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Chemosensory Behavior and Physiology

DOUGLAS GAFFIN
PHILIP BROWNELL

Of all the sensory capacities animals possess, the ability to access chemical content of the surrounding environment is most primitive and influential: visual, auditory, and mechanosensory systems often guide animals to food and prospective mates, but it is the chemistry of contact that most often dominates the release of adaptive behaviors. It is paradoxical, therefore, that of all the senses used by animals, the chemical senses remain least understood at the physiological and biochemical levels. This is perhaps attributable to the enormous diversity of chemical substances in natural environments and to the complexity of peripheral and central nervous systems that process chemosensory information. In this chapter we describe several aspects of scorpion chemosensory physiology and behavior that make these animals favorable models for future research in this area.

Among the metazoa, mandibulate arthropods have already contributed inordinately to our understanding of the chemicals sensed by animals and to the physiological and behavioral processing of this information (Dethier, 1976; Kaissling, 1987; Derby and Atema, 1988). The success of these investigations is due largely to the comparative simplicity of chemical signals used for communication between individuals and the accessibility of their externalized organs for detection, the antennae. In the most familiar examples from insects, female Lepidoptera commonly advertise their reproductive readiness using volatile, species-specific compounds—pheromones—that powerfully activate stereotyped searching behaviors in conspecific males. Many aspects of the sensory anatomy and physiology mediating the male's behavior are known, even to the level of central neuronal circuits conveying sensory input to premotor pathways of locomotion. As we penetrate ever more deeply into studies of the insect-antennal model of chemosensory biology, it should be instructive to study other members of the phylum, particularly those using entirely different strategies and mechanisms for chemical signaling. By comparing these different but related organisms we gain insights into the evolutionary processes that shape the structure and function of chemosensory systems.

Chelicerate arthropods, particularly the several orders of terrestrial arachnids, offer many excellent candidates for comparative study. Aside from the obvious antennalike functions of nonambulatory anterior legs of uropygids and amblypygids, the malleolar organs of solpugids and the pectines of scorpions are now known to be specialized appendages of chemosensory function (Brownell and Farley, 1974; Ivanov and Balashov, 1979; Gaffin and Brownell, 1997a). Although all of these appendages are used for sensing properties of the substrate, the malleoli and pectines are especially important because, unlike any other arthropod chemosensory organ, they extend ventrally from posterior appendages. Both organs (pectines and malleoli) make intimate contact with the substrate and their sensillar microanatomy is appropriate for detection of nonvolatile substances. Thus, by gross anatomical criteria, we can consider the two great successes in terrestrial arthropod evolution (i.e., insects, arachnids) as having followed two lines of chemosensory specialization: a "chelicerate" mode using specialized ventral organs for detecting nonvolatile stimuli on substrates, and a "mandibulate" (insect, decapod crustacean) mode using anterior and dorsal appendages (e.g., antennae) more specialized for detecting olfactants in air or water.

The pectines seem especially advantageous for study of substrate-mediated chemical signaling. Although several properties of these organs suggest they are similar in function to the antennae of insects, they possess unique anatomical and physiological qualities that ensure they will become an important new preparation for basic research.

CHEMOSENSORY BEHAVIOR

In selecting appropriate model systems for study of chemosensory biology, special consideration must be given to the potential for ethological analysis. Natural behaviors are often incompatible with the laboratory environment, even when animal survivorship in captivity is good. We find in sand scorpions an excellent model for combined field and laboratory studies.

Our preferred subject for study is *Paruroctonus mesaensis* from the Mojave Desert of Southern California. This animal is comparatively innocuous, abundant, and easily maintained in the laboratory. Many aspects of its sensory biology, physiology, and ecology are well described because it is easily observed in the field and it withstands physiological manipulations in the laboratory (Stahnke 1966; Hadley 1974; Brownell, 1977; Polis, 1979, 1980). After the first molt, juvenile and adult *P. mesaensis* live singly in burrows, emerging for a few hours at night to forage for insect prey within a meter of the burrow entrance (Polis, 1980; Brownell, 1984). As ambush predators they assume motionless hunting postures and respond to vibrational stimuli created by arthropod prey (including members of their own species) moving nearby (Brownell, 1977). During summer months mature males leave the relative safety of their home burrows and wander over the dune surface in search of potential mates (Polis, 1980). Once a sexually receptive female is located, both sexes display stereotyped reproductive behaviors, including the elaborate *promenade aux deux*, a mating behavior in which the male grasps the female by the pedipalps and leads her to an appropriate substrate where a spermatophore is deposited (see Benton, this volume). Fertilization occurs when the male positions the female over the spermatophore and she takes it into her genital aperture.

Given the nocturnal habits of *P. mesaensis* and the limited visual and auditory abilities of scorpions in general, there is much uncertainty about the sensory information that guides the male scorpion to reproductively mature females. Several anecdotal observations indicate that chemical cues may direct the mate-finding behavior. For example, researchers commonly find clusters of two or more males near burrows of conspecific females, suggesting that species-

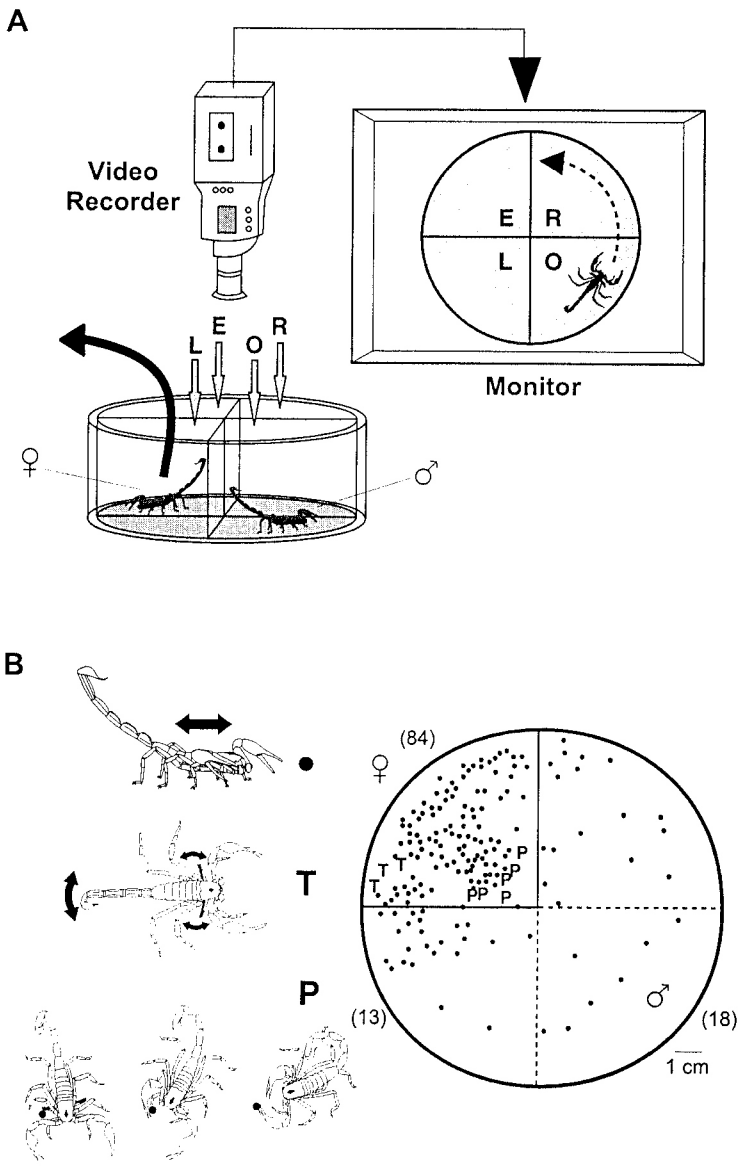

Figure 7.1 Behavioral evidence of chemosensitivity in the sand scorpion *P. mesaensis*. (A) Video monitoring of scorpion movements through quadrants in a round arena. The test animal is restrained behind a fence in the quadrant opposite the test stimuli. A trial is initiated by lifting the partition, allowing the responder access to the remainder of the arena. Stimuli include natural deposits from conspecific females and organic washes from sympatric and conspecific scorpions. Illumination is via a fluorescent black light bulb. (B) Response of male *P. mesaensis* to substrates containing cuticular extracts (chloroform:methanol, 2:1, dried onto 1 g sand) from conspecific female. Occurrence of three stereotyped behaviors, juddering (·), tail wagging (T), and pedipalp reaching (P), are indicated for the first 5 min of animal movement. The frequency of pectine taps on the substrate per minute is indicated by numbers in parentheses for responding male as it first moved from the initial quadrant (O) through quadrant L and into the treated quadrant (E). (Adapted from Gaffin and Brownell, 1992).

specific chemical signals may be the male attractant (G.A. Polis, personal communication). Also, before males make direct contact with females, they commonly display stereotyped courtship behaviors, such as "juddering," a vibrational movement suspected to be a mate-calling behavior at close range. Other workers have observed that males of *Pandinus imperator* and *Bothriurus bonariensis* judder or show other pre-courtship behavior after contact with objects or substrates previously exposed to female conspecifics (Krapf, 1986; Peretti, 1995), further suggesting that chemical signals may stimulate mating behavior. The communicative importance of juddering is unknown, but it may alert the female to an approaching male and subsequently suppress her reflexive predatory behaviors. The risk of predation or cannibalization during mate search (Polis and Farley, 1979a) underscores the importance of accurate and effective communication between these animals.

The importance of chemical signals in guiding mating behavior of *P. mesaensis* can be investigated in the laboratory. One method of study is shown in fig. 7.1A. When males encounter sand substrates previously occupied by conspecific females, their forward steps shorten and their tendency to turn increases (Gaffin and Brownell, 1992). Juddering and other precourtship behaviors (e.g., tail wagging, pedipalp reaching) are released as the response gains intensity near the center of the treated substrate. These behaviors also are elicited in response to organic extracts of female cuticle dried onto sand or filter paper substrates (fig. 7.1B). Moreover, changes in male behavior generally occur only after contact with the treated substrate, a further indication that the chemical signal is of low volatility and requires gustation or near-range olfaction for detection (Gaffin and Brownell, 1992).

CHEMOSENSORY ORGANS

If mate-finding behavior involves chemical signals associated with the substrate, which of the several sensory structures contacting the substrate are important? Numerous pore-tipped sensilla on the pedipalps and tarsal leg segments contact the substrate, where they might mediate chemosensitivity (Foelix and Schabronath, 1983). Behavioral and physiological experiments have already indicated that setaform hairs on the tarsi detect substrate-associated moisture (Gaffin et al., 1992), but their role in detection of intraspecific chemical signals has not been examined. In spiders, a porous cuticular depression on the dorsal surface of each tarsus, called the tarsal organ (Foelix and Schabronath, 1983), may detect sex-specific signals because they respond differentially to extracts derived from male and female cuticle (Dumpert, 1978). In scorpions the

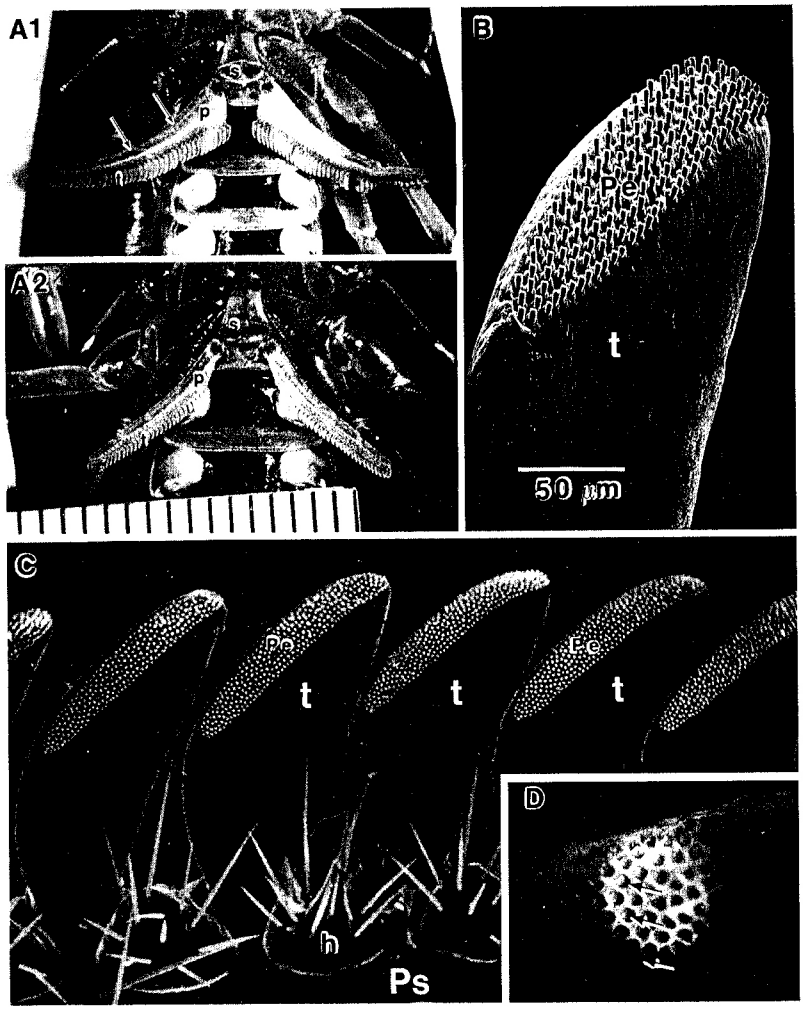

Figure 7.2 External morphology of the pectines. (A) Ventral view of *P. mesaensis* showing the larger size of pectines (p) and greater numbers of sensilla-bearing teeth (t) in males (A1) compared to females (A2) (males = 36.0 ± 1.6; females = 25.5 ± 1.4). (B) Scanning electron micrograph of distal face 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; h, tuft of mechanosensory hairs; Ps, spine of pectine. (D) Epifluorescent illumination of peg sensilla in a live animal. Arrows point to individual pegs.

tarsal organs respond to humid air stimuli (Gaffin et al., 1992), but their sensitivity to pheromonal and other chemical cues remains unexamined.

The largest and most elaborate sensory appendages of scorpions are the pectines (fig. 7.2), two ventromedial appendages that actively sweep over the substrate as the animal walks. Early morphologists considered these comblike extensions to be manipulative or respiratory appendages (see Cloudsley-Thompson, 1955, for summary of early work) until behavioral studies showed they were used as tactile organs to select substrates for placement of the spermatophore (Alexander, 1957, 1959). Neuroanatomically, the pectines were known to rival the innervation density of the antennae of insects and crustaceans (Schröder, 1908) suggesting a more important sensory role than mechanoreception. However, additional behavioral studies of the pectines only confirmed their role as mechanoreceptors (Abushama, 1964). Most important, the initial studies of pectine ultrastructure by Carthy (1966, 1968) failed to reveal pores at the tips of their numerous peg sensilla (a criterion of chemosensitivity), and Hoffmann's (1964) electrophysiological studies found only mechanosensory responses to various modalities of pectine stimulation, including direct application of chemostimulants. These findings dampened interest in further study of the organ, and their exclusive function as mechanoreceptors for substrate texture went unchallenged for almost two decades. In retrospect, these findings contributed strongly to the misconception that scorpions, and perhaps arachnids in general, lacked chemosensitivity comparable to antenna-bearing mandibulate arthropods.

The rebirth of interest in chemosensory biology of scorpions can be traced to the work of Ivanov and Balashov (1979) and Foelix and Müller-Vorholt (1983), who independently reexamined pectine ultrastructure using improved fixation techniques for scanning and transmission electron microscopy. These studies clearly showed an external opening at the terminal end of each peg sensillum in *Mesobuthus eupeus* (Buthidae) *and Euscorpius italicus* (Chactidae), respectively. Pore openings have since been found in representatives of several families, including *Paruroctonus mesaensis* (Vaejovidae), *Hadrurus arizonensis* (Iuridae), and *Pandinus gregoryi* (Scorpionidae) (Brownell, 1988, 1989). In each case the morphology of sensory and accessory cells within the pegs were typical of arthropod contact chemoreceptors, thus inviting reevaluation of their physiological and behavioral functions.

An important advance in understanding pectine function came with the realization that male scorpions may use them to detect pheromones deposited on the substrate by conspecific females. An example of this behavior is illustrated in fig. 7.1B where a male scorpion strongly increased the frequency of pectine "tapping" as it crossed substrate labeled only with the wash from the cuticle of a conspecific female. Similarly, in the example shown in fig. 7.3A, an adult male *H. arizonensis* increased the frequency of its pectine tapping as it entered the vacant home container of male or female conspecifics, but much more dramatically when the previous occupant was a mature female (Brownell, 1988). In another experiment (fig. 7.3B), the precourtship behavior of pectine-ablated males was significantly depressed relative to intact males when both were exposed to substrates treated with cuticular washes from conspecific females (Gaffin, 1994). The simplest explanation of these results is that the pectines are important organs for chemosensory identification of prospective mates.

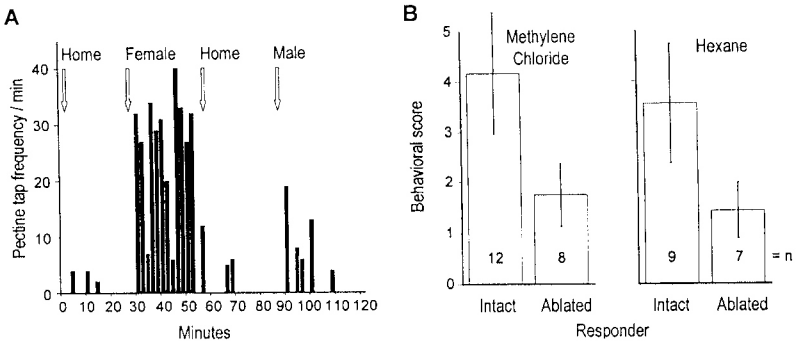

Figure 7.3 Behavioral evidence of pectine involvement in pheromone detection. (A) Frequency of substrate tapping by the pectines of male *Hadrurus arizonensis* as it is moved from its home container to a container previously occupied by a conspecific female. Placement of the male in a container used by a conspecific male failed to evoke intense tapping. (B) Effect of pectine ablation on male precourtship behavioral responses to extracts from conspecific females. Histograms show mean behavioral scores (±SE) of intact and pectine-ablated male *P. mesaensis* to methylene chloride and hexane extracts from conspecific female cuticle. Pooled cuticular extracts from 10 females were dried onto a small volume of sand; see Gaffin and Brownell, 1992, for description of scoring system. Pectines were surgically removed from animals at least 1 week before testing to allow recovery. Locomotory behavior and activity of pectineless animals were otherwise normal at time of testing.

STRUCTURE AND PHYSIOLOGY OF PECTINE SENSILLA

Sensory Morphology

The importance of the male's pectines for mate localization, courtship, and reproduction is consistent with the high degree of sexual dimorphism evident in most species. Pectines are mobile appendages with three flexible segments constituting the "spine," or marginal lamella of the pectine comb (see chapter 2). To the marginal lamellae, as few as five (*Euscorpius*), or as many as 40 (*Parabuthus*), "teeth" are attached. These teeth support dense, two-dimensional arrays of chemoreceptive setae, called "pegs" (fig. 7.2). The pectines of males are longer and typically contain more sensilla-bearing teeth than those of females (table 7.1). Although the number of teeth per pectine does not change from birth, the rate of pectine elongation in adult males may exceed that of females. For example, in *P. mesaensis*, pectine elongation in sexually mature males is greater than in immature males or females at any stage of development (Polis and Farley, 1979b). Most important, in *Paruroctonus* (Vaejovidae) and *Parabuthus* (Buthidae), the number of chemosensory pegs in males increases dramatically relative to females as they become sexually mature (Brownell, 1989) and differs in adult animals even in the few species where males and females have similar numbers of teeth (e.g., *Superstitionia donensis*) (Swoveland, 1978). Such sex-

Table 7.1 Sexual Dimorphism of Scorpion Pectines

Family	Species	Sex	No. teeth/ 2 pectines	No. pegs/ tooth	Total no. pegs	No. pegs (♂)/ no. pegs (♀)	Reference
Vaejovidae	*Anuroctonuis phaiodactylus*	♂	19	201	3,819	2.55	1
		♀	13	115	1,495		
	Nullibrotheus allenii	♂	24	160	3,840	2.75	1
		♀	17	82	1,394		
	Paruroctonus mesaensis	♂	75	1,600	120,000	13.89	1
		♀	48	180	8,640		
	Uroctonus mordax	♂	27	1,100	29,700	9.71	1
		♀	20	153	3,060		
	Vaejovis confusus	♂	34	1,000	34,000	4.31	1
		♀	27	292	7,884		
	Vaejovis spinigerus	♂	46	1,000	46,000	4.01	1
		♀	37	310	11,470		
Buthidae	*Centruroides exilicauda*	♂	50	800	40,000	1.86	1
		♀	44	490	21,560		
	Parabuthus pallidus	♂	72	1,650	118,800	1.82	2
		♀	62	1,050	65,100		
Chactidae	*Superstitionia donensis*	♂	12	800	9,600	1.63	1
		♀	12	490	5,880		
Diplocentridae	*Didymocentrus comondae*	♂	17	1,050	17,850	7.83	1
		♀	15	152	2,280		
Scorpionidae	*Pandinus gregoryi*	♂	36	1,400	50,400	1.65	2
		♀	34	900	30,600		

References: (1)Swoveland, 1978; (2) Brownell, unpublished data.

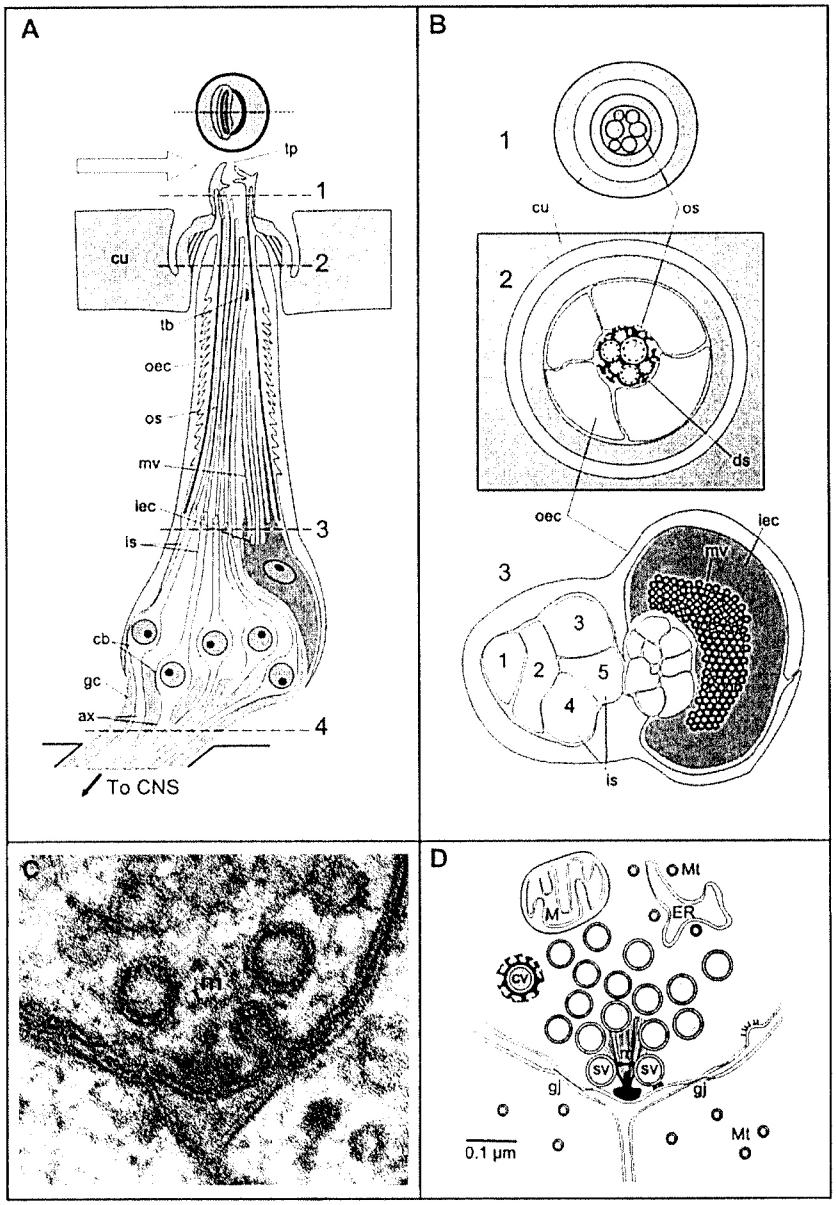

Figure 7.4 Morphology of peg sensilla. (A) Diagram of a longitudinal section through sensillum showing arrangement of dendrites, cell bodies, inner and outer enveloping cells, and structural elements (after Ivanov and Balashov, 1979; Foelix and Müller-Vorholt, 1983). (B) Cross-sections taken from levels 1–3 of panel A showing (1) double-walled shaft containing several dendritic outer segments (os) inside receptor lymph; (2) cuticular base of sensilla, extensions of outer enveloping cells (oec), dendritic sheath (ds), and dendritic outer segments with microtubules arranged at periphery; and (3) regular arrangement of five dendritic inner segments (is) relative to microvillar (mv) projections of inner enveloping cell (iec). (C) Typical dyad synapse and axo-axonic profiles as seen just below sensory cell body layer in pectine (level 4 in panel A) of *Androctonus australis* (×82,500; courtesy of R. Foelix). (D) Diagram of a dyad synapse; m, capping membrane; sv, synaptic vesicles; M, mitochondrion; ER, smooth endoplasmic reticulum; Mt, microtubules; cv, coated vesicles; gj, gap junctions. (From Foelix, 1985 with permission).

ual and developmental differentiation is strongly suggestive of a function related to reproduction.

Blanchard (1853) was first to show that the pectines are richly innervated, and Gaubert (1889) and Schröder (1908) showed that most of the neural elements extend through the teeth and into the peg-shaped sensilla. By comparison to other chemosensory organs of arthropods, Schröder (1908) concluded that the pectines were both mechanosensory and chemosensory in function and were perhaps important in mediating sex recognition in scorpions. Nearly a century later these insightful speculations from histological observations are largely confirmed.

The peg sensilla of scorpions have many structural features in common with chemosensory sensilla of insects (Foelix and Müller-Vorholt, 1983). In external appearance the pegs look like truncated hairs extending from circular sockets spaced 2–10 μm apart. In *P. mesaensis* the pegs are about 2 μm in diameter and 8 μm long, with double-walled shafts that gradually flatten toward the peg tip (Fet and Brownell, unpublished data). Here, a slit-shaped pore opens to a fluid-filled chamber inside the sensillum (fig. 7.4). The reported number of dendrites varies from 10 to 18 per peg (Ivanov and Balashov, 1979; Foelix and Müller-Vorholt, 1983; Brownell and Adams, unpublished data). Most of the bipolar sensory neurons extend through the sensillar chamber to within a few microns of the slit opening. Foelix and Müller-Vorholt (1983) described one neuron as a mechanoreceptor having a characteristic tubular body in a dendrite terminating near the peg base. The dendritic outer segments are surrounded by a dendritic sheath and extensions of outer enveloping cells that appear to isolate the outer segments from hemolymph. At the level of the ciliary regions of the dendritic inner segments are numerous microvillar projections from an inner enveloping cell. The electron-dense inclusions of this microvillar cell are perhaps secretory vesicles containing substances unique to fluids of the sensillar chamber, including putative odorant-binding proteins (Slipher, 1970; Foelix and Müller-Vorholt, 1983; Bulseco and Brownell, 1989). The inner segments of the dendrites are short (<10 μm) compared to the outer segments (20–40 μm). The inner segments of five of the sensory neurons, or about one-third of the sensory cells of each peg, show a highly regular array opposite the microvillar projections of the inner enveloping cell (Brownell, 1989). This reveals a polarity in the arrangement of dendrites within each peg and may provide a means of identifying individual sensory neurons within each sensillum for correlation with physiological properties (see below). The cell bodies of sensory cells are spindle shaped and form a layer or "plate" of somata approximately 50 μm below the cuticular surface.

An interesting feature of peg sensillum morphology is the appearance of chemical synaptic profiles between sensory cell axons. These synapses are lo-

cated just below the somata region where axonal fibers fasciculate into bundles wrapped by glial cell extensions (Foelix and Müller-Vorholt, 1983). The most common pattern of contact is the dyad type, where one fiber is presynaptic to two others (fig. 7.4C,D). Presynaptic cells are distinguished by the presence of synaptic vesicles and bars, capping membranes and presynaptic organelles such as mitochondria and endoplasmic reticulum (Foelix, 1975, 1985). The axons vary markedly in size, and it is not known if all of these sensory axons continue to the central nervous system. It is conceivable that only a subset of these neurons project axons to the scorpion brain, as commonly observed for retinal sensory systems. This question can be resolved by counting the number of neural elements at several levels along the neural projection to the central nervous system.

Morphological evidence of synapses between peripheral sensory neurons is rare among insects, but such synapses may be a common feature for arachnids, having been also described in the antennaform first-leg pair of whip spiders (amblypygids) and harvestmen (Opiliones) (Foelix, 1975). Only recently have there been physiological studies to corroborate this morphology. Below, we describe methods for recording neural activity from pectinal peg sensilla as well as physiological evidence of interactions between active neural elements within these structures.

Electrophysiological Response

Electrical recordings from individual sensory receptors indicate their mode of response and sensitivity to natural stimuli. For peg sensilla on the pectines, the impulse activities of individual sensory neurons can be used to assay the organ's response to potential food and pheromonal stimuli. As a rule, electrophysiological recordings are most useful when applied to sensilla containing few sensory cells, as in pheromone-sensitive hairs of saturniid moth antennae (Kaissling, 1987). Peg sensilla of the pectine contain as many as 18 sensory neurons, but fewer than one-third of these are spontaneously active. Furthermore, these units discharge at low rates (<5 Hz) and can be identified individually by their impulse waveform. These features make it possible to access the most effective stimuli for several of the peg sensillar neurons.

In a typical electrophysiological preparation of scorpion pectine, the animal is anesthetized (by cooling or CO_2) and fixed ventral-side up to a rigid stage. The pectines are further immobilized on a small platform using double-sided tape to arrange the teeth for maximal exposure of the peg fields. A recording electrode with a tip diameter of <0.1 μm is inserted through the flexible cuticle at the peg base (fig. 7.5A), and a reference recording electrode is placed in contact with hemolymph at some distance from the recording site (e.g., soft cuticle at the distal end of the pectinal spine). Conventional electronic instrumentation is used to amplify, visualize, and record the electrical signals for later analysis. In this recording configuration, chemical stimuli can be applied to the sensillar pore directly (gustatory stimulation) or blown across the preparation as a volatile gas (olfactory stimulation). Such recordings are stable for several days without appreciable degeneration of signal form (Gaffin and Brownell, 1997a). The spontaneously active sensory cells produce extracellular action potentials of approximately 1–2 mV, typically with a signal-to-noise ratio >5. Because hundreds of peg sensilla are accessible within small regions of a single tooth, a

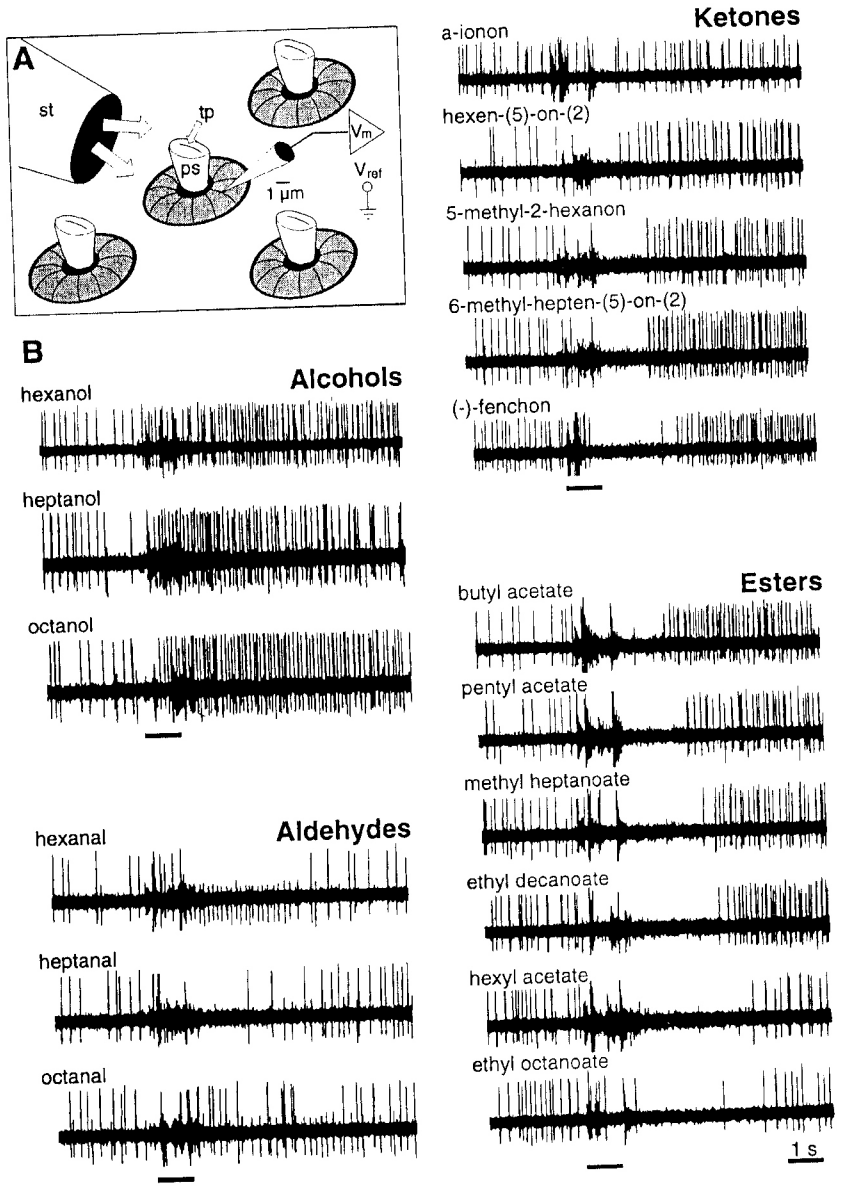

Figure 7.5 Electrophysiological response of peg sensillum to general chemical stimuli. (A) Extracellular recording configuration from peg sensillum. Electrolytically etched tungsten electrode (V_m) is inserted through the flexible cuticle at the base of peg sensillum (ps), allowing stimuli (st) access to terminal pore (tp); reference electrode (V_{ref}) is placed in contact with hemolymph at some distance from recording electrode. (B) Unfiltered, multiunit responses of a single peg sensillum to stimulation by pure substances. Volatile alcohols, aldehydes, ketones, and esters were blown in 1-sec pulses across the sensillum pore. Solid bars at bottom of traces indicate the stimulus duration.

single animal preparation can quickly generate a profile of sensillar responses to many different substances (Gaffin and Brownell, 1997a).

Individual peg sensilla on *P. mesaensis* respond strongly to stimulation with short-chained organic compounds blown across the sensillar pore (Gaffin and Brownell, 1997a). Representative responses of a single peg sensillum to stimuli from four molecular families (alcohols, aldehydes, ketones, and esters) are shown in figure 7.5B. The high threshold for olfactory stimulation suggests that the peg sensillum functions best as a contact chemoreceptor. In some sensilla, simple, straight-chained alkyl-alcohols (C_6–C_8) excite large triphasic units in a dose-dependent manner, whereas aldehydes (C_6–C_8) excite a small, biphasic unit, which appears to inhibit firing of the larger units. Stimulation with ketones and esters of the C_6–C_{11} range initially inhibit, then tonically excite, activity of the large units. In most cases the time course and intensity of a response depends on the concentration and type of stimulus. Thus, although scorpion peg sensilla have morphology typical of contact chemoreceptors and appear to be used for gustation of the substrate, they are capable of responding vigorously to olfactory stimuli. This convenient method of stimulation allows for efficient investigation of their chemosensory response properties.

SYNAPTIC INTERACTION BETWEEN CHEMOSENSORY NEURONS

Arthropod chemoreceptor cells typically fire at rates that vary smoothly over time as a function of stimulus concentration. As clearly evident in fig. 7.5, some neurons within the peg sensilla respond unevenly to stimulation. There is reason to believe that this interrupted pattern of activity is the product of synaptic interactions between these sensory cells. For example, in fig. 7.6, the spontaneous firing pattern of a large unit (labeled A in the figure) is interrupted when a smaller unit (labeled B) begins to discharge. The effect of B on A appears to be inhibitory because the larger spike tends not to fire immediately after the occurrence of the smaller spike. Synaptic interactions between chemosensory neurons of a single sensillum are virtually undescribed for insect olfactory systems where afferents generally make their first synapses only after entering the central nervous system (Homberg et al., 1989).

Cross-Correlation Analysis of Sensory Neuron Activity

Putative synaptic interactions between two neurons, either excitatory or inhibitory, can be examined physiologically by showing dependence of one cell's

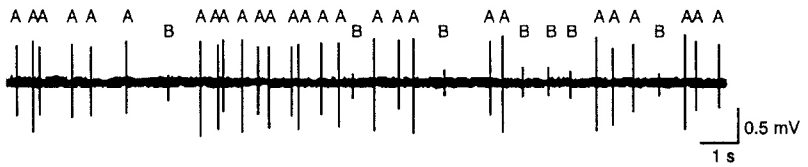

Figure 7.6 Baseline activity from peg sensillum showing interruptions of spiking activity of large unit A by small unit B. From Gaffin and Brownell (1997b), with permission.

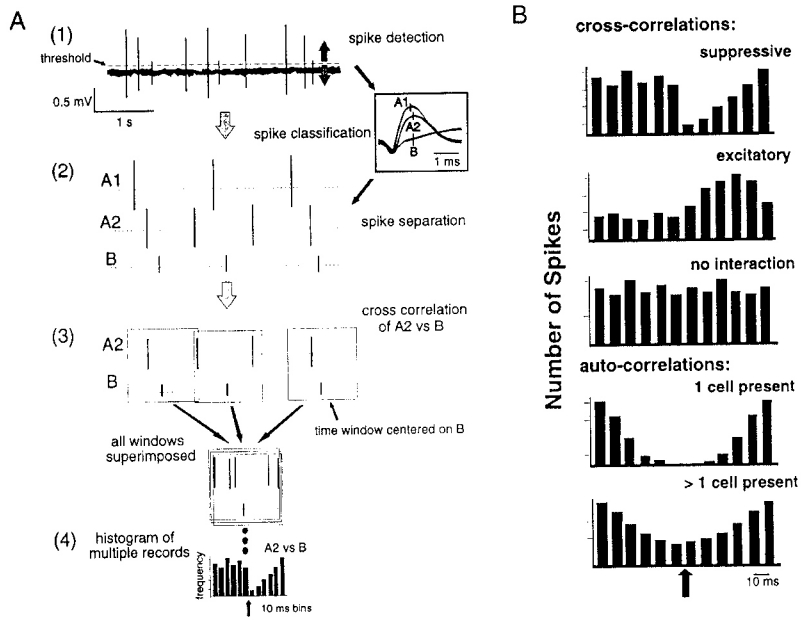

Figure 7.7 Automated spike sorting methods. (A) Flow chart of computer algorithm used in spike sorting and interspike interaction analysis (see text for details). (B) Hypothetical correlation histograms representing various types of cell–cell interactions. Top three profiles are cross-correlations, bottom two profiles are autocorrelations (see text for details). From Gaffin and Brownell (1997b), with permission.

activity on another within a brief (<50 msec) period. To identify single units within the complex multiunit recordings obtained from peg sensilla (fig. 7.7A), spike waveforms for all active units are categorized and parsed by computer so that a template, or averaged waveform, of each cell's electrical output can be identified. Once the integrated activity of the cells in one sensillum is sorted by shape, the activity of individual neurons can be segregated from the multiunit record and displayed as individual traces (fig. 7.7A). Cross-correlation analysis of these traces is then used to reveal interactions between the cells.

For correlation analysis, all spikes of one type, the "reference cell," are detected, recorded, and redisplayed as superimposed impulses in the center of a time window. Spikes from a second class of neuron ("target cell") are then displayed relative to the reference cell's activity, and the number of target cell spikes falling within discrete bins of time before and after the reference spike are counted and displayed in histogram form (fig. 7.7A). In this way, the timing of one sensory neuron's activity can be seen relative to the activity of any other. Possible synaptic interactions are revealed as an increase (excitation) or decrease (inhibition) in spike activity of the target cell relative to the reference cell.

In fig. 7.7B, several hypothetical examples of correlation histograms are shown for possible types of interactions between sensory cells. The top three

histograms show cross-correlation profiles expected for synaptically coupled cells that suppress (inhibit), excite, or have no interaction. The bottom two profiles are correlations of the activity of a spike class referenced against itself (autocorrelation). Autocorrelations provide evidence that all spikes assigned to a particular spike class originate from a single neuron. The first autocorrelation profile shown in fig. 7.7B predicts activity that would be expected if only one cell were active. Autocorrelations for a class containing more than one cell would appear as shown in the bottom profile (see Eggermont, 1990, for further description of correlation analysis). Through such comparisons, the characteristics of synaptic connectivity within a discrete circuit of neurons (e.g., peg sensilla) can be defined.

Evidence of Synaptic Interactions within Peg Sensilla

When correlation analysis is applied to electrical recordings from a single peg sensillum, a clear indication of synaptic interaction between its sensory neurons emerges. In the example of fig. 7.8, the occurrence of a small, biphasic spike of distinctive waveform appears to inhibit the activities of two other sensory cells for a period of about 100 msec. Figure 7.8B shows all possible cross- and autocorrelations of the three classes of spikes for 30 min of unstimulated activity. The small biphasic unit (spike class B in fig. 7.8) clearly suppresses the activity of the two larger triphasic units (A1 and A2), while A1 and A2 have no apparent mutual effect. The immediate suppression of A1 and A2 following activity in B and the appearance of a rebound effect in the activity of the suppressed spike (e.g., see A1 versus B histogram about 200 msec after reference spike B in fig. 7.8B) are further indications of synaptic, as opposed to receptor, level of interaction. Because these records were obtained from a surgically isolated pectine, the site of interaction between these sensory neurons must be local and not involve feedback from the central nervous system (Gaffin and Brownell, 1997b). Although the generality of these sensory interactions in scorpions is unknown, they have been observed in each of five species (representing four families) sampled to date (Vaejovidae, *P. mesaensis;* Iuridae, *Hadrurus arizonensis;* Buthidae, *Parabuthus gregoryi, Androctonus australis;* Scorpionidae, *Pandinus gregoryi;* Gaffin and Brownell, unpublished data).

CONCLUSIONS

It is evident from these initial investigations that scorpions, and probably arachnids as a group, have been neglected as experimental systems for the study of chemosensory biology. As in other arthropods, numerous chemotactic hairs are distributed across the body surface and appendages of scorpions, but these channels pale in comparison to the pectines, which, by numbers of primary sensory neurons, rank among the most densely innervated sensory organs in arthropods. The accumulated findings of anatomical, behavioral, and physiological research indicate that these unique structures mediate at least two crucial roles in the reproductive biology of scorpions: chemosensory identification and localization of potential mates, and tactile discrimination of suitable substrates for spermatophore deposition. A strong sexual dimorphism of the pectines in most species reflects this role in mating. But other behaviors, such as feeding,

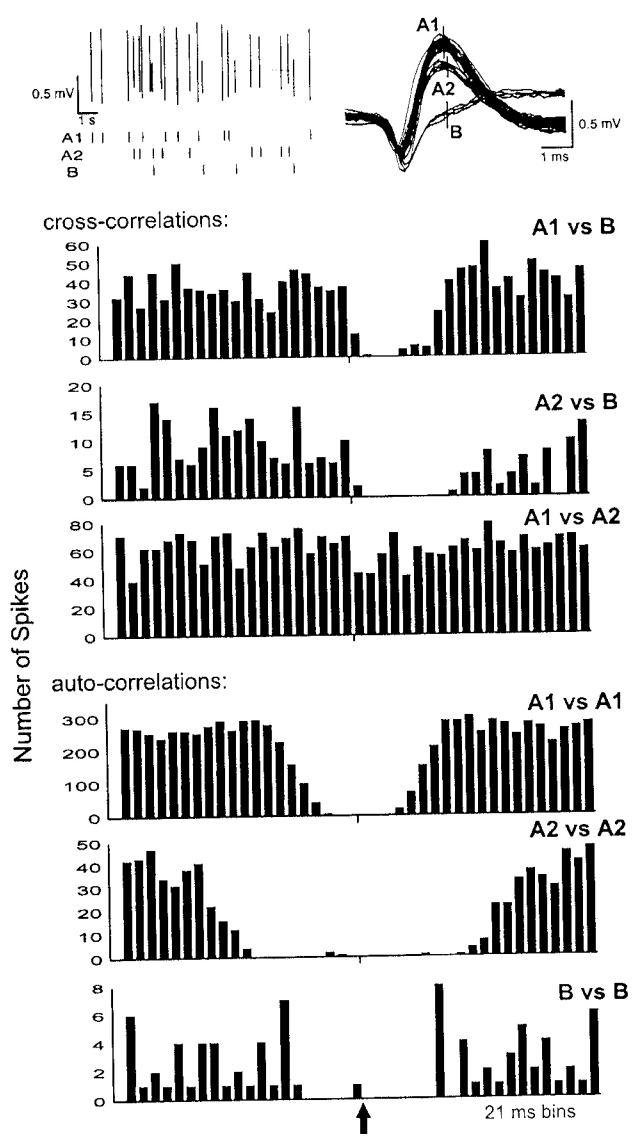

Figure 7.8 Physiological evidence of interaction between spiking units in a single sensillum. (A) Sample of baseline recording from peg sensilium from a detached pectine of *P. mesaensis.* This record has been filtered and categorized by computer algorithm into three spike classes (A1, A2, and B). Sixty of these 10-sec records, sampled from 30 min of continuous baseline activity, were used to create the histograms shown in panel B (see fig. 7.7 for procedure). (B) Histogram profiles of inter- and intraclass interactions grouped by 21-msec bins. The nomenclature "A1 vs. B" indicates that B is the centered (reference) spike. Note the period of suppression of activity of spike types A1 and A2 in reference to the activity of B (top two profiles). Spike types A1 and A2 show no apparent effect in relation to each other (third profile). Bottom three profiles are autocorrelations, the result of referencing each spike type against itself. Adapted from Gaffin and Brownell (1997b).

predator avoidance, and navigation, are also influenced by chemical cues, raising the possibility that future studies will expand our knowledge of the organ's greater utility to the animal. Finally, in thinking about their function, it is noteworthy that pectinal appendages have retained similar form in all scorpions since their first appearance on Silurian aquatic species (Kjellesvig-Waering, 1986). Whatever the pectines do for modern terrestrial scorpions, it is possible that they worked as well on water-submerged substrates with little or no alteration of form.

Because of their clear specialization for use on substrates, the pectinal organs are an important addition to the arsenal of experimental systems used for research in chemical ecology. Compared to odorants transported by air or water, we know little about the nature of chemical signals deposited on substrates or their mode of detection. For example, nonvolatile chemical stimuli attached to solid surfaces could have physical shape that conveys useful information about the signal source. Such spatial information may be detectable by a two-dimensional array of sensors, such as the fields of peg sensilla on the scorpion's pectines, which project to ordered glomeruli in the scorpion brain (Brownell, 1998; Brownell, this volume). Chemical signals transported in fluid media—air or water—have unstable spatial structure, and this may account for the general absence of topography in the olfactory pathways of most animals. By developing a model system for investigating substrate-mediated chemical signaling, we may begin to understand how physical structure of chemical deposits is encoded by nervous systems.

Apart from its value as an arachnid model for comparative chemosensory studies, the pectinal sensory system has utility for basic studies of information processing. Peripheral synaptic connections have been recognized morphologically and/or physiologically in several arthropods including Xiphosuran chelicerates (Griffin and Fahrenbach, 1977; Hayes and Barber, 1982), various arachnids (Foelix, 1985), and insects (Moulins and Noirot, 1972; Steinbrecht, 1989; Gödde and Haug, 1990; White et al., 1990), but in scorpion pectines these connections are particularly prevalent and accessible for physiological analysis. Each peg sensillum contains 10–18 sensory cells, a rather large number compared to other chemotactic sensilla in arthropods (Slipher, 1970; Foelix and Müller-Vorholt, 1983). The potential complexity of response from each peg is increased by the apparent synaptic interaction between several of these cells. Yet the electrical signature of individual cells is sufficiently different that the activity of any one unit can be resolved within a multiunit extracellular record (Gaffin and Brownell, 1997b). This makes possible a physiological dissection of the integrated response from complex chemosensory circuits outside the central nervous system.

The morphological and physiological evidence of synaptic connectivity within individual peg sensilla suggests new lines of research on the neurobiology of processing of chemical and mechanical information. Among the important questions to ask are: What is the role of sensory cell interaction in processing sensory input from natural chemical signals (e.g., pheromones, potential food, or predators)? Do the synaptic interactions between neurons of the same sensillum extend to neurons of neighboring sensilla? Are mechanoreceptors within the sensillum gating or otherwise affecting the response of chemosensory cells? Development of methods to directly stimulate individual pegs (chemically and mechanically) will be useful for characterizing and quantifying response patterns to more natural modes of stimulation. Of paramount importance will be the identification and purification of the species-specific pheromonal substances detected by the pectines. Apart from their obvious importance to the coordination of reproductive behavior of these animals, pheromonal stimulants would provide a powerful tool for analyzing circuit-level response and the encoding of information of great behavioral significance to the animal.

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