontaneously active and may require more specific stimuli for activation.

The dorsally-located tarsal organ does not come in direct contact with the substrate although its proximity to the surface and high sensitivity to humid air clearly leave open the possibility of indirect detection of substrate moisture. Foelix and Schabronath (1983) describe the organ in buthid scorpions as a two-pored structure of unknown function situated inside a small oval depression of the cuticle (16 x 22 μm). We found a similar

depression in the dorsal tarsal cuticle of *P. mesaensis* and a double-pored opening (approx 1 x 3 μm) of unique appearance. This organ is most likely to be the source of hygrosensitivity detected in our physiological recordings. Direct contact of these pores with water (saline-filled electrodes as used in hair-tip recordings) produced vigorous multi-unit responses with summed spiking frequencies of approximately 180 nerve impulses/s for the first 200 ms which abruptly decreased to about 50 impulses/s for the following few seconds. Though such direct stimulation of the tarsal organ is unlikely to occur in freely behaving animals, the number of discrete units seen following strong stimulation of this kind is consistent with morphological description of 6 to 9 sensory neurons per pore (Foelix and Schabronath 1983).

Reversible blocking of the most elaborate chemosensory structure of scorpions, the pectines, had little to no observable effect on their water-sensing behavior. However, normal animals were observed to repeatedly sweep dampened areas with their pectines suggesting these mid-ventral appendages may be used secondarily to assay moisture content of the sand once it has been detected by the tarsi. Electrophysiological studies of individual chemoreceptive sensilla on the pectines are consistent with this view (Brownell 1988,1989; Gaffin & Brownell 1990).

Thus, the sand scorpion, *P. mesaensis*, appears to have several independent sensory channels capable of detecting substrate-associated water and humid air. The tarsal chemosensory hairs are a small and particularly

interesting subset of these receptors in that they are highly effective triggers for stereotyped behaviors associated with imbibition. Future neuroethological analysis of this sensory-motor association may reveal the integrative processes that direct and coordinate water-orienting behavior in this animal.

Table 3.1 Occurrence of water-stimulated behavior (Response Index) in scorpions with normal (unblocked) and selectively disabled sense organs (blocked pectines, tarsi or tarsal organs).

Treatment	n	Average Response Index (mean ± SE)	Z-value[1]
Unblocked (controls)	32	0.65 ± 0.11	-
Pectines blocked	7	0.47 ± 0.26	0.231
Tarsi blocked	14	0.25 ± 0.08	0.023
Tarsal organs blocked	11	0.49 ± 0.20	0.265

[1] From Mann-Whitney test comparing experimental and control groups.

Figure 3.1 Behavioral responses of *P. mesaensis* to dry sand (n=6) and sand moistened with water (n=18) or paraffin oil (n=9). For each treatment group, histogram bars show percentage of animals displaying each of ten behaviors: Walk-through (W); Pause (P); Turn (T), Pedipalp-pull (PP); Rototiller-digging (RD); Stop (S), Headstand (H); Wall-climbing (C), Backing-up (B); Rest (R).

Figure 3.1

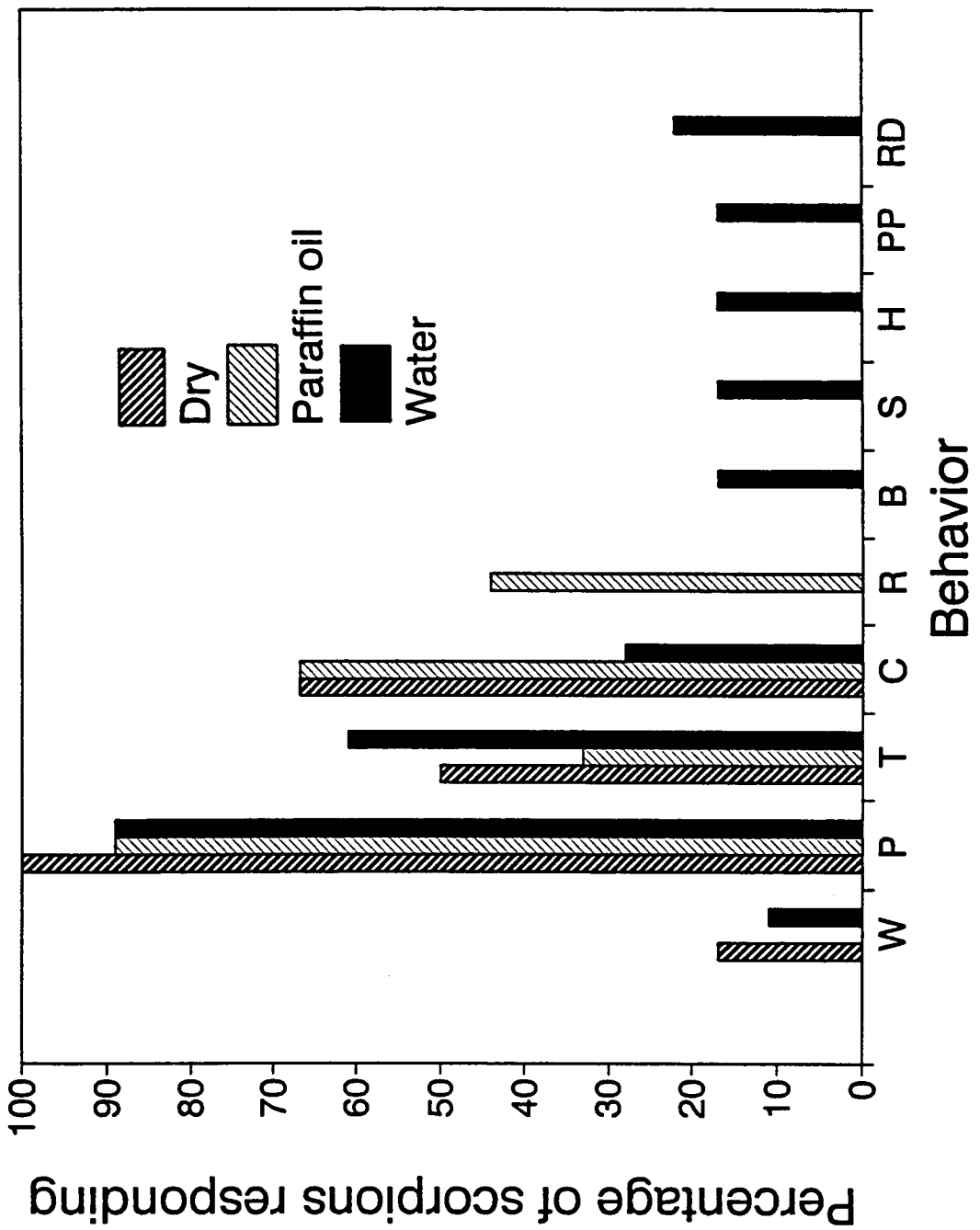

Percentage of scorpions responding
100
90
80
70
60
50
40
30
20
10
0
Dry
Paraffin oil
Water
W
P
T
C
R
B
S
H
PP
RD
Behavior

Figure 3.2 Behaviors associated with water detection in sand scorpions. **A** In "Headstand" behavior, scorpions elevate posterior body segments and tail above the substrate, pressing the pre-oral cavity into the sand while chewing it with the chelicera. **B** In "Pedipalp-pull" behavior the pedipalps sweep surface sand toward mouth.

Figure 3.2

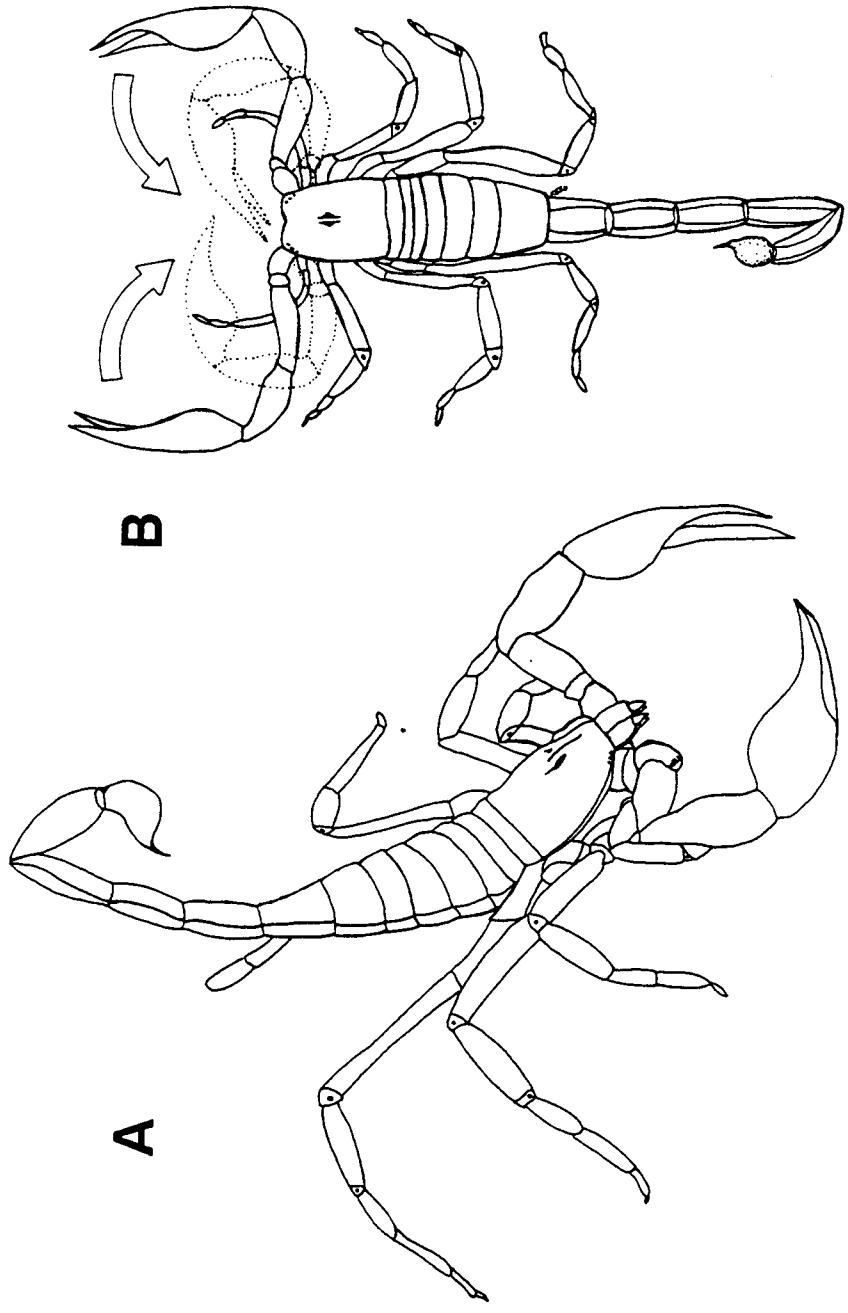


Figure 3.3 Scanning electron micrographs of tarsal chemosensory sensilla in *P. mesaensis*. **A** Tarsus leg 2, ventral view. **B** Chemosensory hair sensillum (arrow in A) showing characteristic curve in hair shaft and single terminal pore (**C**). **D** Tarsus leg 2, dorsal view. **E** Expanded view of tarsal organ (arrow in D) showing two distinct pore morphologies. The larger pore, possibly a wax secreting duct, appears open while the second double-pored opening (**F**) has fine structure of a chemosensory pore. c, chemosensory hairs; to, tarsal organ; m, mechanosensory hairs; bt, basitarsus; b, bristle hairs; cl, claws

Figure 3.3

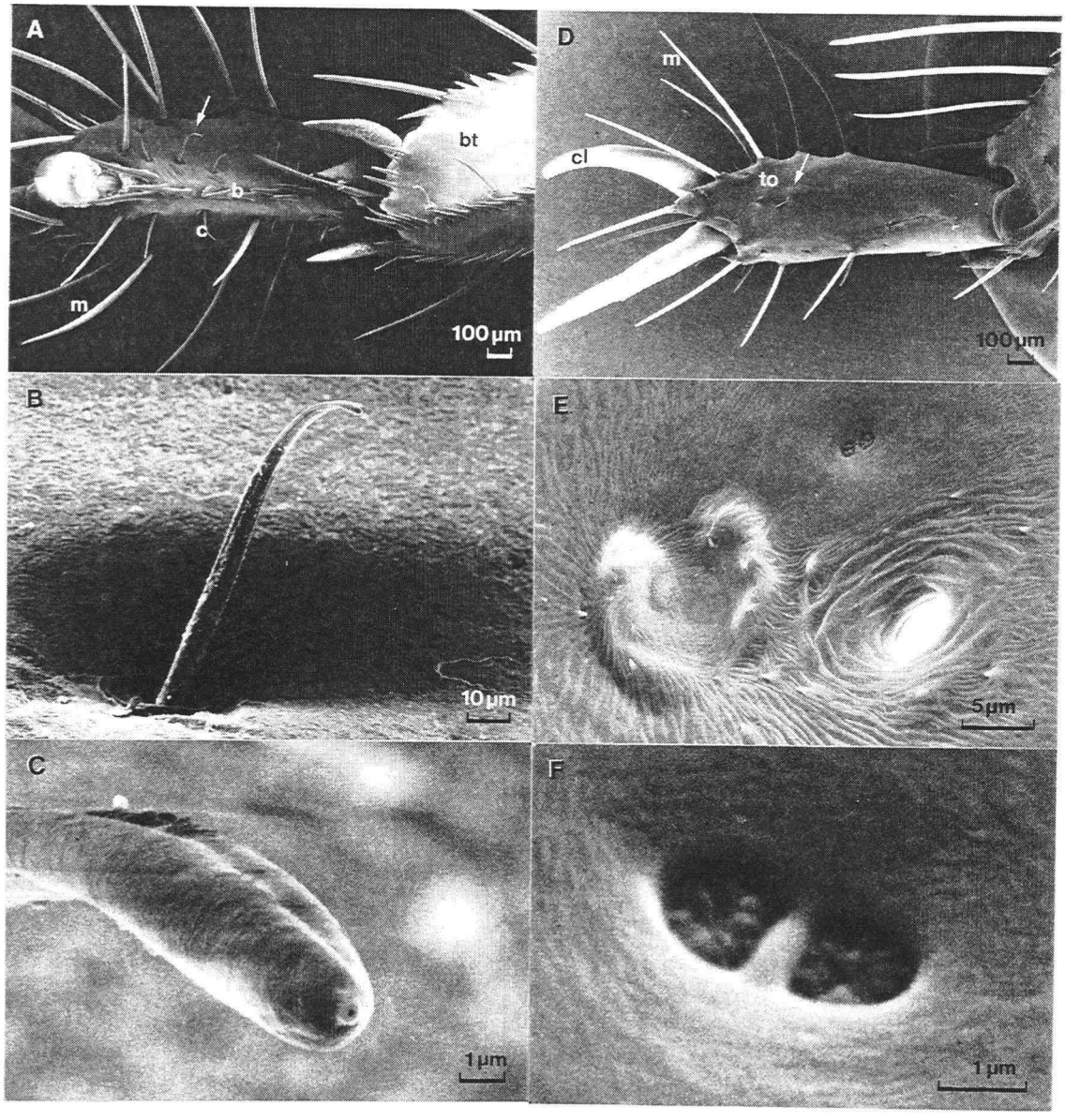


Figure 3.4 Electrophysiological response of tarsal chemosensory hairs. **A** Ventral-posterior view of left first tarsus showing location of chemosensory hairs (c) and placement of single hair within bore of saline-filled recording pipette. m, mechanosensory hairs; bt, basitarsus; b, bristle hairs. **B** Initial sensory response of sensory hair to contact with 150 mM NaCl solution in recording pipette. Top trace shows first 3 s of electrical activity; bracket indicates initial 0.5 s of response expanded and displayed in bottom trace. Artifact at beginning marks time of stimulus application. A fast adapting (<200 ms) unit of large amplitude can be seen in lower trace along with brief activity of a smaller unit (indicated by dots). A third unit, showing a tonic pattern of excitation, becomes active 0.5 s after stimulus application.

Figure 3.4

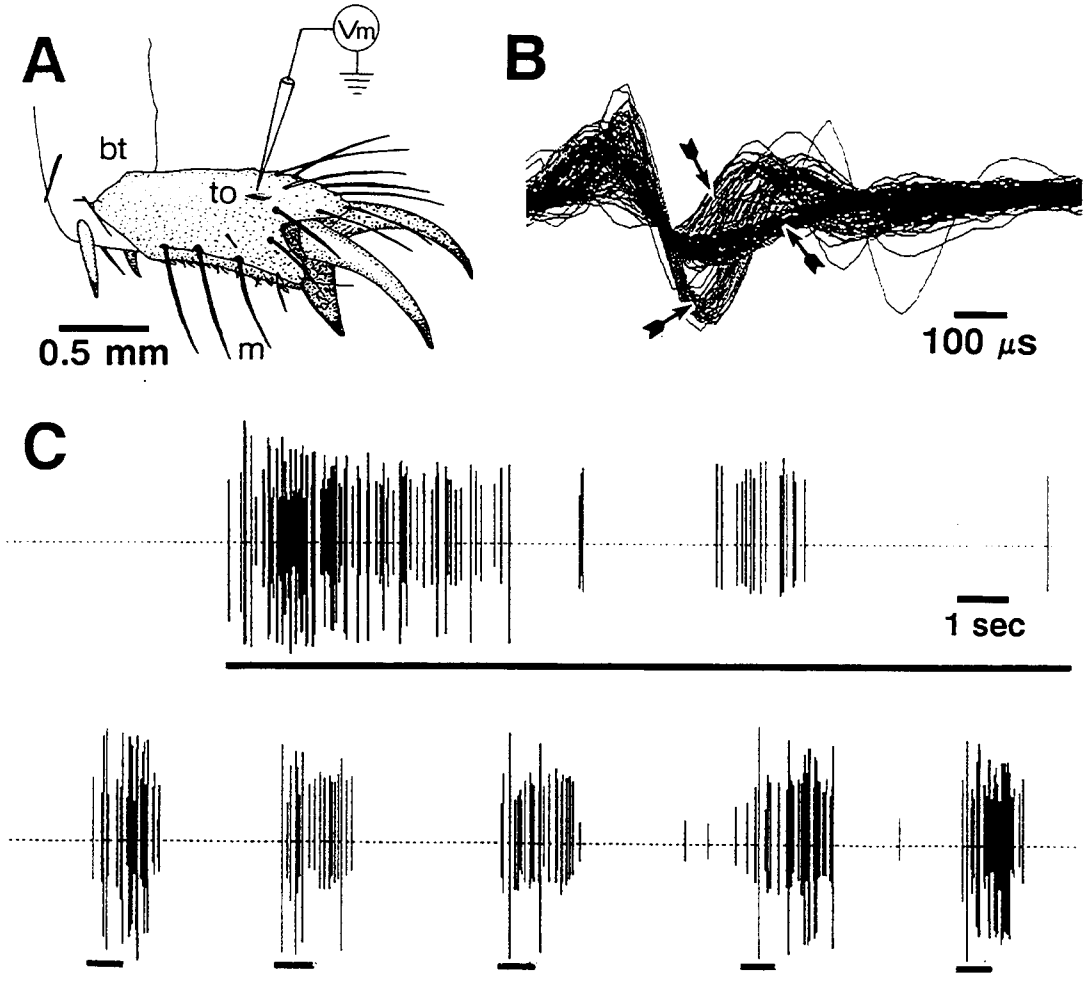
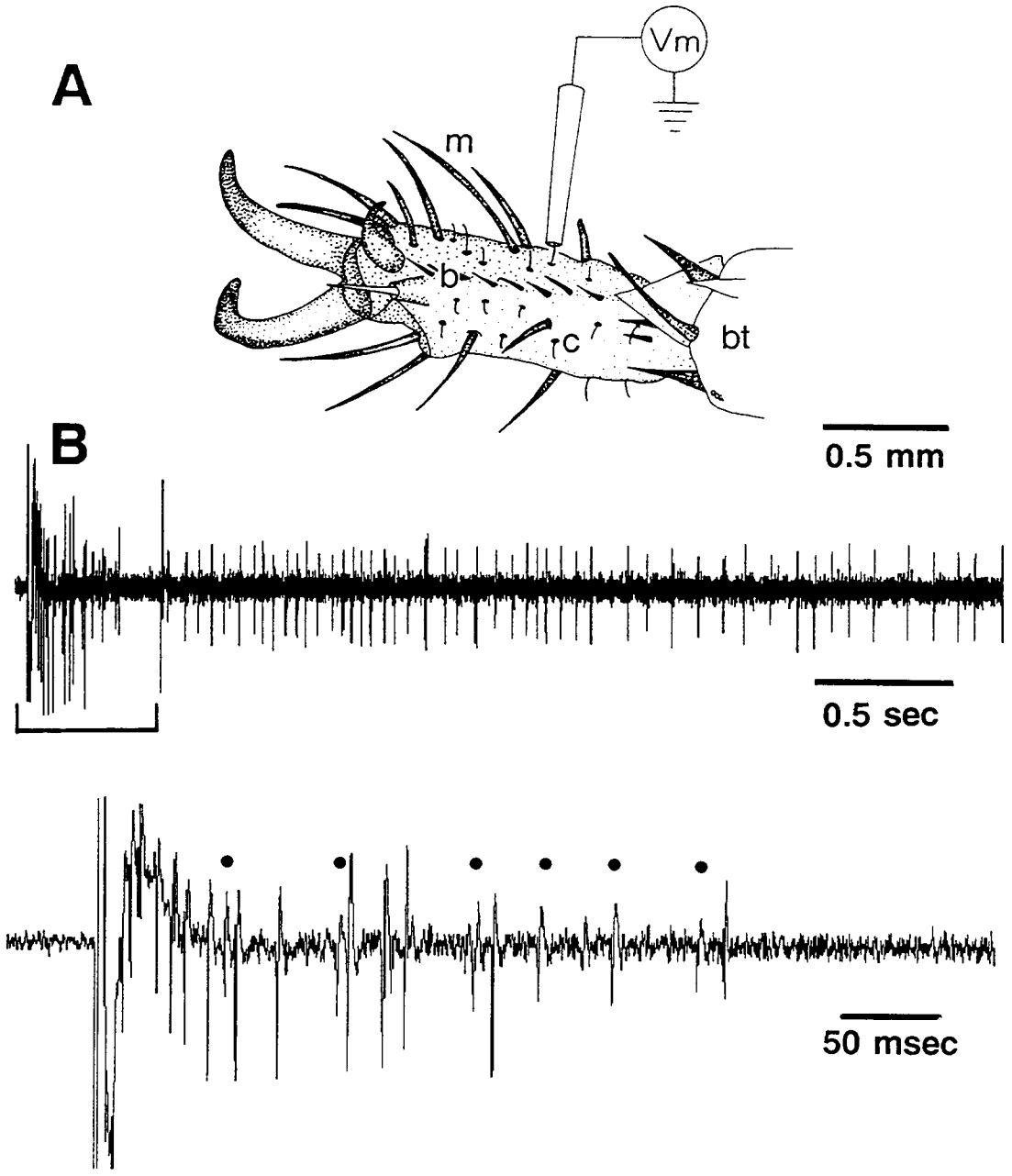


Figure 3.5 Electrophysiological responses of tarsal organ stimulated by humid air. **A** Dorsal-posterior view of right second tarsus showing placement of metal electrode through cuticle near tarsal organ (to). m, mechanosensory hair; bt basitarsus. **B** Superimposed spike wave forms recorded from tarsal organ during stimulation with humid air. Arrows indicate the presence of at least three different spike classes within these records. **C** Computer filtered records (20 s duration) of spiking activity of tarsal organ to near-range water stimulation. Top trace: Water on tip of a paper swab held stationary 1 cm from the tarsal organ. Bottom Trace: Water on swab tip brought within 1 cm of organ, withdrawn, and returned for five repetitions. Black bars indicate approximate duration of stimulus.

Figure 3.5



CHAPTER 4

CHEMOSENSORY RESPONSE OF PEG SENSILLA ON PECTINES OF THE DESERT SAND SCORPION

ABSTRACT

Behavioral and anatomical studies indicate that the pectines of scorpions have both chemical and mechanosensory functions. The elementary sensory units on these comb-like, midventral appendages are the tens of thousands of minute, peg-shaped sensilla arranged in dense, 2-dimensional arrays on the ventral surface of these organs. In this study we used extracellular recording techniques to examine spontaneous and stimulated activity of sensory units within individual peg sensilla on scorpion pectines. Long-term recordings (up to several days) showed a diurnal rhythm in the spontaneous spiking frequency of units in peg sensilla. Several types of units were identified by their stereotypical waveforms. Near-range olfactory stimulation by volatile alcohols, aldehydes, ketones, esters, and carboxylic acids produced distinguishable, dose-dependent patterns of response in specific sensillar units. Responses were also seen in contact stimulation by chemicals, including water, and mechanical deflection of the peg tip. This study represents the first physiological evidence that peg sensilla on scorpion pectines are broadly sensitive to odorants and tastants applied near or on the sensillum tip. These findings are consistent with the hypothesis that

pectines are major chemosensory appendages of scorpions and the functional equivalent of antennae in mandibulate arthropods.

INTRODUCTION

The pectines are bilateral appendages extending ventrally from the ninth body segment of all scorpions. Although the existence of these organs can be traced in the fossil record to aquatic scorpions in the Devonian (Kjellesvig-Waering 1986), their functional significance has been enigmatic (see Cloudsley-Thompson 1955 for early references). These comblike appendages are composed of a flexible spine and a series of movable, ground-directed teeth. The most conspicuous sensory feature of the pectines are the 10^3-10^5 (species-dependent) peg-shaped sensilla on the distal surfaces of the pectinal teeth (Schröder 1908; Carthy 1966; Swoveland 1978; Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983; Brownell and Locket unpubl.). These dense peg arrays appear to make contact with the substrate as the animal periodically taps its pectines against the ground (Brownell and Gaffin unpubl.).

Structurally, the peg sensilla of scorpions have features in common with contact chemosensory sensilla of insects (Slifer 1970). Individual pegs are cylindrical structures, approximately 1 μm in diameter (at their base) and 2-5 μm in length, depending on species. Each peg is composed of a double-walled cuticular shaft that protrudes from a circular socket in the surface of the tooth and terminates in a broad and flat tip. A single, slit-shaped pore is located at the distal end of each peg. This minute opening connects the external environment to a fluid-filled chamber inside the sensillum. The

dendritic outer segments of several (11-18) bipolar sensory neurons extend into this sensillar chamber within a few microns of the slit opening (Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983; Brownell unpubl.). An additional cell terminates near the peg base and possesses a tubular body characteristic of arthropod mechanosensory cells (McIver 1975; Foelix and Müller-Vorholt 1983).

Behavioral evidence supports the morphological interpretation of a dual mechanosensory / chemosensory function for the pectines. Several studies imply that the pectines are used as discriminators of surface texture (Abushama 1964; Carthy 1966, 1968; Boyden 1978), particularly by males during location of sites for spermatophore deposition (Alexander 1957,1959). Krapf (1986) showed the pectines respond to general chemostimulants in the detection of food items and the relocation of stung prey. Evidence for detection of substrate-borne pheromones by male pectines was presented in Chapter 2.

Prior to the work presented in this chapter, the chemosensory role of scorpion pectines had not been demonstrated physiologically. In the only previous electrophysiological report to appear, Hoffmann (1964) showed that units in individual peg sensilla on *Euscorpius carpathicus* and *E. italicus* (Chactidae) responded to mechanical deflection of the peg tip, but not to various organic chemicals applied to the sensillum. In this study we used electrophysiological techniques to examine the response characteristics of

cells in pectinal peg sensilla of scorpions. We demonstrate for the first time that neurons in these sensilla are responsive to chemical stimulation and confirm the presence of at least one mechanosensory unit in each peg. Preliminary reports of some of these findings have appeared as abstracts (Gaffin and Brownell 1990, 1992a).

MATERIALS AND METHODS

Animals

Male and female *P. mesaensis* collected during summers of 1989 and 1990 from sandy regions of the Mojave Desert near Indio, CA were the subjects of these experiments. Only animals judged to be sexually mature (i.e. instar stage 5 or older) were used (Stahnke, 1970). Animals were kept individually in plastic containers and maintained on a diet of grasshoppers and wax worms. In all, the results presented in this chapter and the next are based on more than 300 h of recording time from 54 peg sensilla on 11 *P. mesaensis* (8 ♂, 3 ♀).

Electrophysiology

Scorpions were anesthetized by cooling and immobilized ventral side up to a rigid plexiglass stage using a mixture of carnauba and bee's wax. The pectines were further secured on a metal platform (20 mm x 5 mm x 0.5 mm) using double sided tape; the teeth were arranged for maximal exposure of the peg sensilla. Extracellular recordings were made using electrolytically (1 M $NaNO_3$) sharpened tungsten wires (17 μm diam., tips sharpened to < 0.5 μm) inserted in the flexible cuticle at the base of single peg sensilla (Fig. 4.1A); a tungsten reference electrode was placed in contact with hemolymph, usually through soft cuticle at the distal end of the pectinal spine. Impaled sensilla were allowed to recover for 15 min before delivery of the first

stimulus. Recorded signals were amplified 100 x (WPI Model DAM-5A) and displayed on an oscilloscope (Tektronix Model 5115). Chemosensory responses were stored as 1 min records (15 s pre-stimulus baseline, 45 s post-stimulus response) on audio magnetic tape for subsequent playback (Sony Cassette Recorder TC-W320), digitization and analysis.

Long-term fluctuations in spontaneous spike activity were automatically monitored by computer for periods of several hours to several days. Samples (10 s and 20 s duration) were obtained directly from recorded sensilla at 20 min intervals; samples obtained from the same sensillum and hour on successive days were averaged to obtain baseline spiking frequency per hour per animal.

Stimuli

General chemosensitivity

Volatile organic compounds, known to elicit responses in other arthropod chemoreceptors, were presented as puffs of air to test the sensitivity of peg sensilla to odorants. Stimuli were delivered via an olfactometer (Kafka 1970) where the 1 ml of stimulus was contained within a 10 ml vial inside a 20 ml syringe (volume of stimulus pulse was 10 ml). Individual stimulus syringes were re-equilibrated for at least 80 s. The syringe nozzle (3.0 mm diam.) was positioned about 5 mm from the tip of the impaled sensillum. Due to the design of the recording platform and the

orientation of stimulus syringe, only sensilla on middle to distal teeth of the right pectine were used. The air stream velocity was approximately 3 m/s, stimulus duration approximately 1 s. An exhaust fan continually flushed clean air over the preparation. Experiments were performed at room temperature (21-28°C). A complete list of compounds applied during these studies is given in Table 4.1.

Response variability in single peg sensilla

Response patterns inherent to individual peg sensilla were studied by stimulating with C6-C10 n-alkanes, n-alcohols, n-aldehydes, n-esters, and ketones. Three additional ketones ((+)-fenchon, (+)-carvon, and α-ionon) were also tested. Substances were used pure and/or diluted in paraffin oil (Merck DAB 7) to log molar concentrations ranging in half steps from 0.5 to -3.0. Stimuli were presented at three minute intervals in the following log molar concentration order: -1.0, -3.0, -0.5, -2.5, 0.0, -2.0, 0.5, -1.5, pure. Triplicate applications were given for concentration series of alcohols and aldehydes; duplicate applications were given for straight-chained ketone series. Alkanes, esters and ring-structured ketones were presented only at pure and 1 M concentrations (single application). A pure paraffin oil control was given after every fifth stimulus to monitor fluctuations in baseline spiking frequency.

Contact chemosensitivity and mechanical stimulation

Water stimulus was reversibly applied to the tips of individual sensilla by immersing the pectine in paraffin oil and extruding a droplet from a glass micropipette until it contacted the recorded sensillum. The stimulus was removed by retracting the droplet back into the syringe. Recordings made in this manner were stable and allowed for precise manipulation of the stimulus. An electrical contact artifact marked stimulus onset and removal.

Chemical stimuli miscible in paraffin oil were applied in air directly to the peg tip by a micropipette in order to demonstrate contact chemosensitivity of peg sensilla. Occasionally, clean, reversible applications of stimuli were achieved (e.g. Fig. 4.5b), but difficulties in control of stimulus application and removal precluded its use as an effective means for assaying gross sensillar sensitivities.

For mechanical stimulation of pegs we delivered puffs of air from a pasteur pipette mounted within 1 mm of the peg tip. Although not a quantifiable method, this means of stimulation produced minimal stimulus artifact and selectively evoked mechanosensory unit responses.

Spike identification and analysis

Discrimination of sensory units in our recordings required high-resolution monitoring of spike activity. For this we digitized electrical recordings of sensillar responses through an IBM DACA analog-to-digital

converter (settling time approx. 30 μs) and displayed on the computer screen. TSG ("Turbo Spike Grabber", Gaffin and Jubran 1991) software was used to identify and group spikes of similar waveforms from multi-unit recordings (Fig 4.1B). In this program all spike events exceeding an adjustable threshold voltage are stored to disk along with their time of occurrence in the recording. The digitized waveforms of each event are then redisplayed at high sweep speed for automatic (waveform template matching algorithm) or interactive-manual sorting into discrete classes. The activity of each identified spike type is reconstructed in separate traces so that its activity over time relative to the stimulus can be easily visualized (Fig 4.1B).

RESULTS

General electrical characteristics of peg sensilla

The peg sensilla of *P. mesaensis* were numerous and easily penetrated by electrodes for extracellular recording. Although each sensillum is small (approx. 2 μm, base diam.), the sleeve-like structure of the peg socket facilitated penetration of sharpened metal electrodes through flexible cuticle at the peg base. Morphologically, each peg sensillum contains about 14 sensory neurons (Brownell 1989), but only two or three these were spontaneously active at low frequencies (combined firing rate approx. 1 - 2 Hz). Extracellular action potentials were approximately 1-2 mV in amplitude with signal to noise ratio >10 for the largest amplitude spikes. Recordings made through the peg base were stable, on average, several hours and facilitated by sealing of cuticle around the electrode. In this regard, tungsten electrodes secured better than glass electrodes and were preferred for long-term recordings of these experiments. On occasion these recordings were stable for several days, showing no appreciable degeneration of signal form. Most preparations were reusable for several days thereby allowing extended experimentation on pectines from individual animals.

Identification of sensory units in peg sensilla

In recordings of spontaneous electrical activity from peg sensilla of *P. mesaensis*, three distinct types of spiking units were typically observed. Two

of these units, spike types 'A1' and 'A2' (Fig. 4.2A,B), gave large-amplitude, biphasic impulses that were generally distinguishable by waveform. In baseline recordings where units 'A1' and 'A2' were coactive, one unit ('A1') was generally more active by at least 2-fold, thus providing a basis for identification. A third identifiable unit, type 'B' cells, had smaller amplitude and a characteristic triphasic waveform (Fig. 4.2A,B). 'B' units discharged spontaneously at low frequency (typically one-tenth the frequency 'A2') and displayed some burstiness in its pattern of activity. Peak firing frequency of 'B' spikes was approximately 7 - 8 hz, even when maximally excited by chemical stimuli (e.g. pure hexanal).

Two other units frequently observed were excited by mechanical deflection of the sensillum ('M' type units) or particular chemical stimuli ('C' type units). Spikes of type 'C' were characterized by waveforms of smaller and more variable amplitude (< 1 mV), which were commonly difficult to observe above background electrical noise. These spikes fired at high frequencies (up to 30 Hz in hexanol stimulation) in bursts of short duration (approx. 0.5 s), bursts during or toward the end of stimulus application. The waveforms of type 'M' spikes had larger negative-phase amplitudes than other observed waveforms and their positive-phase potentials were longer lasting. On rare occasions, activity of a second unit with waveform similar to type 'M' was induced during mechanical stimulation, raising the possibility that another neuron in the peg is capable of responding to this modality.

Other spike waveforms were occasionally observed in our recordings and three of these occurred frequently enough (> 10 observations within a single sensillum preparation) to be identified by waveform and pattern of activity. Spike type 'X' (Fig. 4.2C,D) had the largest peak-to-peak amplitude (approx. 3 mV) of any spike recorded in this study. This spike occurred very rarely and did not appear to be coupled to any of our test stimuli. Spike types 'Y' and 'Z' (Fig. 4.2C,D) occurred together in one recording as a single, high frequency burst. As with spike type 'X', the causal stimulus was unknown. The shapes of these two spike types were uniquely characterized by the initial positive phase of their waveforms, with spike type 'Y' having a larger amplitude than spike type 'Z'. Although pectines have muscle fibers, the waveforms described here were distinct from the larger (>5 mV) and longer duration (> 20 ms) discharges characteristic of motor units.

Diurnal fluctuation in electrical activity of peg sensilla

Electrical recordings from the base of individual peg sensilla were sufficiently stable to permit monitoring of spike activity over several days. Figure 4.3 indicates that spontaneous electrical activity of peg sensilla increased steadily during the four-hour period between 1800 h and 2200 h and decreased by a commensurate amount between 2200 h and 0200 h (n = 4; 3 ♂, 1 ♀). A similar fluctuation in activity with time of night was observed when sensilla were stimulated with paraffin oil controls (via

olfactometer) during assays of chemosensitivity. It is noteworthy that *P. mesaensis* are normally most active beginning a few hours after sunset until a few hours after midnight (Polis 1980).

Modes of response

Mechanosensory

Mechanical deflection of peg sensilla elicited high-frequency, fast-adapting spike discharges from type 'M' neurons (Fig. 4.4). Figure 4.4 shows a segment of recording where spikes classified as type 'A', 'B', and 'M' fired in temporal proximity. The presence of discrete spike waveforms and absence of gradation between them suggests that class 'M' cells represent a unique class of neurons in the peg. On occasion spikes of 'A' and 'M' types fired within a few milliseconds of each other (Fig. 4.4, inset), further indicating their independence of action. The peak firing frequency of type 'M' spikes was notably higher (> 100 Hz) than that observed for type 'A' spikes, and type 'M' spikes showed fast adaptation and recovery as is typical of mechanosensitive units in other arthropod sensilla (McIver 1975). Furthermore, type 'M' units were not responsive to chemical stimulation of the peg unless such stimulation caused the peg to deflect.

Contact chemosensitivity

Sensory neurons in the peg sensilla of *P. mesaensis* responded to stimulation by water when droplets were applied to the sensillum tip (Fig. 4.5A). It is noteworthy that peg sensilla were unresponsive to water in humid air presented as water-moistened filter paper brought within 1 mm of the sensillum pore.

Figure 4.5B shows the gross multi-unit response of a peg sensillum to successive contacts with pure octanol. This record is condensed from more than 2 min of response during which the stimulus was applied and withdrawn several times; the duration of each contact was approximately 1-2 s. The three traces are 20 s sections from the beginning, middle, and end of the record. The initial response showed immediate phasic-tonic excitation of large-amplitude spikes ('A' type). With repeated applications (middle trace), type 'A' response become more phasic while firing of type 'B' spikes increased between contacts. Type 'A' spikes adapted by the end of the record (bottom trace) while type 'B' spikes showed selective recruitment.

Olfactory sensitivity

General chemosensitivity. Individual peg sensilla displayed a range of excitatory and inhibitory responses to stimulation by various organic substances applied as puffs of vapor across the preparation. The threshold for response to stimulatory substances was on the order of 10^{-3}M. The

pattern of the response was strongly correlated with the chemical classification of the organic substance tested and in general was strongest for molecules with 6 to 10 carbons. A notable exception was seen in fatty acids, where the stronger excitation corresponded to C1 to C3 compounds and the compounds of longer carbon chain lengths (C5 to C9). Since these short fatty acid molecules could affect the pH balance of the sensillar lymph, the strong C1-C3 responses may be an artifact. Representative olfactory responses of peg sensilla to stimulation by substances representing four molecular types are shown in Fig. 4.6.

Response patterns from single peg sensillum. Within a single peg, identifiable units showed repeatable, dose-dependent responses to specific substances. However, absolute comparability between pegs was not apparent, nor appropriate, using the method of olfactory stimulation in these experiments. This variability in response between pegs is likely attributable to differences in orientation of the peg tip in relation to the directed pulse of stimulus vapor. As such, the effective amount of stimulant that accesses the slit-shaped terminal pore could not be normalized between recordings. To avoid this problem we analyzed and cross-compared unit chemosensory responses within individual peg sensilla during single, continuous recordings. Under these conditions stimulus presentation remained relatively constant between successive applications, allowing high-resolution comparisons of the time course of unitary responses.

Figures 4.7, 4.8, and 4.9 show the segregated responses of spike types 'A', 'B', and 'C' from a single peg sensillum to olfactory stimulation with a concentration series of primary alcohols and aldehydes (C_6 - C_8), a series of pure esters (C_6 - C_9), and a series of ketones (straight-chained and ring-structured). In these tests, units 'A1' and 'A2' were ambiguously resolved by waveform analysis so they were combined in the composite spike 'A' class. All responses shown were obtained between 2000 h and 0100 h (except for hexanol series 1530 h in Fig. 4.7).

Alcohols generally gave a simple pattern of excitation in peg sensory neurons. Olfactory application of alcohols (Fig. 4.7) to the sensillum produced dose-dependent, phasic excitation of spike types 'A' and 'C'. Hexanol also stimulated activity of type 'B' spikes with peak firing frequency occurring sooner with decreasing concentration. Peak firing frequency of type 'A' spikes decreased and occurred later with increasing carbon chain length as did high frequency bursts of type 'C' spikes. The sensitivity of peg sensilla to chemical stimulation appears also to depend on the time of stimulus application. The response of spike types 'A', 'B', and 'C' to a concentration series of hexanol given at 1530 h and 2200 h is shown in Fig. 4.7. Peak frequency of type 'A' spikes in the 2200 h series was higher and more phasic in pattern than the 1530 h series. Frequency of type 'B' spikes increased in the 2200 h series with the onset of their firing moving earlier in the response compared to the 1530 h series. Peak firing frequency of type

'C' spikes did not appear to change, but the onset of their burst was approx. 0.5 s earlier in the 2200 h response than in the 1530 h series.

In contrast to alcohol stimulation, aldehyde application (Fig. 4.8) produced dose-dependent suppression of type 'A' spikes. Notably, stimulation with hexanal also showed dose-dependent excitation of type 'B' spikes and a distinct absence of type 'C' spikes. In contrast, heptanal and octanal stimulation elicited early bursts of type 'C' spikes and delayed, moderate activity of type 'B' spikes.

The response to ester stimulation (Fig. 4.9) was characterized by high frequency firing of type 'C' spikes and delayed excitation of type 'A' spikes following an initial period of inhibition; type 'B' spikes showed delayed excitation in some responses. Activity of spike type 'C' and inhibition of spike type 'A' increased in response to stimulation with acetate esters with longer alcohol chains (Fig. 4.9, top three esters). Between the two heptate esters tested, greater activity was evoked in type 'C' spikes with stimulation by ethyl heptate vs methyl heptate; ethyl heptate produced a slightly shorter inhibition and lower peak firing frequency of type 'A' spikes than ethyl hexate (Fig. 4.9, bottom three esters).

Stimulation of the peg sensillum by ketones of straight-chain vs. ring-structure revealed the sensitivity of some peg neurons to detect gross differences in molecular structure. Both groups elicited inhibition with delayed tonic excitation of type 'A' spikes, but straight-chained compounds

also excited bursts of type 'C' spikes. The inhibition of type 'A' spikes was considerably greater with (+)-fenchon stimulation as compared with (+)-carvon or α-ionon stimulation.

DISCUSSION

Our results show that several units within peg sensilla of scorpion pectines can be identified and discriminated by their electrical signals and responses to mechanical and chemical stimulation. Most of these units are chemosensory as judged by their responses to chemicals applied directly to the peg tip or blown across the sensillar preparation as puffs of volatile substance. These findings are the first physiological confirmation of a chemosensory function for peg sensilla and they support morphological evidence that the pegs are chemotactic sensilla (Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983). This study also compliments behavioral studies which implicate the pectines are involved in the detection and orientation of scorpions to food substances (Krapf 1986) and pheromonal signals (Gaffin and Brownell 1992a).

The high threshold for olfactory responses ($>10^{-3}$M) indicates that these sensilla are probably best classified as gustation or near-field olfactory structures. Behavioral observation of the pectines support this conclusion. During normal locomotory movements the pectines are swept or tapped intermittently against the substrate. During chemically stimulated behavior (as in the experiments described in Chapter 2), tapping frequency increased as the pectines swept over the contaminated surface. High-speed video photography shows that these "sniffs" bring the distal, sensilla-bearing faces of the pectinal teeth in very close approximation to the substrate. It appears

that mediation of chemically-induced behavior by the pectines is achieved by direct contact with or close-range transmission of substrate borne-chemicals.

Coding of chemosensory response

The encoding of chemosensory information by primary afferents is complicated by the number of substances of potential importance to animals. Therefore the mechanism probably requires each receptor to be sensitive to many substances. (Boeckh 1967; Boeckh and Ernst 1983, 1987; Derby and Ache 1984; Derby and Atema 1988; Dethier 1974, 1976; Kauer 1991; Maes and Harms 1986; Maes and Ruifrok 1986; Sass 1976, 1978; Seelinger 1983; Selzer 1981, 1984; Tichy and Loftus 1983). Single peg sensilla of scorpion pectines are consistent with multiple receptor models.

Although scorpion peg sensilla have a morphology typical of contact chemoreceptors and are probably used by the animal to taste the substrate, they are also responsive to near-range olfactory stimulation. This provided us with a convenient method for assessing the chemosensitivity of sensory neurons within individual pegs to a broad spectrum of chemostimulants. We found that chemicals of various class (e.g. aldehydes, alcohols, ketones, esters) were distinguishable by the composite firing patterns they evoked in three responsive neurons, the 'A', 'B', and 'C' cells. For example, 'A' cells showed phasic excitation to alcohol stimulation and inhibition to aldehydes, ketones, and esters; 'B' cells showed immediate excitation to six-carbon

aldehyde and delayed activity to alcohols and longer chained aldehydes; 'C' cells fired with immediate bursts in all stimulations except hexanal and ring-structured ketones. Other cells (e. g. 'X', 'Y', 'Z') may participate in more specific detection (e.g. pheromones). These selective patterns of neuronal response suggest that primary neurons in the pectine sensory system encode at least the general composition of complex natural chemostimulants they encounter on the substrate.

In Table 4.2 the unitary responses of 'A', 'B', and 'C' neurons are compared to suggest how such a code might operate. For clarity, only the responses to the pure substance (Figs. 4.7, 4.8, 4.9) are shown here and these are rated qualitatively as having excitatory (+), inhibitory (-), or no response (0). The relative strengths of response are represented subjectively by the number of symbols (e.g. + = weak excitatory response; +++ = strong excitatory response) and the dynamics of each response is classified ('A' and 'B' units only) by early (first 4 s post-stimulation) and late (> 4 s) phases. Using this scoring system, seven distinct 'codes' are evident in the pattern of unitary response to the nine types of substances presented. For simplicity, all ester responses are grouped and ketones are grouped by straight and linear forms.

Sensory innervation of peg sensilla

By morphology, each peg sensillum in *P. mesaensis* is innervated by an average of 14 bipolar sensory neurons (Brownell 1989). In these studies we have identified eight units ('A1','A2','B','C','M','X','Y','Z') by their spike waveforms and pattern of response to mechanical and chemical stimulation. Our evidence for a second mechanosensory unit was insufficient to determine whether this neuron was a unique cell.

Among all of the units we recorded, type 'B' spikes were unique in the complexity of spike waveforms they generated In particular, the rising phase of their spike signature contains an unusual inflection (at arrow in fig. 4.10), suggesting that a second site of regenerative spike initiation may be present. Because this inflection was such a predictable feature of this spike class, we consider the possibility that it results from summation of spikes from two electrically-coupled cells. In Figure 4.10. the generalized type 'B' waveform has been drawn along with hypothetic spike 'B1', which is drawn in the form of a typical type 'A' spike. Hypothetic spike 'B2' was generated by subtracting, at each point in time, the voltage of 'B1' from 'B'. In theory, the electrical signature of spike type 'B' could result from addition of two large-amplitude events of opposite phase and may therefore represent the existence of an another sensillar unit or a second spiking region in the same cell.

Additional units may also exist within the small-amplitude class we labeled type 'C' units. These spike waveforms had small amplitudes (i.e. low signal to noise ratio) and variable waveforms, leaving open the possibility that two or three neurons contribute to this class. Future investigations using cross-correlation analysis (see Chapter 5 and Eggermont 1990) should help resolve this. However, even if all of these suppositions are correct, only 10-12 units are accounted for, suggesting additional units remained quiescent throughout these experiments or fail to elicit recordable regenerative spikes when stimulated.

In this study we have shown that peg sensilla of scorpion pectines function primarily as chemoreceptors. When considered with recent behavioral and anatomical reports, this research supports the hypothesis that the pectines are the principal chemosensory organs of scorpions and fill similar roles to antennal sensory systems of mandibulate arthropods.

Table 4.1 Pure chemicals used in tests of chemosensitivity of peg sensilla.

Alkanes
- hexane
- heptane
- octane
- nonane
- decane

Carboxylic acids
- formic acid
- acetic acid
- proprionic acid
- butyric acid
- pentanoic acid
- hexanoic acid
- heptanoic acid
- octanoic acid
- nonanoic acid

Alcohols
- pentanol
- butanol
- pentanol
- hexanol
- heptanol
- octanol
- nonanol
- decanol
- undecanol
- dodecanol

Aldehydes
- hexanal
- heptanal
- octanal
- nonanal
- decanal

Ketones
- hexene-(5)-on(2)
- 6-methyl-5-hepten-2-on
- 5-methyl-2-hexanon
- (+)-fenchon
- (+)-carvon
- α-ionon

Esters
- butyl acetate
- pentyl acetate
- hexyl acetate
- ethyl hexanate
- methyl heptanate
- ethyl heptanate
- methyl octanate
- ethyl octanate

Table 4.2 Chemostimulant encoding by identified sensory units of peg sensilla.

Stimulus	Unit Type					Response Class*
	A		B		C	
	Early	Late	Early	Late		
Alcohols						
Hexanol	+ +	0	0	+	+ + +	a
Heptanol	+ +	0	0	+	+ +	b
Octanol	+	0	0	0	+	c
Aldehydes						
Hexanal	——	0	+ +	0	0	d
Heptanal	—	0	0	+	+	e
Octanal	—	0	0	+	+	e
Ketones						
Linear	—	+ +	0	0+	+ +	f
Ring	—	+ +	0	0	0	g
Esters	—	+ +	0	0+	+ +	f

+ = excitatory, — = inhibitory, 0 = no response

* Letters indicate distinguishable responses to stimulation.

Figure 4.1 Extracellular recording configuration and computer processing of multi-unit records from scorpion peg sensilla. **A** Recording electrode is inserted through the flexible cuticle at the base of peg sensillum to record its electrical activity (V_m) relative to a reference electrode (V_{ref}). This configuration allows olfactory stimuli (olfactometer) direct access to the terminal pores (tp) of peg sensilla (ps). **B** Computer segregation of spiking units: spike events exceeding an adjustable threshold (B1) are classified by waveform (B2) and segregated as reconstructed displays in separate traces (B3).

Figure 4.1

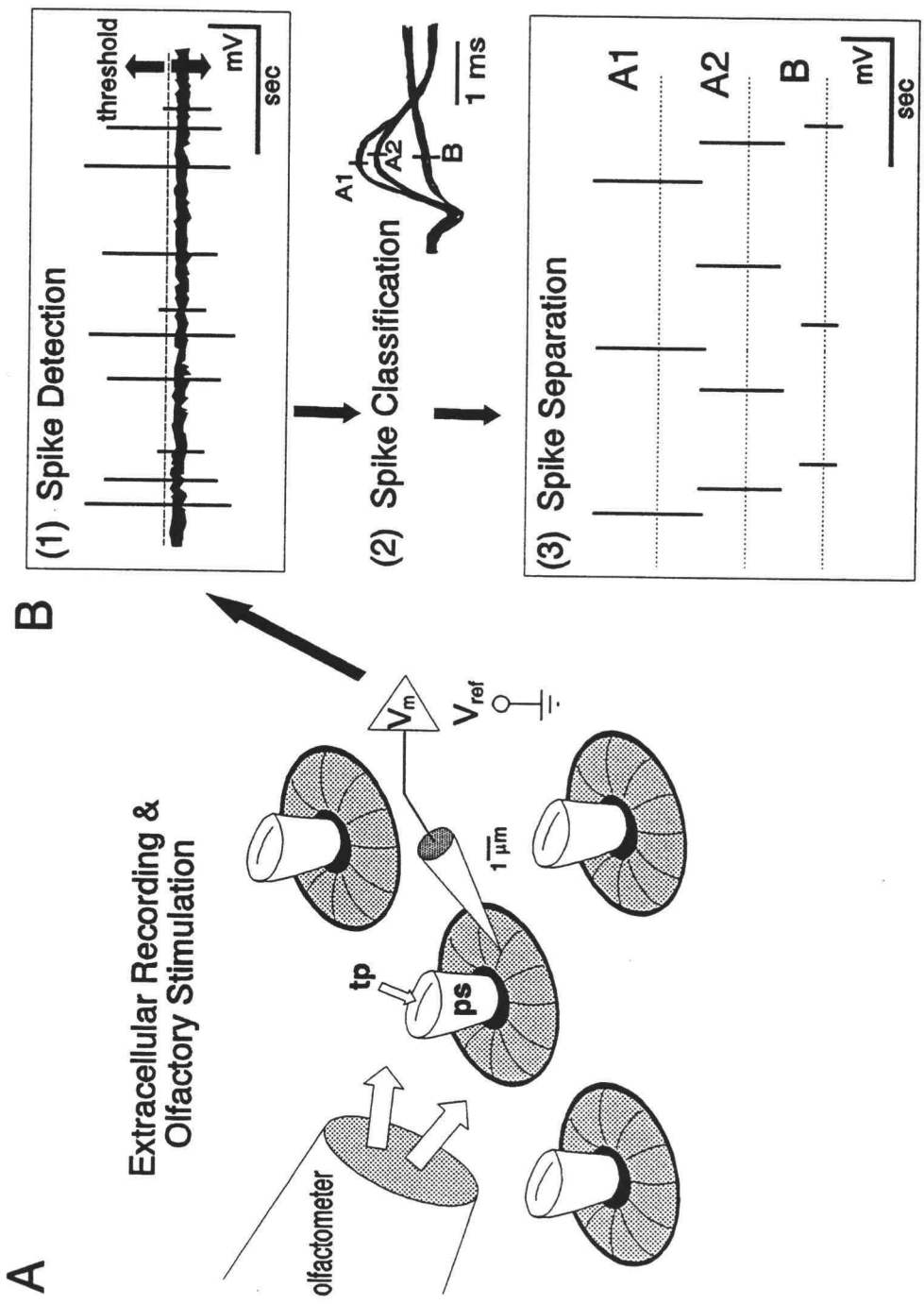

A
Extracellular Recording &
Olfactory Stimulation
olfactometer
tp
ps
1 µm
V_m
V_{ref}
B
(1) Spike Detection
threshold
mV
sec
(2) Spike Classification
A1
A2
B
1 ms
(3) Spike Separation
A1
A2
B
mV
sec

Figure 4.2 Sensory units observed in peg sensilla of *P. mesaensis*. **A** Waveforms of five spike types have been averaged from multiple records and superimposed here to show relative amplitudes and phase relationships. Spike types 'A1', 'A2' and 'B' are spontaneously active units recorded from one sensillum; 'C' and 'M' spikes were taken from other recordings and normalized by amplitude to the 'A' and 'B' units co-occurring in their records. 'C' and 'M' units were stimulated chemically ('C') or by mechanical deflection of the peg tip ('M'). **B** Samples of > 10 superimposed spikes showing consistency of their waveforms. **C** Expanded waveforms of 'A1'/'A2', 'X', 'Y', and 'Z' units; superimposed 'A' type units are shown for reference to larger amplitude 'X', 'Y', and 'Z' units. The large type 'X' spikes were observed infrequently as isolated spikes; bursts of spike types 'Y' and 'Z' were observed only once. **D** Samples of superimposed spikes of types 'A1/A2', 'X', 'Y', and 'Z' show consistency of their waveforms. 'X', 'Y', 'Z' have unique stereotypical waveforms that cannot be attributed to coincident summation of small amplitude spikes.

Figure 4.2

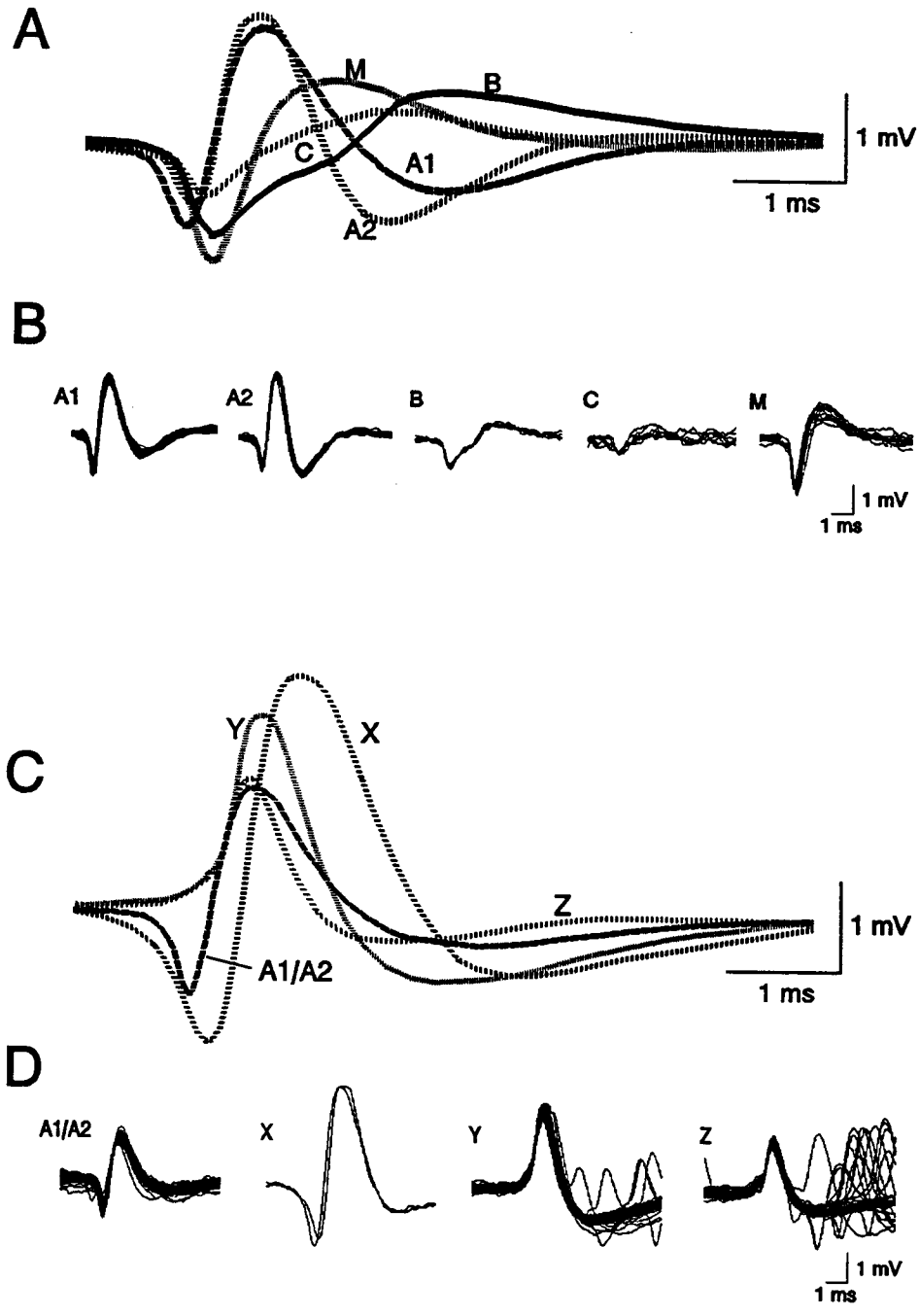

A
M
B
C
A1
A2
1 mV
1 ms
B
A1
A2
B
C
M
1 mV
1 ms
C
Y
X
Z
A1/A2
1 mV
1 ms
D
A1/A2
X
Y
Z
1 mV
1 ms

Figure 4.3 Diurnal fluctuations in impulse activity recorded from single peg sensilla in *P. mesaensis*. Mean ± SE of normalized spiking frequencies (n=4). Spike frequency was normalized individually to the peak spiking frequency of each record. Highest activity coincides with time these animals would normally forage for food and mates (late evening to early morning).

Figure 4.3

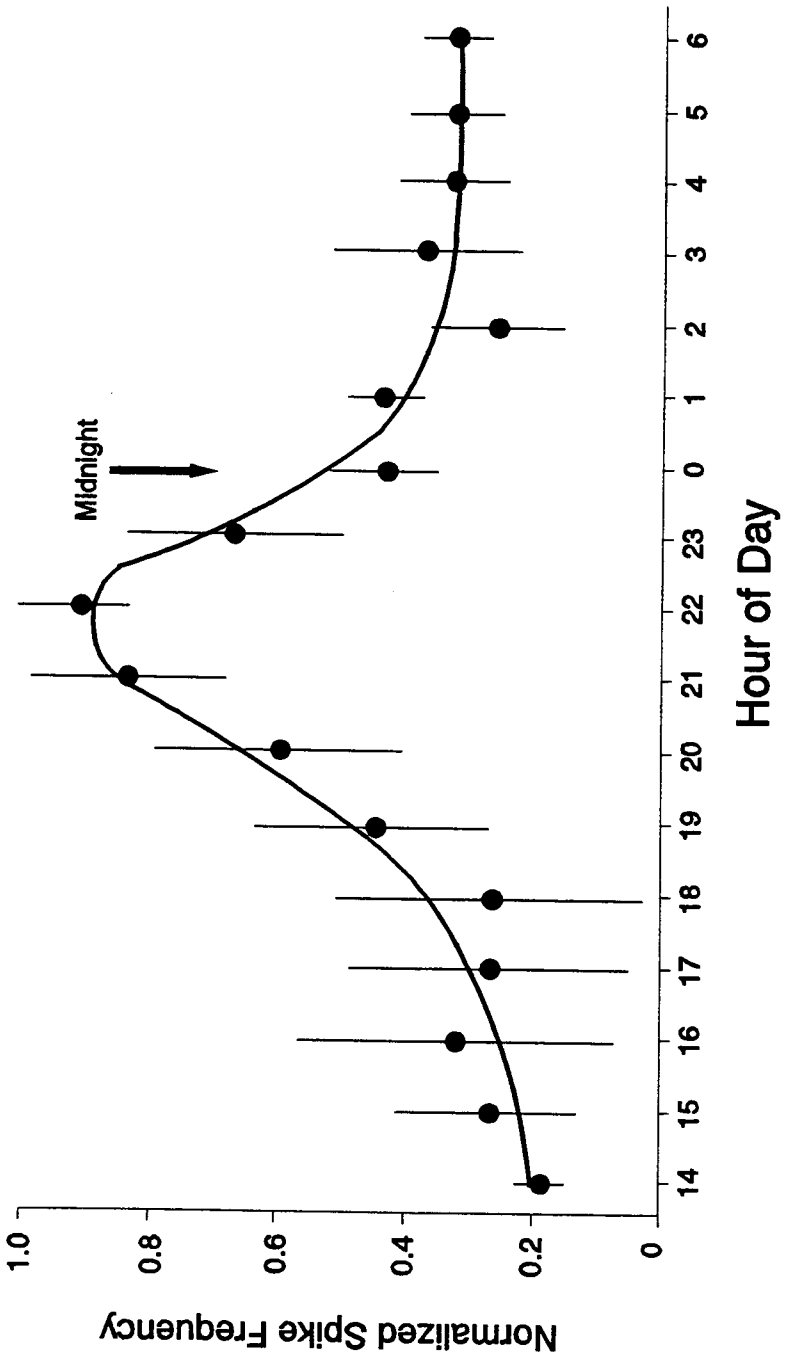

Midnight
Hour of Day
Normalized Spike Frequency
0
0.2
0.4
0.6
0.8
1.0
14
15
16
17
18
19
20
21
22
23
0
1
2
3
4
5
6

Figure 4.4 Electrical response of a peg sensillum to mechanical stimulation. Deflection of peg sensillum of *P. mesaensis* by directed puffs of air (at arrows) elicited bursts of spikes (type 'M') amidst spontaneously active 'A' and 'B' units. Inset: Presence of doublet spikes show near-temporal firing of type 'M' and type 'A' spikes.

Figure 4.4

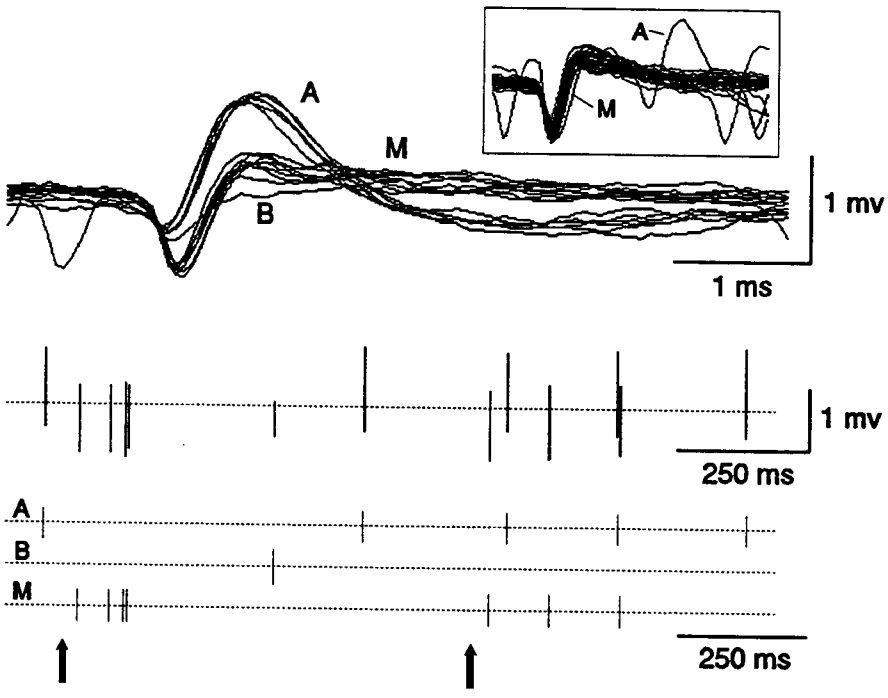


Figure 4.5 Electrical responses of peg sensilla in *P. mesaensis* to contact with water and octanol. **A** Peg sensillum submerged in paraffin oil contacts a droplet of water (up arrow). Prior to contact, peg sensilla showed normal (low frequency) spike activity. When the terminal pore is contacted with a droplet of water extruded from a micropipette, several units discharge at high frequency until the droplet is removed (down arrow). **B** Electrical response of peg sensillum in air to successive contacts with pure octanol. This record is condensed from many contacts over a period of > 2 min. Arrows indicate approximate time of contact; duration of each contact was approximately 1-2 s.

Figure 4.5

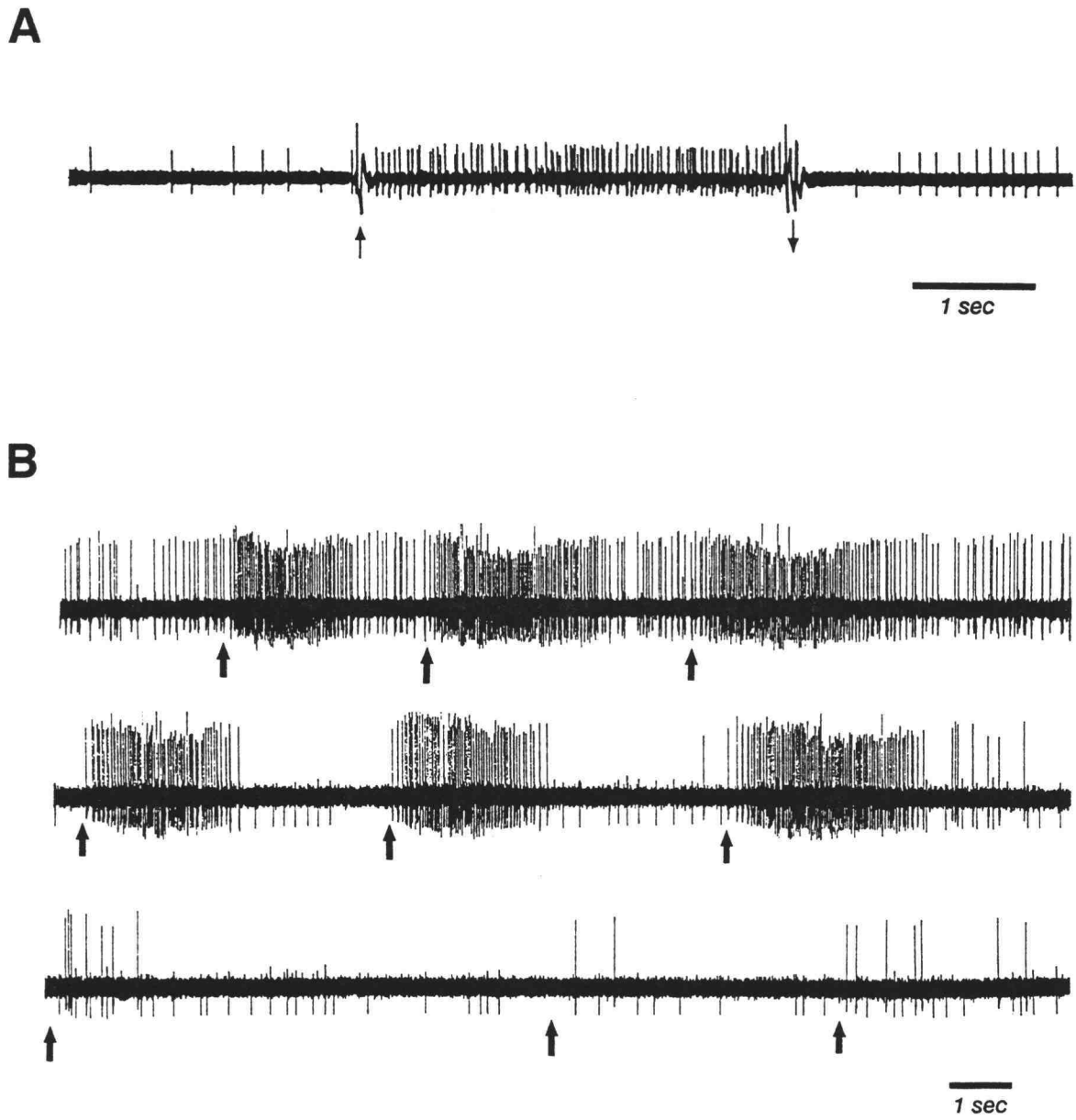


Figure 4.6 Representative examples of electrophysiological response of peg sensilla to chemical stimuli. Olfactory stimulation of the pectines by pulses of air (bar) saturated hexanol (upper trace) excited large-amplitude spikes ('A' type). The six carbon aldehyde (hexanal) excited small-amplitude spikes ('B' type) and suppressed activity of 'A' units. The hexyl ester and six carbon ketone suppressed type 'A' units without evoking type 'B' activity. The arrow in ketone stimulation indicates rapid firing of small-amplitude type 'C' units.

Figure 4.6

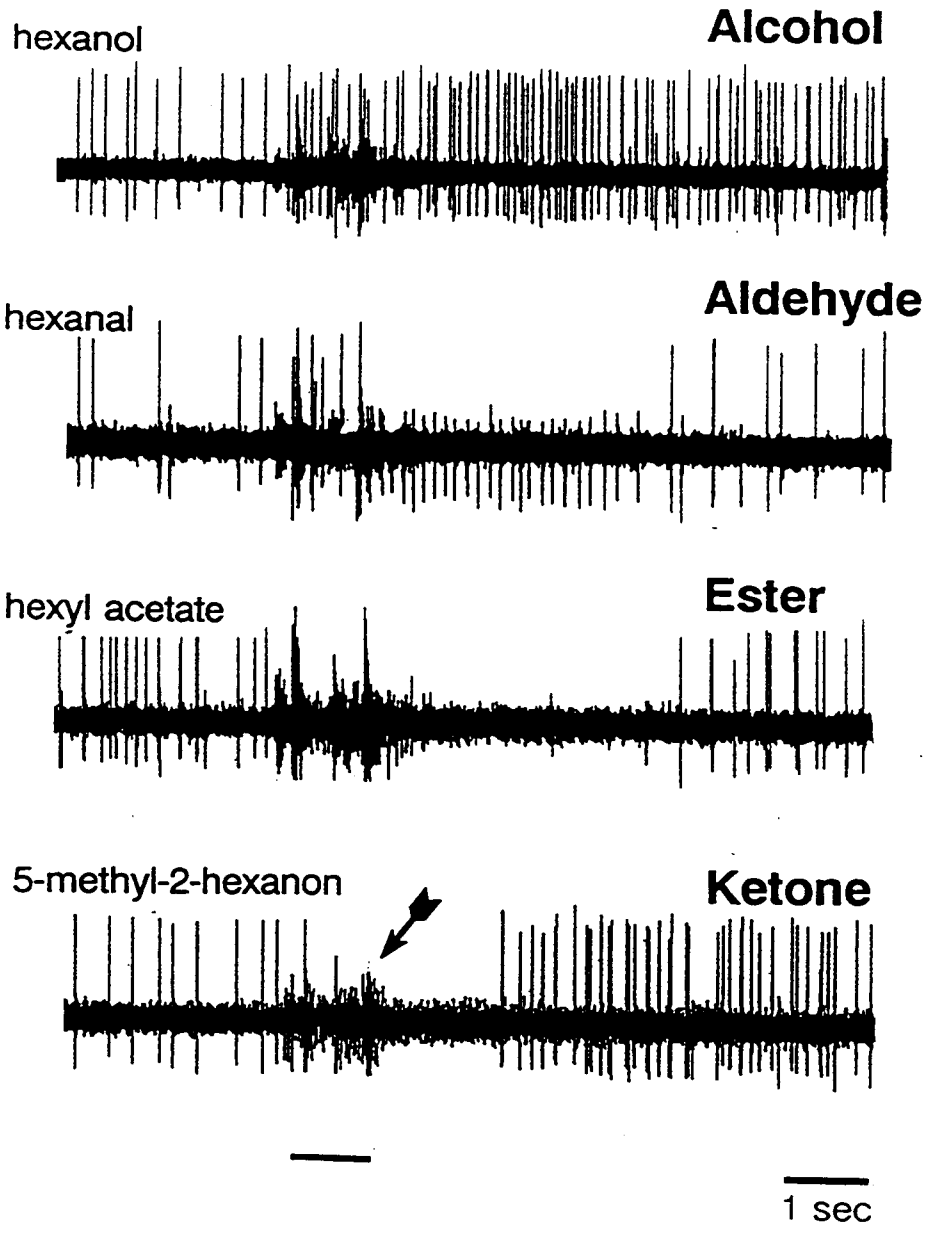

hexanol
Alcohol
hexanal
Aldehyde
hexyl acetate
Ester
5-methyl-2-hexanon
Ketone
1 sec

Figure 4.7 Dose-dependent responses of a peg sensillum to olfactory stimulation by C6 - C8 primary alcohols. Graphs show 4 s of pre-stimulus baseline activity (frequency of spiking) and 12 s of post-stimulus response to 1 s pulses (indicated by solid bars) of stimulus blown across the preparation. Each curve was normalized by subtraction of averaged pre-stimulus spiking frequency. Curves for spike types 'A' and 'B' represent 5 point running averages of spiking frequencies in 0.25 s bins (i.e. running average spans 1.25 s of activity). Histograms of spike type 'C' represent absolute frequency values within 0.25 s bins. Hexanol responses obtained at 1530 h and 2200 h show change of sensitivity of units in this peg sensillum with time of application. Heptanol and octanol responses were obtained between 2100 h and 2300 h.

Figure 4.7

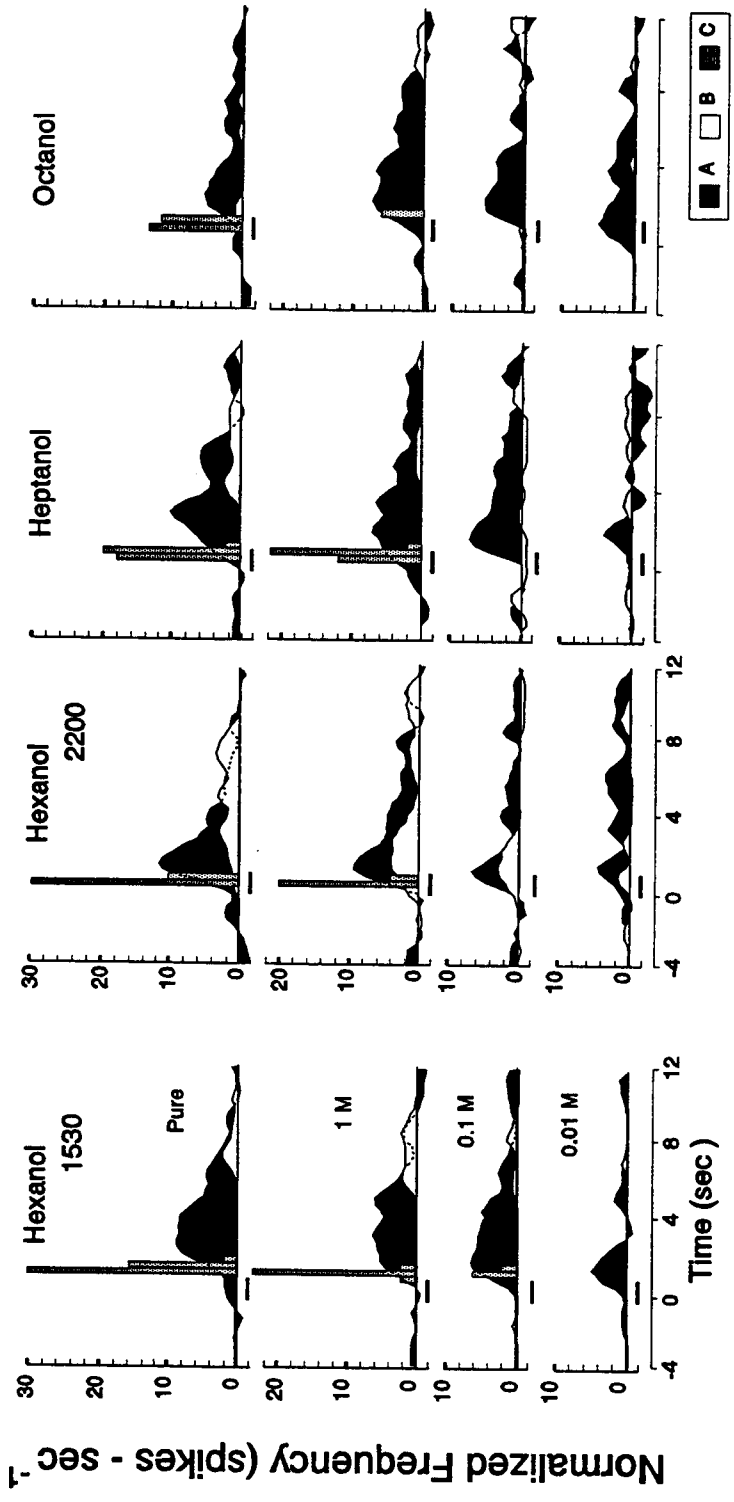

Hexanol
1530
Pure
1 M
0.1 M
0.01 M
Hexanol
2200
Heptanol
Octanol
A
B
C
Time (sec)
Normalized Frequency (spikes - sec-1)

Figure 4.8 Dose-dependent responses of a peg sensillum to olfactory stimulation by C6 - C8 primary aldehydes. Graphs show 4 s of pre-stimulus baseline activity (frequency of spiking) and 12 s of post-stimulus response to 1 s pulses (indicated by solid bars) of stimulus blown across the preparation. Each curve was normalized by subtraction of averaged pre-stimulus spiking frequency. Curves for spike types 'A' and 'B' represent 5 point running averages of spiking frequencies in 0.25 s bins (i.e. running average spans 1.25 s of activity). Histograms of spike type 'C' represent absolute frequency values within 0.25 s bins. All responses were obtained between 2300 h and midnight.

Figure 4.8

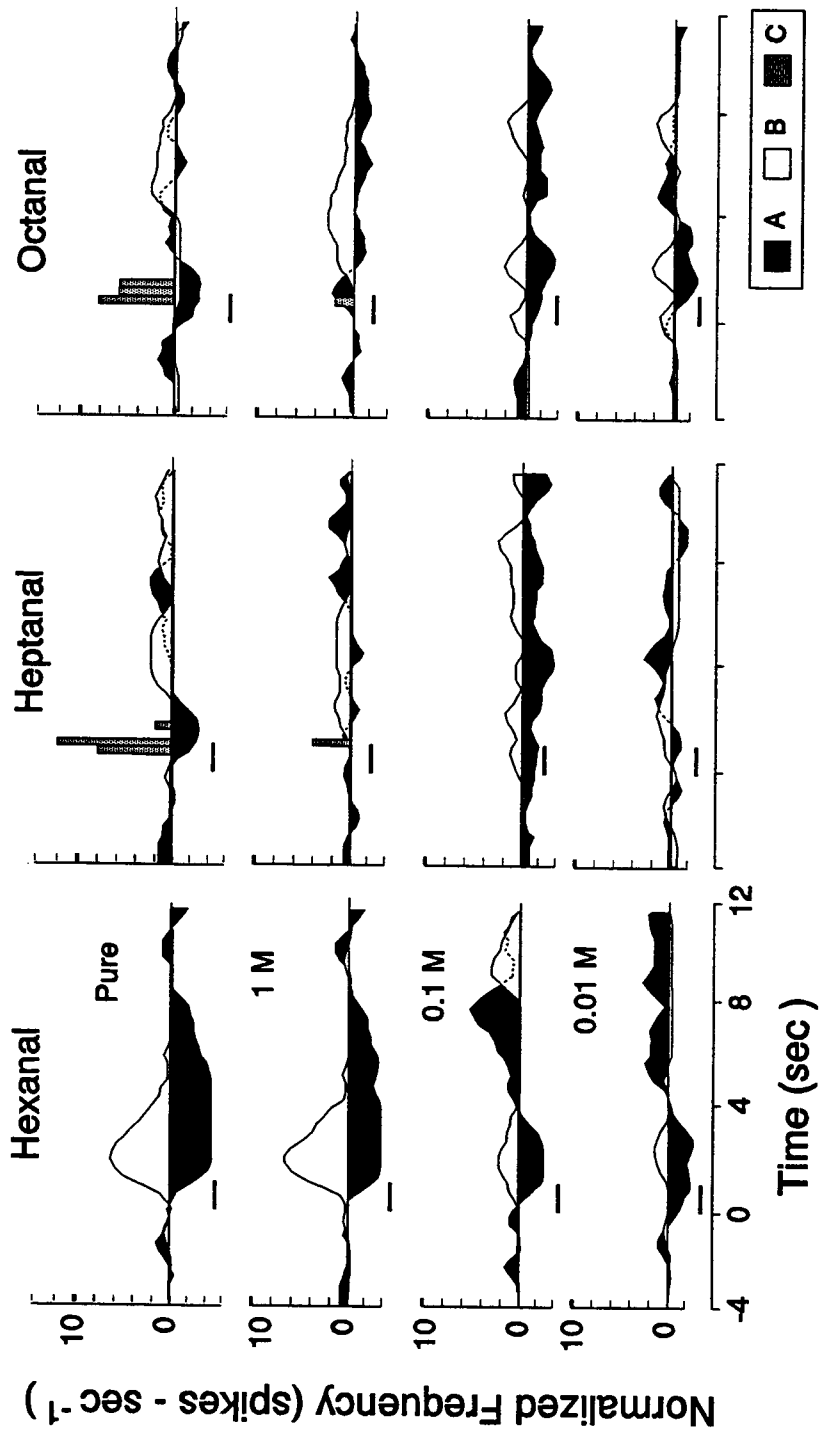

Octanal
Heptanal
Hexanal
Pure
1 M
0.1 M
0.01 M
A
B
C
Normalized Frequency (spikes - sec⁻¹)
Time (sec)
10
0
-4
0
4
8
12

Figure 4.9 Responses of a peg sensillum to olfactory stimulation by pure esters and ketones. Graphs show 4 s of pre-stimulus baseline activity (frequency of spiking) and 12 s of post-stimulus response to 1 s pulses (indicated by solid bars) of volatile chemical substances blown across the preparation. Each curve was normalized by subtraction of averaged pre-stimulus spiking frequency. Curves for spike types 'A' and 'B' represent 5 point running averages of spiking frequencies in 0.25 s bins (i.e. running average spans 1.25 s of activity). Histograms of spike type 'C' represent absolute frequency values within 0.25 s bins. Ketone responses were obtained between 2300 h and midnight; ester responses were obtained between midnight and 0100 h.

Figure 4.9

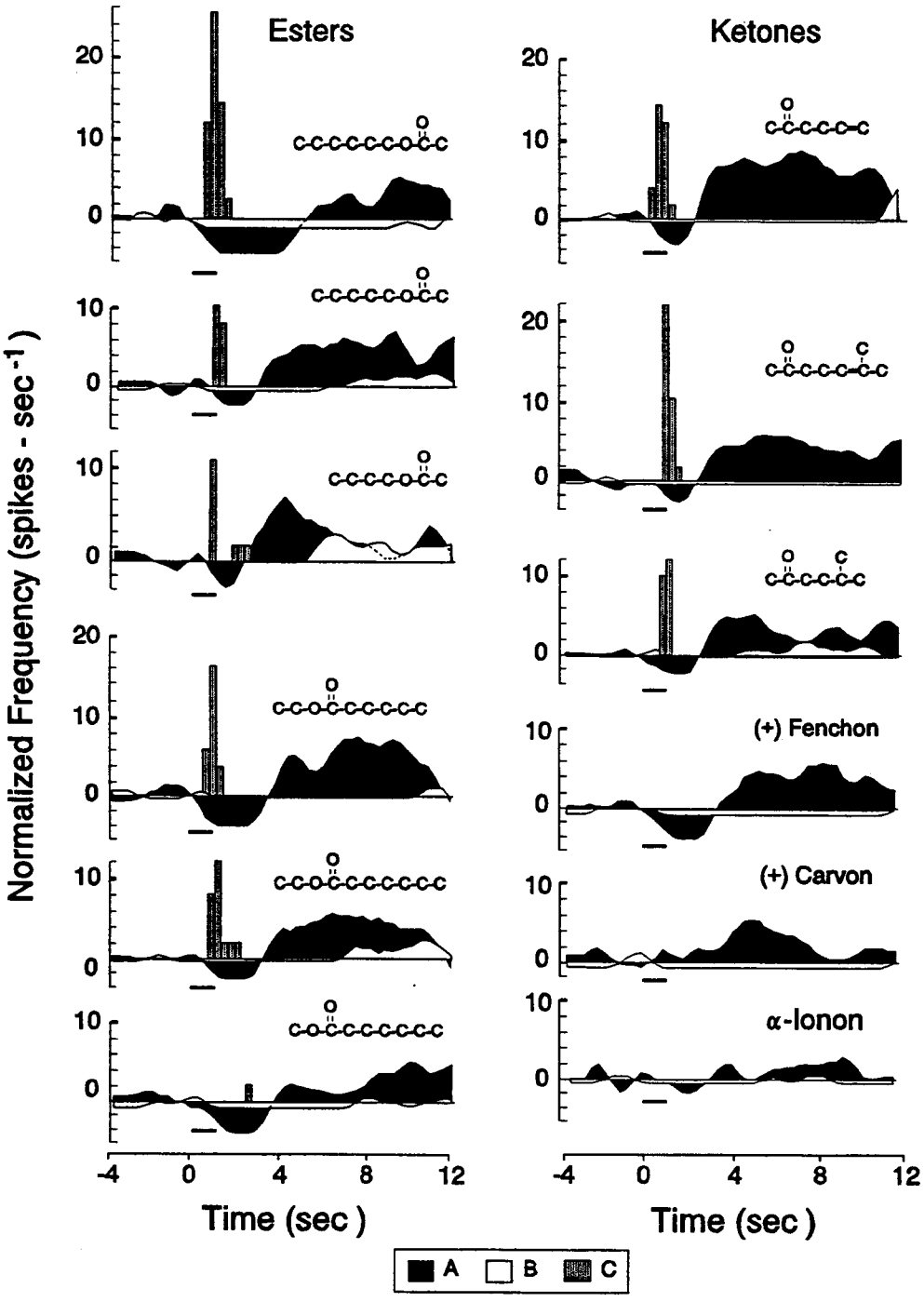
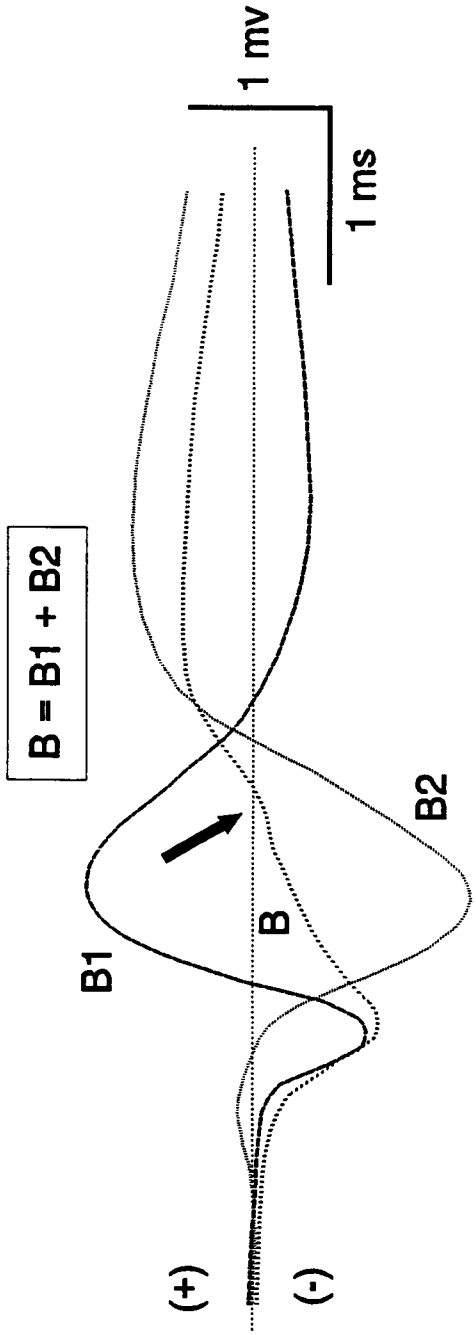

Esters
Ketones
Normalized Frequency (spikes - sec-1)
(+) Fenchon
(+) Carvon
α-Ionon
Time (sec)
Time (sec)
A
B
C

Figure 4.10 Hypothetic summation of two spike waveforms to produce the multiphasic type 'B' waveform. Time expanded waveform of spike type 'B' shows a consistent inflection (arrow). Summation of two hypothetical spike types ('B1' and 'B2') of normal waveform gives the complex waveform. 'B1' was assigned the shape of a typical type 'A' spike; inverted spike 'B2' results from subtraction of 'B' from 'B1'.

Figure 4.10



CHAPTER 5

ELECTROPHYSIOLOGICAL EVIDENCE OF SYNAPTIC INTERACTIONS IN SINGLE PEG SENSILLA OF SCORPION PECTINES

ABSTRACT

The pectines of scorpions are a useful model for the study of peripheral integration of chemosensory information. In extracellular recordings from individual peg sensilla, individual sensory units can be identified and segregated on the basis of spike shape and firing frequency by a computer algorithm. Cross-correlation analysis of spiking activity during non-stimulated baseline and chemosensory response show that at least two units of distinguishable spike-form (spike types 'A1' and 'A2') are inhibited during the 100 ms period immediately following spiking activity of a third unit (spike type 'B'). This apparent interaction between units in a single sensillum is seen in the isolated pectine for several hours, indicating that it does not involve efferent feedback from the central nervous system (CNS). Other potential forms of synaptic interactions are observed between type 'A' units and units of type 'Y' and 'Z'. Firing of type 'Z' units appears to excite firing of type 'Y' units which, in turn, excite firing of type 'A' units. Type 'A' spikes then appear to exert an inhibitory influence of type 'Z' spikes to complete a negative feedback circuit. Morphological studies of the pectine show multiple synaptic contacts between axons of sensory neurons, a potential substrate for such complex interactions. Thus, chemosensory

information appears to undergo some form of processing within individual sensilla prior to its relay to the CNS. Taken together with their accessibility to electrophysiological investigation, scorpion peg sensilla appear to be a useful model for study of peripheral integration of chemical based information.

INTRODUCTION

With rare exceptions (White et al. 1990), the first synaptic interaction between neurons in the chemosensory pathways of mandibulate arthropods (insects, crustaceans) occurs within the central nervous system. In the antennae of insects, for example, the axons of primary sensory neurons pass from chemosensory hairs on the antenna through the antennal nerve to the olfactory lobe where they converge on dendrites of second order neurons in glomerular neuropile (Bullock and Horridge 1965; Kaissling 1986, 1987).

The chemosensory pathways in chelicerate arthropods (e.g. horseshoe crabs, arachnids) appear to depart significantly from this design. Morphological studies of sensory systems in several chelicerate arthropods show abundant cell-to-cell contacts in the peripheral nervous system of these animals (Hayes and Barber 1982; Griffin and Fahrenbach 1977; Foelix 1985). In scorpion peg sensilla, axo-axonal synaptic profiles exist between chemosensory neurons just below the cell body layer, about 50 - 100 μm beneath the cuticular surface of the pectine tooth (Foelix and Müller-Vorholt 1983; Foelix 1985). An example of these profiles in *Parabuthus pallidus* is shown in Fig. 5.1 (Brownell unpubl.).

In this study we use cross-correlation analysis of unit spiking activity in scorpion peg sensilla to demonstrate the functional existence of synapses in these chemosensory structures. This represents the first physiological support of synaptic interactions in any arachnid chemosensory organ. The

significance of these synaptic interactions in scorpions is discussed in terms of their potential contribution to peripheral processing of chemosensory information. Preliminary reports of some of these findings have appeared (Gaffin et al. 1991; Gaffin and Brownell 1992a).

MATERIALS AND METHODS

Adult sand scorpions (*Paruroctonus mesaensis*) from the Mojave Desert in Southern California were the subjects of these investigations. Animals were kept in plastic containers with natural sand substrates and maintained at capture weight by regular feedings of wax worm larvae. Animals were housed within an environmental chamber that maintained constant temperature of 27°C and a photoperiod of 15 L / 9 D. Electrophysiological techniques and olfactory stimulations were as described in Chapter 2.

Simultaneous extracellular recordings from neighboring sensilla were used to investigate possible inter-sensillar interactions. Stable extracellular recordings obtained from adjacent or nearby sensilla were amplified then combined to form a single data record. To facilitate resolution and identification of spike classes, one of the two electrode outputs was inverted prior to combining the records. Electrode output was turned off individually to identify the source sensillum of each class of unit.

Synaptic interaction between neurons is expressed by dependence (either excitatory or inhibitory) of one cell's activity on another within a brief time frame. To reveal and describe patterns of interaction between neurons, we have written software to isolate and cross-correlate activities of specific spiking units (Gaffin and Jubran 1991). In this program, multi-unit traces obtained by extracellular recording are digitized for relay to the computer where a user-adjustable discrimination window is set to identify spiking

events from background noise. Detected events are captured and stored along with a time marker to encode their time of occurrence. Spiking events are then superimposed on the computer monitor to show their waveforms on an expanded time base (approx. 3 ms). Spikes from different cells can usually be grouped into discrete classes and redisplayed independently of the activities of other spikes in the record.

As separated records, the activity patterns of particular cells can be compared with others in the recording. For correlation analysis, all spikes of one class are centered in windows of a defined time width and spikes of a second class are displayed relative to this "centered" or referenced spike type. The number of spikes falling within discrete bins of time before and after the centered spike are counted and displayed in histogram form for multiple records (see bottom of flow chart in Fig. 5.2A). The resultant histogram profiles indicate interactions between the spike classes, as displayed for several hypothetical interactions in Fig. 5.2B. The bottom two profiles of Fig. 5.3B are auto-correlations, where spikes within one class are referenced against themselves. Auto-correlations are important for verifying that spikes assigned to a particular spike class originate from a single source. The top profile shows the type of activity predicted in an auto-correlation of a class that has only one cell represented. Here, the action potential recovery period of the cell results in a clearing around the referenced spike. Auto-correlations of classes containing more than one cell would appear as

shown in the second profile. The top three histograms in Fig. 5.2B are cross-correlation profiles for spikes from two different classes. The histograms show patterns expected for inhibitory, excitatory, and no interaction (for greater detail on correlation analysis see Eggermont 1990).

RESULTS

Sensory units in peg sensilla of *P. mesaensis* show variable electrophysiological patterns of response to different classes of stimulus substances (Chapter 4). The frequency of any one unit's discharge appears to be a product of not only receptor-level events, but also local (sensilla-level) interactions between sensory neurons. An indication of sensory cell interaction comes from examination of spiking patterns during spontaneous spiking activity. In baseline recordings, we noticed interruptions of spiking immediately following firing of type 'B' spikes. These periods can be seen in Fig. 5.3 where the activity of the large-amplitude type 'A' spikes appears interrupted by the activity of the small-amplitude type 'B' spikes. The effect of type 'B' on type 'A' appears to be inhibitory since type 'A' spikes tend not to fire immediately after the firing of type 'B' spikes. This simple observation is important because it suggests some form of physiological interaction between chemosensory neurons at the sensillar level.

To examine this apparent interaction between units in peg sensilla, we used cross-correlation analysis (see Materials and Methods) to resolve the near-temporal influences between units during long-term baseline recordings. A series of 54, ten-second samples from 20 minutes of non-stimulated baseline was decomposed by computer algorithm into the three discrete spike classes, 'A1', 'A2', 'B'. The summed near-temporal environments, as derived from cross- and auto-correlation analysis of these three spike classes,

are represented by the histogram profiles of Fig. 5.4. In each case, the referenced spike class was centered and activity of the other classes during the 140 ms before and after the referenced spike was assessed. The activity of spike types 'A1' and 'A2' was suppressed in the 14 ms following the firing of spike type 'B' (Fig. 5.4, cross-correlations: top two profiles) with the period of influence lasting at least 100 ms after the firing of spike type 'B'. The activities of spike types 'A1' and 'A2' appear unaltered when referenced against each other (Fig. 5.4, cross-correlations: third profile). The activities of spike classes 'A1' and 'A2' prior to firing of spike type 'B' appear uniform, suggesting that these spike types do not exert an effect on the firing probability of spike type 'B' during spontaneous activity (Fig. 5.4, cross-correlations: top two profiles). By referencing each of the spike types against itself (Fig. 5.4, auto-correlations: bottom three profiles), each of the three spike classes was determined to contain only one type of spiking unit (the lack of random spiking near the arrow serves to verify this).

Analysis of the spiking patterns of a chemically-stimulated sensillum showed a pattern of unit interaction similar to the unstimulated sensillum. Four successive records representing stimulation of the sensillum with pure hexane (Fig. 5.5) were examined with respect to interactions between spike types; hexane moderately excited both 'A' type and 'B' type spikes in this sensillum. The spiking units of this recording were resolved into two distinct classes (labeled A and B, Fig. 5.5). Referencing class 'B' against itself (Fig.

5.5, third profile) produced the characteristic clearing around the centered spike, confirming that only one type of spiking unit was present in this class. Referencing of class 'A' against itself (Fig. 5.5, second profile) did not produce the same clearing, indicating at least two different types of spiking units were present ('A1' and 'A2'). The top profile of Fig. 5.5 shows the activity of spike class 'A' is suppressed in the period immediately following the firing of spike 'B'. By expanding the time scale, as in the bottom profile of Fig. 5.5, the suppression of 'A' by 'B' was seen to occur within the first 7 ms; the period of strong influence lasted at least 80 ms post-firing of 'B'. An apparent rebound effect occurred approximately 140 ms post-firing of 'B' followed by a return to normal activity around 160 ms (Fig. 5.5, top profile). Some evidence of a facilitative effect of spike class 'A' on the probability of firing of spike type 'B' is indicated by the increased activity of 'A' just prior to firing of 'B' (see * in bottom profile, Fig. 5.5).

We next investigated whether the inhibitory action of spike type 'B' on 'A1' and 'A2' was local to the pectine or a result of feedback from the central nervous system. A stable recording was made of a peg sensillum in which the three spike classes 'A1', 'A2', and 'B' were resolvable. After one hour of baseline recording, the pectine spine was severed just distal to its point of attachment to the ninth body segment. The signal recovered to its original, pre-cut intensity following a brief electrical interference (approx. 20 s) caused by contact of the scissors with the preparation. The same 'A1',

'A2', and 'B' spike types present in the intact pectine were present in the dissociated pectine; spontaneous spiking activity persisted approx. 5 h. Sixty, 10 s samples representing 30 min of post-cut spiking activity were categorized into the three discrete spike types and examined for interactions by correlation analysis. Figure 5.6B shows the histogram profiles generated by this analysis; spiking activity before and after referent spikes was grouped by 21 ms bins. As in previous examples, spike type 'B' inhibits the activity of spike types 'A1' and 'A2' while 'A1' and 'A2' show no apparent influence in regards to each other. Auto-correlations indicated that each spike class consisted of single spiking units and gave an indication of the frequency and firing dynamics of each spike type. Auto-correlations of type 'A' spikes showed that spike 'A1' fired at five times the frequency of spike 'A2' and approximately 30 times that of type 'B'. Spike type 'B' shows a tendency to burst as suggested by the two, disproportionately large frequency bins near the center of the profile.

Inhibitory interactions may extend beyond single pegs to affect neighboring sensilla. The possibility of inter-sensillar interactions was investigated by cross-correlating spiking activity of units from adjacent and nearby pegs. Figure 5.7B shows a sample of the complex record obtained by combining electrode outputs (V1 and V2) from adjacent sensilla; the output of V2 was inverted to help resolve spike classes from the two electrodes. Auto-correlation analysis indicates that spike types 'A1' and 'A2'

superimposed in the outputs from both V1 and V2 to form the composite spike class labeled 'A'. Cross-correlation of units within each of the two sensilla showed the familiar inhibition of type 'A' spikes by type 'B' spikes (Fig. 5.7C: V1A vs V1B and V2A vs V2B). The four histogram profiles generated by cross-correlations of units from adjacent sensilla showed no indication of interactions between sensilla (Fig. 5.7C: V1A vs V2A, V1B vs V2B, V1A vs V2B, and V1B vs V2A).

Cross-correlation analysis of 'A', 'Y', and 'Z' spike types suggest that additional types of interactions may exist between sensillar units. In Chapter 2 we noted an isolated burst of spike types 'Y' and 'Z' during a section of unstimulated activity of spike types 'A1' and 'A2'. This 7 s burst is shown in Fig. 5.8A. Cross-correlation analysis (Fig. 5.8B) indicates the activity of spike type 'Z' is inhibited by type 'A' spikes (composite of 'A1' and 'A2' as indicated by auto-correlation) with a time course similar to the inhibitory interaction of type 'B' and type 'A' cells. Cross-correlation of spike type 'Y' with spike type 'A' indicates that spikes of type 'Y' are more likely to fire immediately before firing of type 'A' spikes (i.e. type 'Y' has an excitatory or facilitative influence on the firing of type 'A'). Cross-correlation of spike type 'Y' with spike type 'Z' also indicates an excitatory interaction (type 'Z' excites firing of type 'Y'), but with a delayed effect compared to the type 'Y' and type 'A' interaction. Auto-correlations of type 'Y' and type 'Z' indicate these classes contain single units which discharged at high frequency. The

activity interactions indicated by the cross-correlation profiles suggest a simple feedback circuit, such as depicted in Fig. 5.8C.

DISCUSSION

In this study we have shown that spiking activity of some units in scorpion peg sensilla are affected by the activities of other sensillar units. These interactions do not involve feedback from the CNS and appear to be restricted to individual sensilla. When coupled with morphological evidence showing putative axo-axonic synaptic profiles just proximal to sensory cell bodies (Foelix and Müller-Vorholt 1983; Brownell unpubl.), the simplest interpretation is that chemosensory cells in peg sensilla interact synaptically.

The significance of these interactions may lie in the temporal encoding of stimulus quality. In Chapter 4 we showed that various chemostimulants were distinguishable by the patterns of unit activity they evoked in single peg sensilla, with different response patterns being observed for substances differing only in functional group. In particular, the activity patterns of 'A' and 'B' units differed in response to six-carbon alcohol (hexanol) and six-carbon aldehyde (hexanal; Fig. 5.9A). The suppression of type 'A' activity by the aldehyde can be modeled as an inhibitory interaction between units 'A' and 'B' (Fig. 5.9B). This simple circuitry may modify the temporal activity of primary neurons and encode subtle differences in chemical quality prior to relay to the CNS.

The synaptic connectivity of sensory neurons in peg sensilla is complicated. Each peg is innervated by an average of 14 neural elements (Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983; Brownell 1989)

and several types of synaptic configurations connect these cells into a complex neural plexus (Foelix and Müller-Vorholt 1983). The most common interaction observed morphologically was the "dyad" synapse, where one presynaptic fiber contacts two postsynaptic fibers. Also observed were serial connections (a postsynaptic fiber was presynaptic to a third fiber) and reciprocal synaptic contacts (Foelix and Müller-Vorholt 1983; Foelix 1985). Each of these configurations is represented in physiological interactions observed in this study. The dyad synapse may be reflected in the suppression of 'A1' and 'A2' units by 'B' units (Figs. 5.4 - 5.7). Serial connectivity was observed in the interactions between 'A', 'Y', and 'Z' units (Fig. 5.8). For example, 'A' units inhibited 'Z' units, which in turn excited 'Y' units. Finally, a possible reciprocal interaction between 'A' and 'B' units is shown in Fig. 5.5, where 'A' units appear to simultaneously excite, and be inhibited by, 'B' units. An alternative type of reciprocal interaction may exist in the coupling of cells to form the spike signature of typical 'B' spikes as discussed in Chapter 4 (Fig. 4.10).

Despite the complexity of the peg sensillum plexus, the physiological evidence presented here and in Chapter 4 have allowed us to draw a tentative diagram (Fig. 5.10) of the apparent connections between sensillar units. In this diagram, the interactions involving 'Y' and 'Z' units have been expanded in complexity to show connections with both 'A1' and 'A2' units;

unit 'B' is modeled as an electrical coupling between two units, 'B1' and 'B2'. Further details of this circuit are described in Fig. 5.10.

It has been a general conception that first order synapsing of arthropod chemoreceptors occurs in the brain. While this may be more or less true for olfactory systems in mandibulate arthropods (thermo-/hygrosensitive sensilla may be represent an exception (Steinbrecht 1989; Gödde and Haug 1990)), it can not be generalized to chelicerate arthropods and perhaps not to gustatory systems as a whole (Moulins and Noirot 1972; White et al. 1990). Peripheral synaptic densities have been recognized in chelicerate systems for almost 20 years since the first descriptions by Foelix (1975). It is surprising, therefore, that physiological investigations of these interactions have lagged so far behind the morphological accounts. This is likely due to the lack of tractable systems for electrophysiological manipulations and the difficulty of manual segregation of multi-unit records. In this report we have shown how computer-based techniques can be used to "tease" apart a natural and accessible neural network, the scorpion peg sensillum. The high fidelity of electrophysiological recordings obtained from these structures make this an excellent model system for continued study of peripheral processing of chemosensory information. Future identification of specific pheromonal moieties will be of increasing importance to the analysis of how this circuit responds and encodes behaviorally meaningful chemical signals.

Figure 5.1 Axo-axonic synaptic densities in a peg sensillum. **A** TEM of axonal processes just proximal to cell body layer (approx. 50 μm below cuticular surface) in peg sensillum of *Parabuthus pallidus* showing sensory cell bodies (sc) and axon bundles (ax). **B** Enlargement of area within box in A reveals profiles of putative chemical synapses (syn) between sensory axons.

Figure 5.1

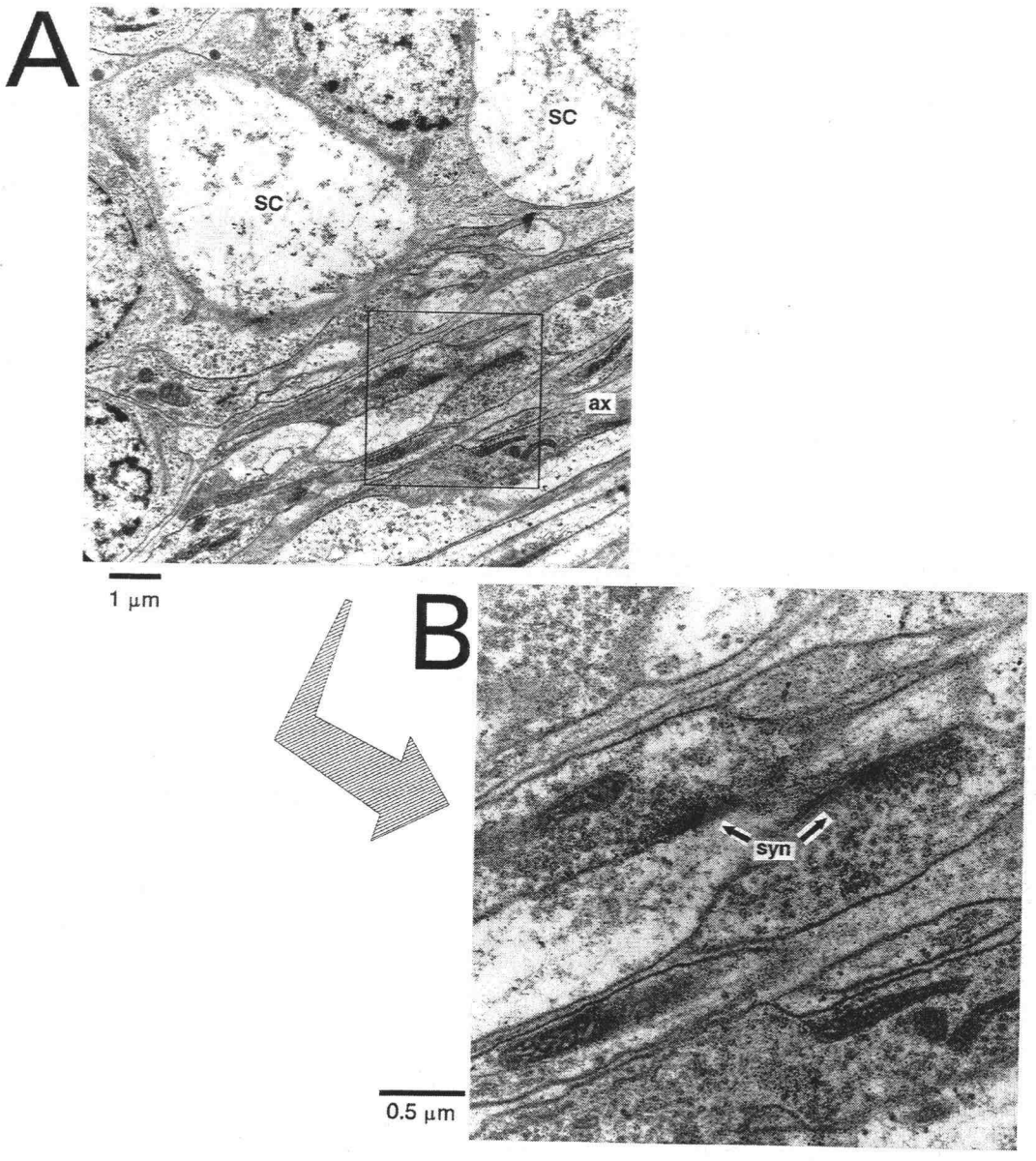


Figure 5.2 Spike correlation analysis of electrophysiological records. **A** Schematic summary of spike analysis by TSG ("Turbo Spike Grabber") software. (1) Original or playback recordings are digitized and spikes are detected by adjustable threshold discrimination. The digitized waveform of each captured spike is stored with its time of occurrence and these are played back (through DAC to computer screen) at high sweep speed for automatic (waveform template matching algorithm) or interactive-manual sorting into discrete classes. (2) Each identifiable spike type ('A1', 'A2', 'B' in this example) is displayed separately in reconstructed traces to reveal their spontaneous activity or response to stimulation. (3) Cross-correlation analysis is performed to reveal interactions between units in the original recording. The occurrence of a spike (unit 'B' in this example) is centered in a time window and another spike ('A2') is displayed in proper temporal relation to it. (4) By summing several of these windows, the frequency of one neuron's activity ('A2') relative to the referent spike ('B') can be displayed in histogram form. **B** Types of histograms generated by cross-correlating activities of neurons with inhibitory, excitatory and no interaction. Auto-correlation analysis (correlating a unit with others within its class) reveals whether a given class of spikes is produced by one or more neurons.

Figure 5.2

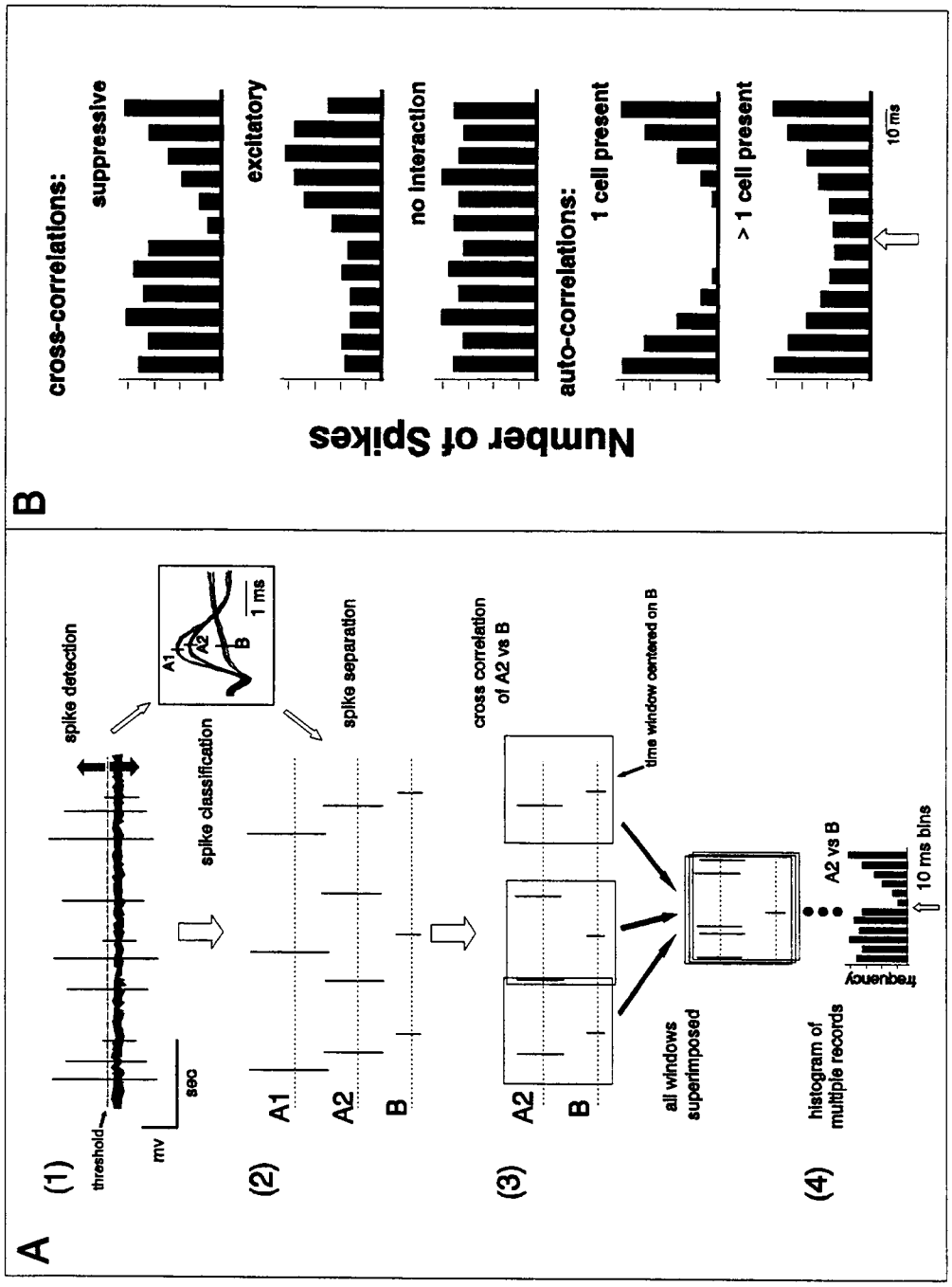

A
(1)
threshold
mv
sec
spike detection
spike classification
(2)
A1
A2
B
spike separation
1 ms
cross correlation of A2 vs B
(3)
A2
B
time window centered on B
all windows superimposed
(4)
histogram of multiple records
A2 vs B
frequency
10 ms bins
B
Number of Spikes
cross-correlations:
suppressive
excitatory
no interaction
auto-correlations:
1 cell present
> 1 cell present
10 ms

Figure 5.3 Baseline recording of spontaneous neural activity from peg sensillum of *P. mesaensis*. Units with large-amplitude spikes (labeled 'A') appear to be inhibited when smaller-amplitude units ('B') discharge.

Figure 5.3

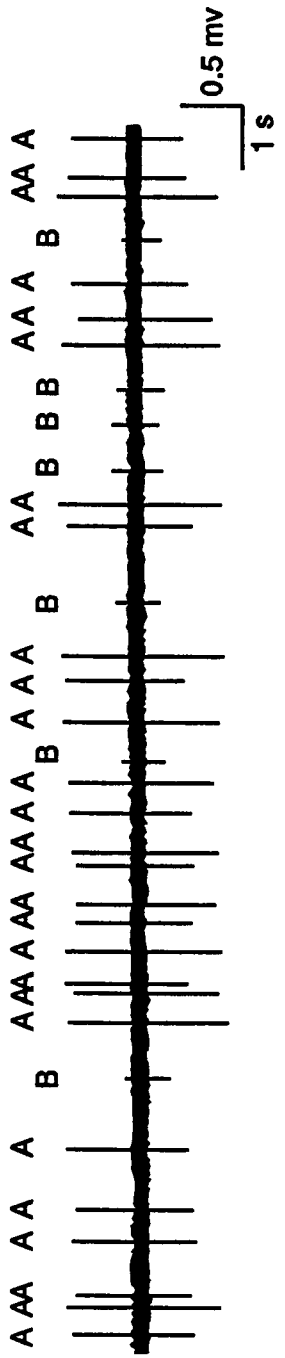


Figure 5.4 Interaction of spiking units in the absence of stimulation. Fifty-four, 10 s samples from 20 min of continuous baseline recordings (45% of total record) were categorized by computer algorithm into three separate spike classes, 'A1', 'A2', and 'B'. Histogram profiles of all possible auto- and cross-correlations of these three spike classes are shown. Upper three graphs are cross-correlations between neurons. In each case, one spike type is centered in the profile (arrow). The nomenclature 'A1' vs 'B' indicates that 'B' is the centered spike. Note the suppression of spike types 'A1' and 'A2' in period following activity of spike type 'B' (top two profiles). Spike types 'A1' and 'A2' show little to no effect in relation to each other (third profile). Bottom three graphs are auto-correlations where each spike type in a single class is referenced against itself. Patterns produced in these profiles verify that each category represents only one spiking cell.

Figure 5.4

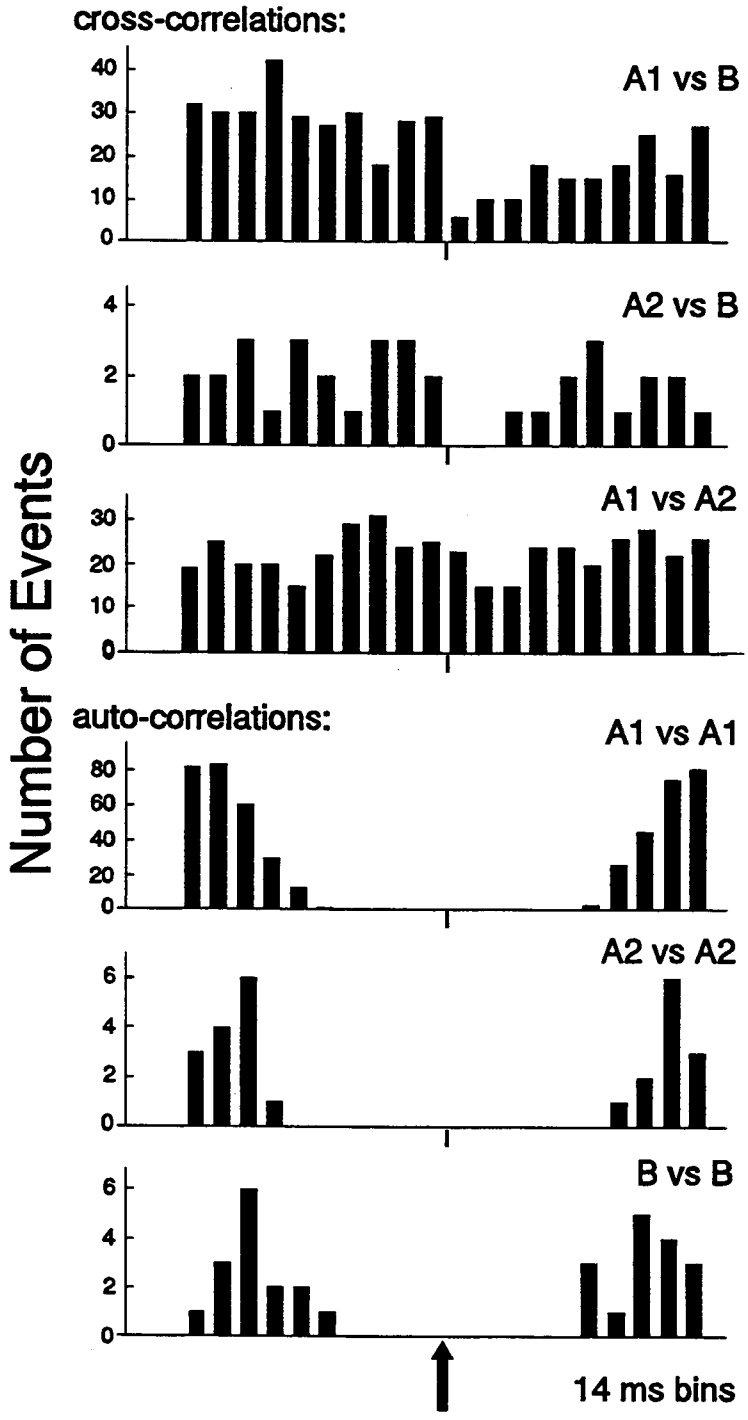

cross-correlations:
A1 vs B
A2 vs B
A1 vs A2
auto-correlations:
A1 vs A1
A2 vs A2
B vs B
Number of Events
14 ms bins

Figure 5.5 Interaction of spiking units during chemically stimulated activity. **A** Four stimulations of the sensillum by hexane were categorized by computer algorithm into two spike classes (**B**). Class 'A' probably contains more than one active unit as evidenced by auto-correlation analysis of spike class 'A' ('A' vs 'A' graph) showing lack of clearing around referenced spike. Auto-correlation of spike class 'B' ('B' vs 'B' graph) indicates only one cell as the source of these spikes. Histogram profiles are grouped by 21 ms bins. Note the inhibition of activity of type 'A' spikes immediately after the firing of spike type 'B' (top profile) and the post-inhibitory rebound in activity of type 'A' spikes beyond this period (arrow in top profile). The bottom profile is an expansion of 'A' vs 'B' into 7 ms bins to show the time course of inhibition of spike class 'A'. Asterisk indicates increased activity of 'A' immediately prior to spiking of 'B', suggesting a possible facilitative effect of unit 'A' on spiking probability of unit 'B'.

Figure 5.5

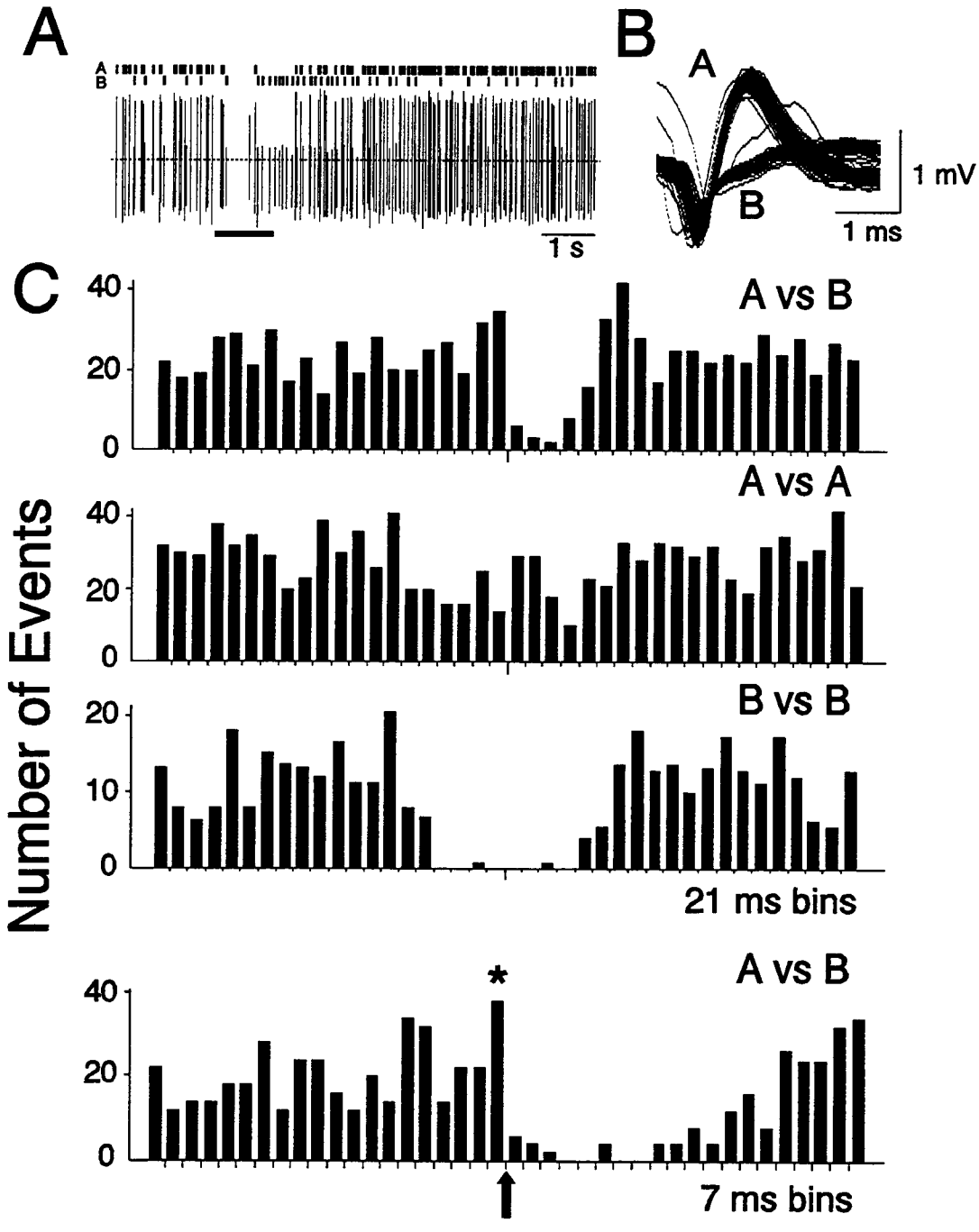

A
A
B
1 s
B
A
B
1 mV
1 ms
C
Number of Events
40
20
0
A vs B
A vs A
20
10
B vs B
21 ms bins
*
A vs B
7 ms bins

Figure 5.6 Interaction of spiking units in peg sensillum of an isolated pectine. Sixty, 10 s samples from 30 min of continuous baseline were categorized by computer algorithm into spike classes 'A1', 'A2', and 'B'. Histogram profiles are of cross- and auto-correlations of these three spike types, referenced as described in Figure 5.4. A period of suppressed activity in 'A1' and 'A2' spike types follows activity of spike type 'B'. The auto-correlation profiles verify that each spike class represents only one spiking cell and also gives an indication of the relative spiking frequencies of the three spike types.

Figure 5.6

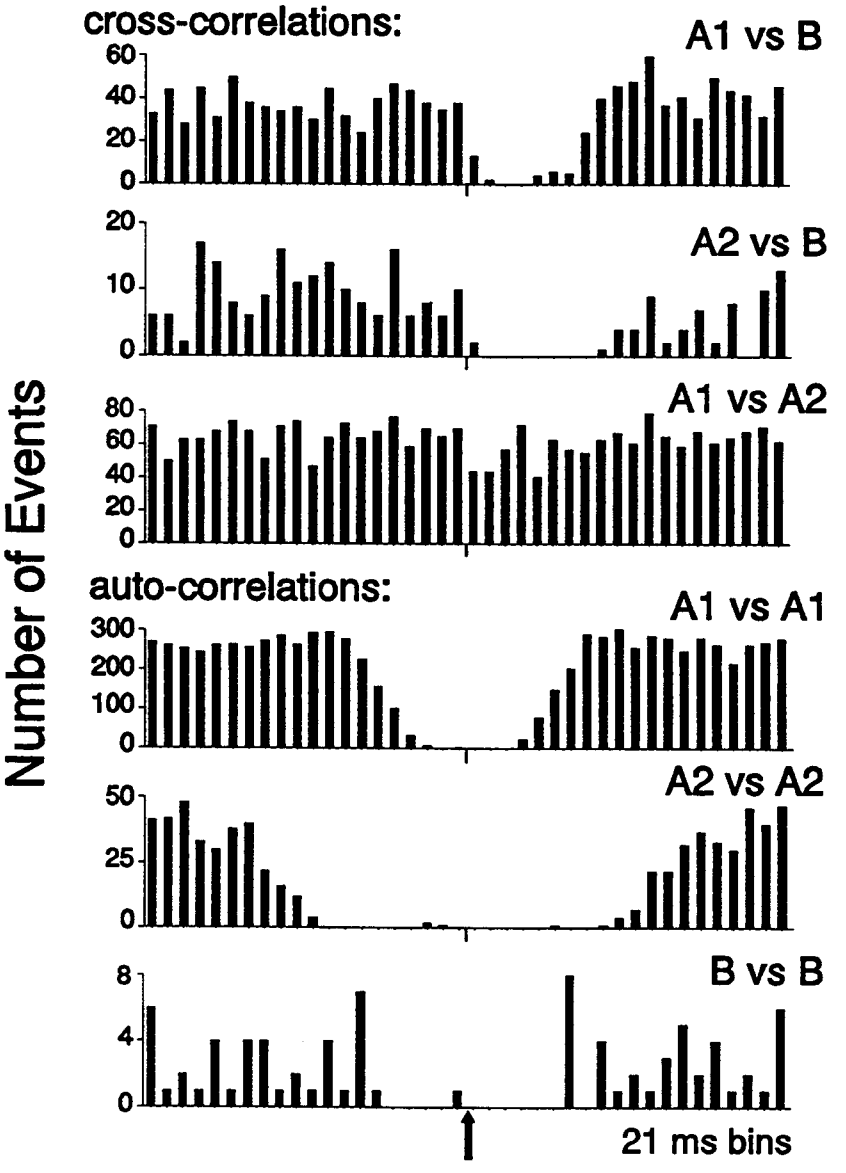

cross-correlations:
A1 vs B
60
40
20
0
A2 vs B
20
10
0
A1 vs A2
80
60
40
20
0
auto-correlations:
A1 vs A1
300
200
100
0
A2 vs A2
50
25
0
B vs B
8
4
0
21 ms bins
Number of Events

Figure 5.7 Cross-correlation of unit activities in adjacent peg sensilla. **A** Simultaneous extracellular recordings were obtained from adjacent peg sensilla (V1 and V2) and combined to produce a single data record (**B**) for correlation analysis by the procedures described in Figs. 5.4, 5.5, and 5.6. The larger-amplitude spike class (labeled 'A') from both sensilla contains more than one spiking unit as indicated by auto-correlation analysis (see histogram profiles V1A vs V1A and V2A vs V2A). **C** Histogram profiles generated from auto- and cross-correlation analysis of the four spike classes. The data show the presence of an inhibitory effect of spike type 'B' on the activity of the type 'A' spikes within each sensillum (V1A vs V1B and V2A vs V2B profiles) but no apparent interaction between units from adjacent sensilla (V2A vs V1B, V1A vs V2B, V2A vs V1A, and V2B vs V1B).

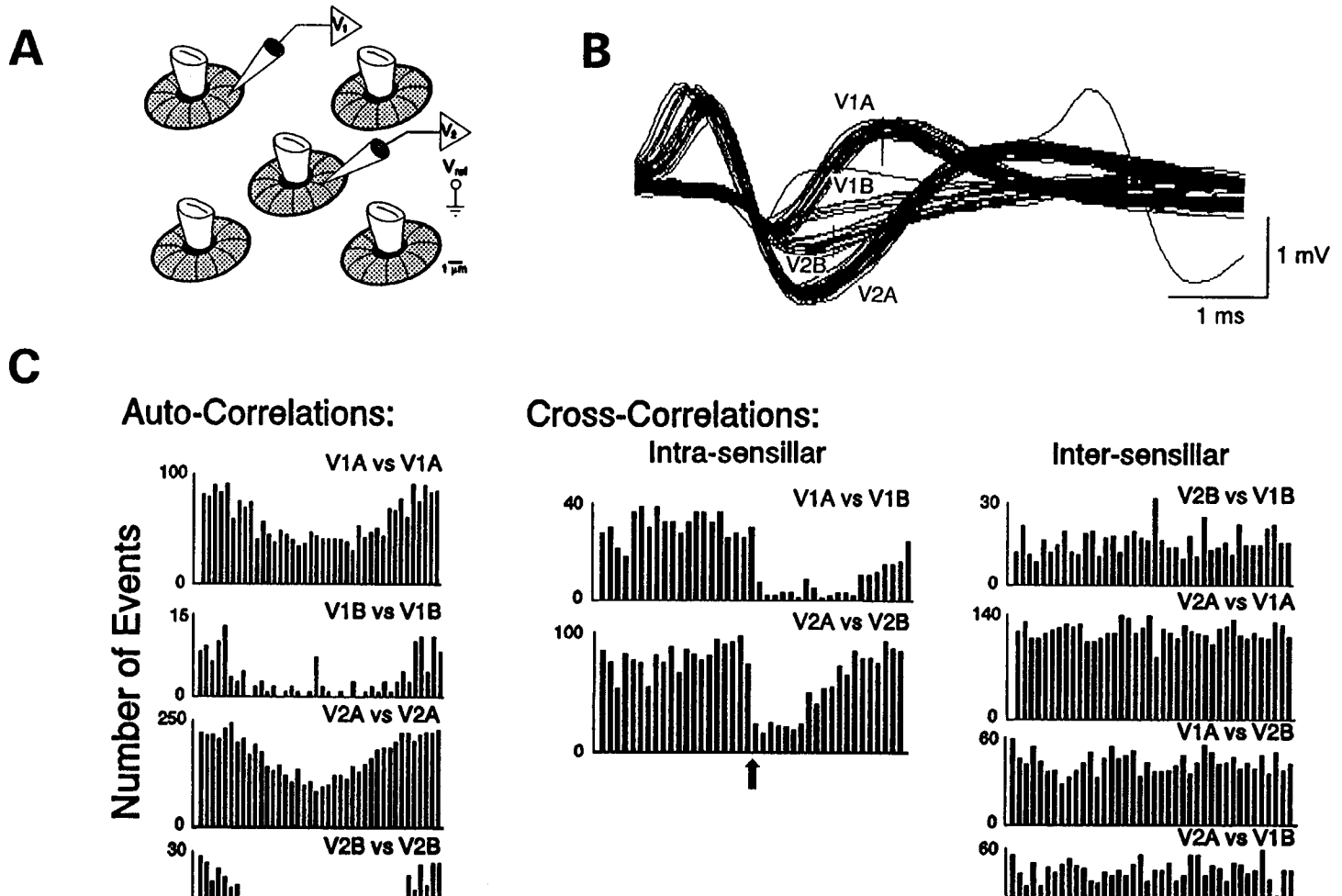

A
B
V1A
V1B
V2B
V2A
1 mV
1 ms
C
Auto-Correlations:
Cross-Correlations:
Intra-sensillar
Inter-sensillar
Number of Events
V1A vs V1A
V1B vs V1B
V2A vs V2A
V2B vs V2B
V1A vs V1B
V2A vs V2B
V2B vs V1B
V2A vs V1A
V1A vs V2B
V2A vs V1B
14 ms bins

Figure 5.7

Figure 5.8 Correlation analysis of spike bursts from type 'Y' and 'Z' units. **A** Top: Segregated record showing 7 s burst of spikes from units of type 'Y' and 'Z' amid continuous activity of spike class 'A' ('A1/A2'). Bottom traces show superimposed waveforms of each spike type. **B** Histogram profiles generated by cross- and auto-correlations of spikes within burst in record A above. Auto-correlations show composite spiking pattern of type 'A' spikes and patterns of high frequency, bursty type 'Y' and 'Z' spikes. Insets in cross-correlation profiles indicate the type of interaction suggested by each pattern. **C** Diagram of neural circuitry indicated by the patterns of activity observed in A and B.

Figure 5.8

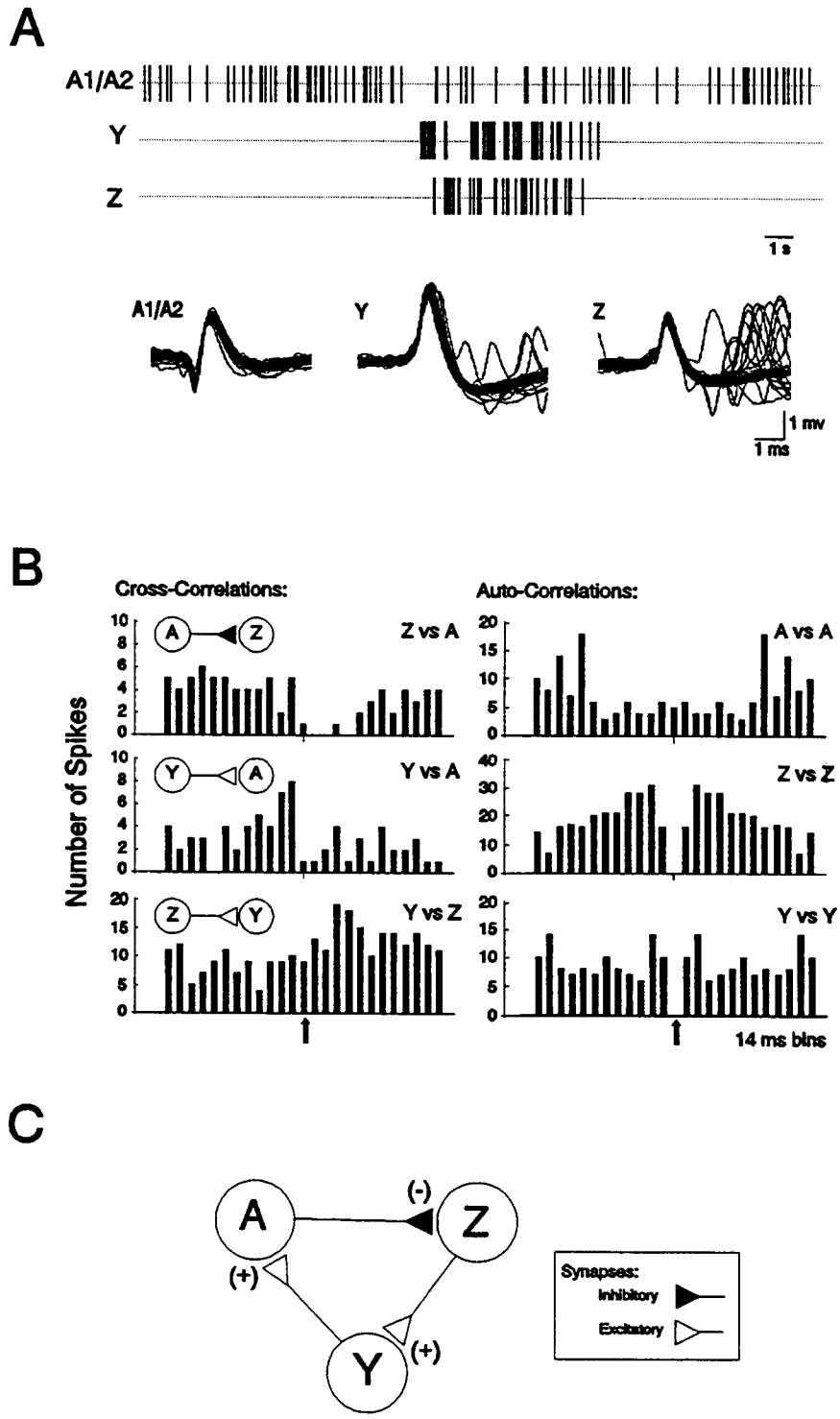

A
A1/A2
Y
Z
1 s
A1/A2
Y
Z
1 mv
1 ms
B
Cross-Correlations:
Auto-Correlations:
Number of Spikes
A
Z
Z vs A
A vs A
Y
A
Y vs A
Z vs Z
Z
Y
Y vs Z
Y vs Y
14 ms bins
C
A
Z
Y
(-)
(+)
(+)
Synapses:
Inhibitory
Excitatory

Figure 5.9 Differential sensitivity of type 'A' and 'B' units and possible function for inhibitory synaptic interaction between these cell types. **A** Segregated spike recordings of hexanol and hexanal responses showing strong response of type 'B' unit to the aldehyde (which inhibits firing of 'A') but not the alcohol (firing of 'A' continues). Solid bar indicates pulse duration of volatile pure substances blown across sensillum pore. **B** Proposed sensillar circuitry and dynamics of chemosensory interaction based on observations in A and Figs. 5.4 - 5.6.

Figure 5.9

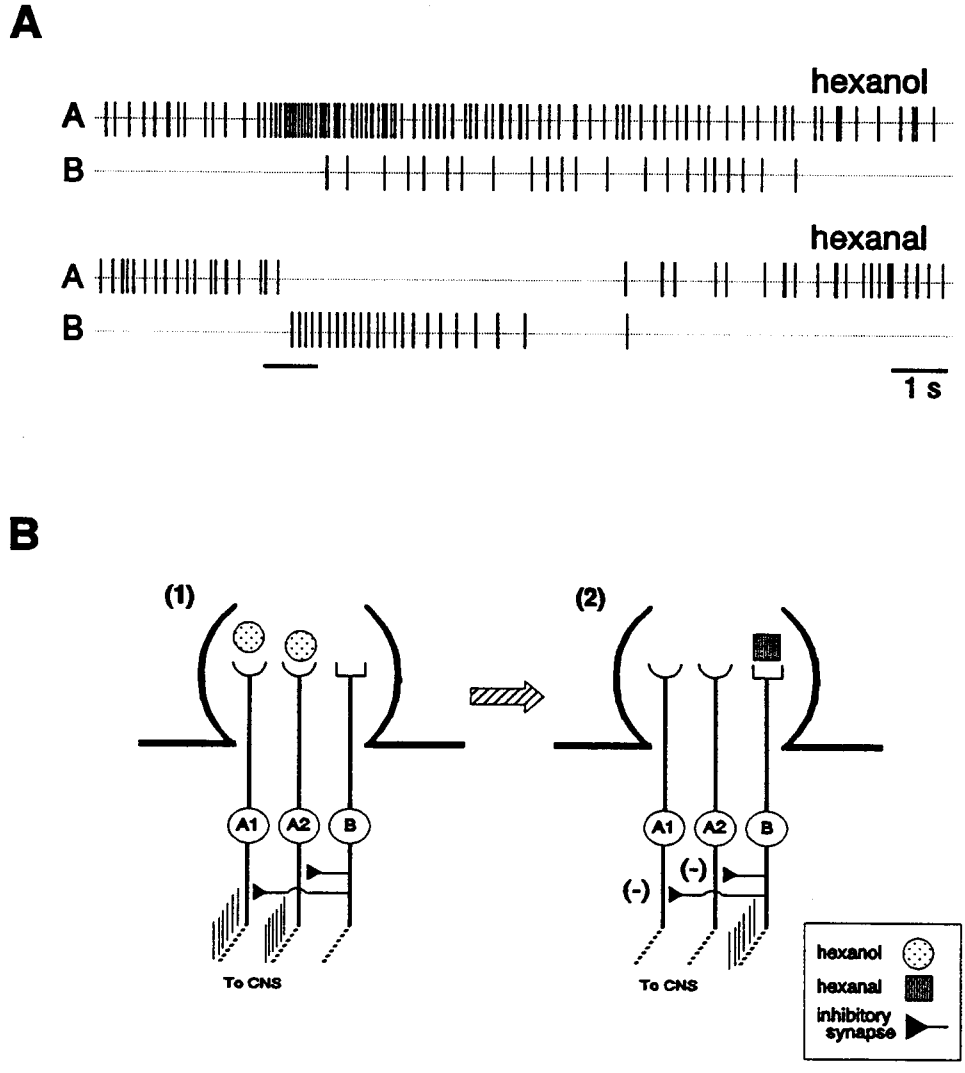
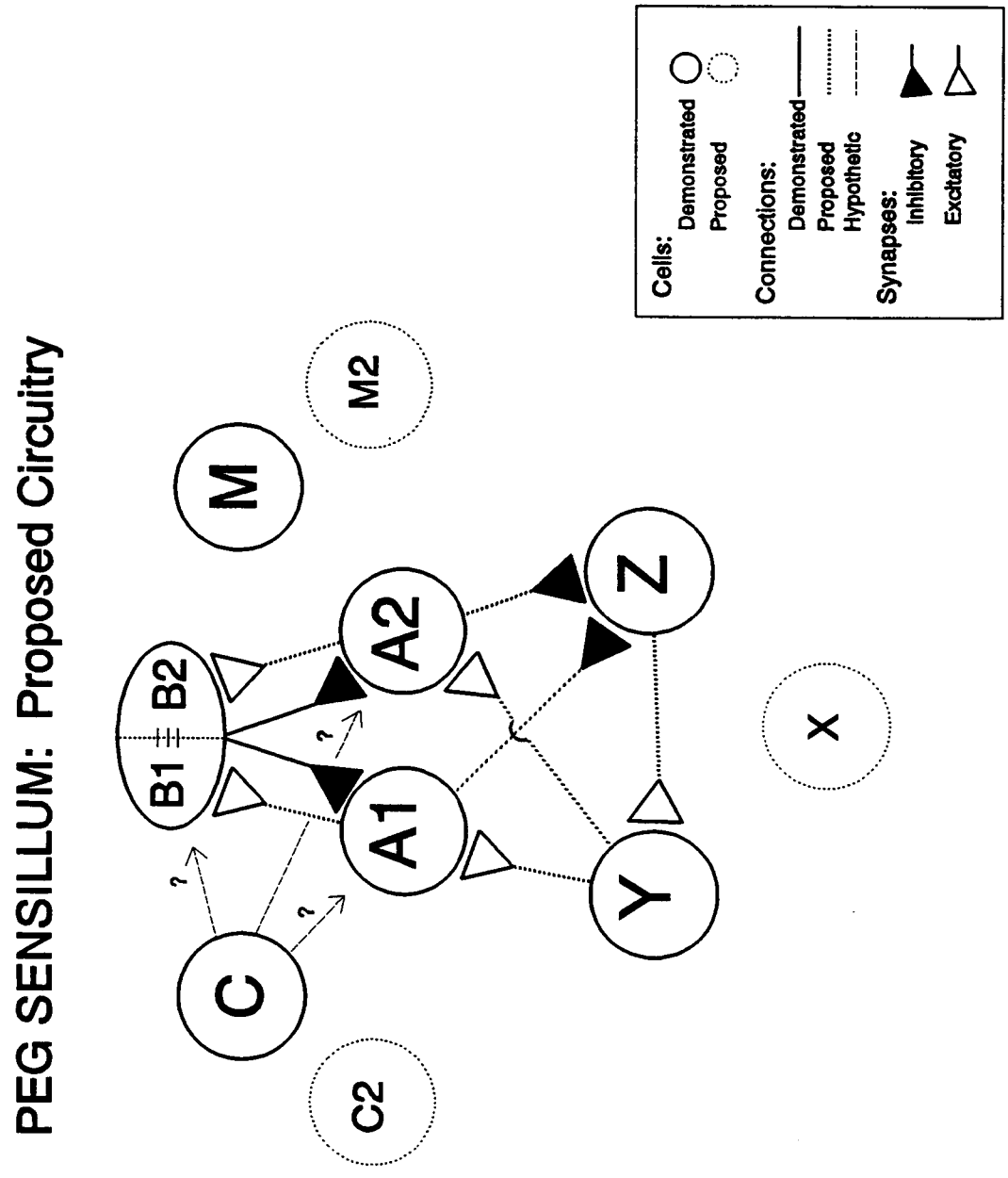

A
hexanol
A
B
hexanal
A
B
1 s
B
(1)
(2)
A1
A2
B
To CNS
(-)
hexanol
hexanal
inhibitory synapse

Figure 5.10 Proposed circuitry of peg sensillum plexus. In this diagram, proposed neurons are represented by circles and proposed connections by lines. Circles with solid borders indicate cells for which the physiological evidence is relatively strong, broken borders indicate the evidence is tentative. Unit 'B' is depicted as an electrical interaction between units 'B1' and 'B2' (see Fig. 4.10). Inhibitory (closed triangles) and excitatory (open triangles) interactions between sensillar units are drawn based on the results of cross-correlation analyses presented in this chapter; the connections are drawn as solid, dotted, or dashed to indicate the strength of the evidence for the particular interaction (see figure key).

Figure 5.10


PEG SENSILLUM: Proposed Circuitry
Cells:
Demonstrated
Proposed
Connections:
Demonstrated
Proposed
Hypothetic
Synapses:
Inhibitory
Excitatory
M
M2
B1
B2
A1
A2
Z
Y
X
C
C2
?
?
?

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