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Analysis of sensory processing in scorpion peg sensilla

Douglas D. Gaffin: Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019-0235, USA. E-mail: ddgaffin@ou.edu

Abstract. Primary chemosensory afferents within each peg sensillum on scorpion pectines contain a dense plexus of synaptic contacts of unknown importance to informational processing within this simple sensory structure. These connections probably contribute to the processing of chemical signals from the substrate to the encoded pattern of spike activity ascending the pectinal nerves to the CNS. A key finding of earlier studies of this system was the apparent existence of strong and long-lasting inhibitory interactions between one identifiable unit – type "B" cells – and at least two other sensory neurons – identified as "A1" and "A2" – cells within the same sensillum. Because peripheral synaptic interactions are rarely observed between primary sensory neurons, it is important to reject the alternative non-synaptic mechanism to account for the unusual spike waveform of inhibitory B units, namely, that it is derived from coincident discharge of the A1 and A2 units it is presumed to inhibit. High resolution waveform analysis of two or more units firing in close temporal proximity (within about 5 ms) showed unequivocally that type B units occur within the post excitatory period when the A units would be refractory to re-excitation. Furthermore, the number of these B/A1 or B/A2 doublets was in line with the number predicted for the observed spontaneous firing frequency of the B, A1, and A2 units in the peg. This analysis corroborates the original conclusion that B unit activity is the electrophysiological signature of an inhibitory processing event, one that strikingly transforms the information encoded and passed from each peg sensillum to the central nervous system.

Keywords: Synaptic interaction, chemosensory, electrophysiology, pectines

Synaptic coupling between peripheral sensory neurons is uncommon and especially rare among chemosensory afferents (Foelix 1975; Hayes & Barber 1982). For example, in the well-studied antennal systems of insects, the first synaptic interaction between cells appears to be in the antennal lobe of the brain (Bullock & Horridge 1965; Ernst & Boeckh 1983; Kaissling 1987). In non-scorpion arachnids, there is evidence of extensive peripheral synaptic interaction among mechanosensory neurons in spiders (Fabian-Fine et al. 2002) and whip spiders (Foelix et al. 2002; Spence & Hebets 2006), but no indication of interaction between chemosensory cells. The major chemosensory organs of scorpions, the pectines (Cloudsley-Thompson 1955; Ivanov & Balashov 1979), are organized differently. Morphological studies (Foelix & Müller-Vorholt 1983; Foelix 1985) and physiological evidence based on cross-correlation analysis of unit activity (Gaffin & Brownell 1997a; Gaffin 2001) suggest the presence of cell-to-cell synaptic interactions at the level of the first order chemosensory neurons. Further, it appears that these synapses are important in the processing of information prior to relay to the scorpion CNS (Gaffin & Brownell 1997b; Gaffin 2002).

While the morphological and physiological evidence makes a compelling case for the existence of chemical synapses in peg sensilla, the physiological evidence is correlative and indirect. For example, the pattern of inhibition in cross-correlograms could result from indirect effects of other undetected cells in the circuit (Perkel et al. 1975). Alternatively, an electrical coupling of two cells (Hestrin & Galarreta 2005) could generate a novel waveform in extracellular electrophysiological recordings. This novel waveform, when analyzed relative to the two contributing cells, would produce the semblance of an inhibitory effect in cross-correlograms. Could this be the case in scorpion peg sensilla? A further curiosity in peg recordings is that the putative inhibitory cell with the type B waveform has a peculiar inflection or notch in its otherwise highly repetitive waveform, suggesting that it could result from the combination of two coincident and subordinate events (Fig. 1).

To check the validity of previous assumptions, I looked closely at high-resolution recordings from peg sensilla of two species of scorpions where all three units (A1, A2, B) were clearly resolved. I mathematically combined idealized A1 and A2 waveforms, offset at various time intervals, to see if the B waveform is derived by simple summation of the former. I also looked for evidence of A1 and A2 unit discharges in close temporal proximity of B cell firing. If the B waveform is the expression of coupled A1 and A2 activity, then the refractory period of A1 or A2 should preclude their appearance within the B waveform. I calculated an expected number of contaminations of B by A1 and/or A2 based on spiking frequencies of the three cells and compared this to empirical observation. Based on these observations, I conclude that the B waveform does not result from a coupling of A1 and A2. This is consistent with the idea that B is a separate entity from A1 and A2 and that B exerts an inhibitory synaptic influence over A1 and A2 events in scorpion peg sensilla.

METHODS

A high quality, archived recording from a peg sensillum of *Smeringurus mesaensis* Stahnke 1957 (Scorpiones: Vaejovidae; formerly *Paruroctonus mesaensis*) and a new recording from a peg sensillum of *Paruroctonus utahensis* Williams 1968 (Scorpiones: Vaejovidae), collected near Kermit, Texas, USA (31°57′46.44″N, 102°58′53.59″W) formed the data set for this paper. A voucher specimen of *P. utahensis* has been deposited at the Sam Noble Oklahoma Museum of Natural History. The specific methods of the recording techniques are in Gaffin & Brownell (1997a, 1997b). The archive recording was relayed from an audiocassette tape through digitizing hardware (1401-plus, Cambridge Electronic Design (CED), Cambridge, UK) at 20 kHz sampling rate to a computer for analysis. I relayed the new *P. utahensis* recording directly from the preparation

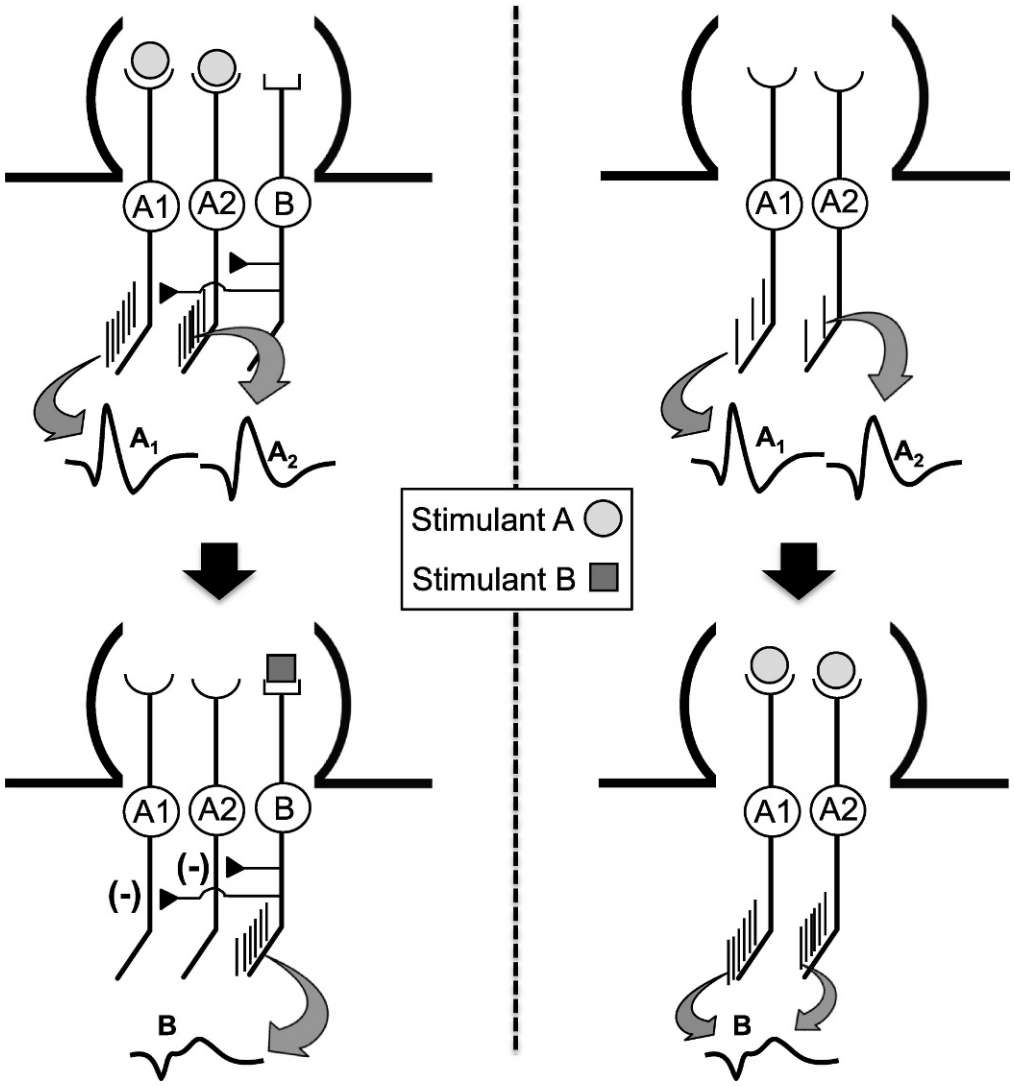
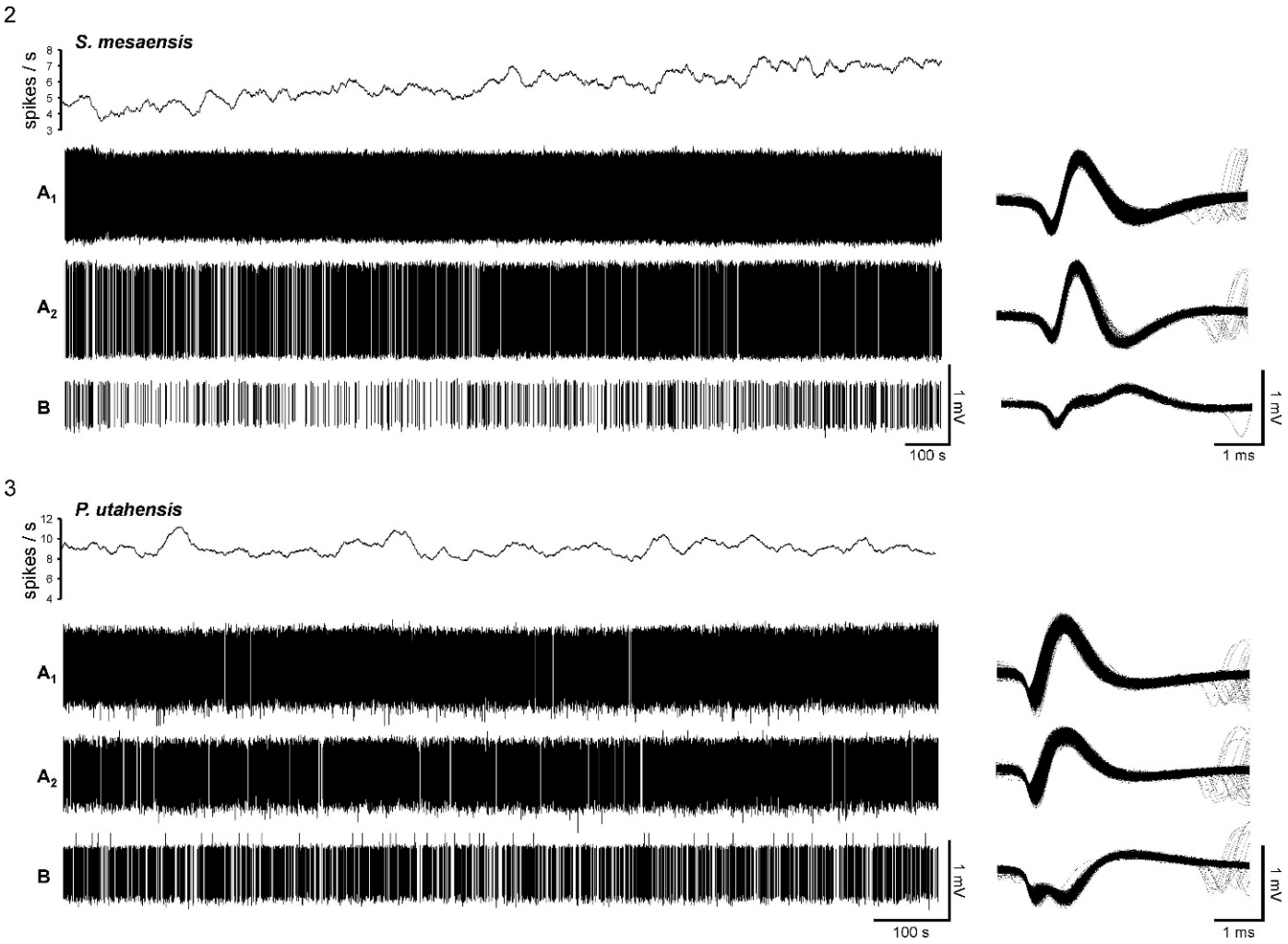


Figure 1.—Alternative interpretations of electrophysiological recordings from peg neurons. At left is a situation with three cell types A1, A2, and B where B has an inhibitory influence on both A1 and A2; each of the three neurons produce their own distinct waveforms. When the B cell is stimulated, it inhibits A1 and A2 and produces its own triphasic type B waveform. At right is a situation with only two active cells, A1 and A2. When A1 and A2 firings are coincident (as with simultaneous stimulation) their waveforms merge to produce the triphasic type B waveform.

through the digitizing software to the computer using the same settings. I used Spike 2 software (version 3.21, CED) to capture and analyze the spiking events in the records.

High-quality spike classification was necessary to support the findings of this study. The Spike 2 template matching parameters most effective in resolving sensillar waveforms included: 1) at least five similar spikes for a new template; 2) new template width 20% of amplitude; 3) no templates rarer than 1 in 150; 4) 20% maximum amplitude change for match; 5) minimum of 75% of points in template; and 6) linear waveform interpolation method. I reclassified unresolved waveforms (type 0 in Spike 2) by restricting the waveform comparison window to the first half of the triggered spike, and visually comparing and assigning each spike to the best-matched wave class. I produced auto-correlograms (Eggermont 1990), which captured same-waveform activity in the 0.5 s before and after each event, for each wave class to check the purity of spike assignments.

Once assured of accurate event classifications, I further analyzed the parsed records. I ran cross-correlograms (Eggermont 1990) to cross-reference activity between spike classes and detect activity-dependent interactions between waveforms. I averaged all classified spikes (minus those initially classified as type 0) to determine the average 75-point waveforms for the three classified spike types: A1, A2, and B. These values were then copied to an Excel spreadsheet for summation analysis. I added the 75 points forming the A1 and A2 waveforms point by point to derive a resultant waveform for comparison to the B waveform. Then, I offset the A1 and A2 waveforms by a point relative to each other and recalculated the resulting waveform. I repeated this process for 30 points positive and negative displacement of A1 and A2 relative to each other. Each one-point offset represented 50 μs of time displacement because of the 20 kHz sampling frequency (1/20,000 = 0.00005 s or 50 μs). The family of summed waveforms spanned ± 0.75 ms or 1.5 ms overall displacement relative to each other.



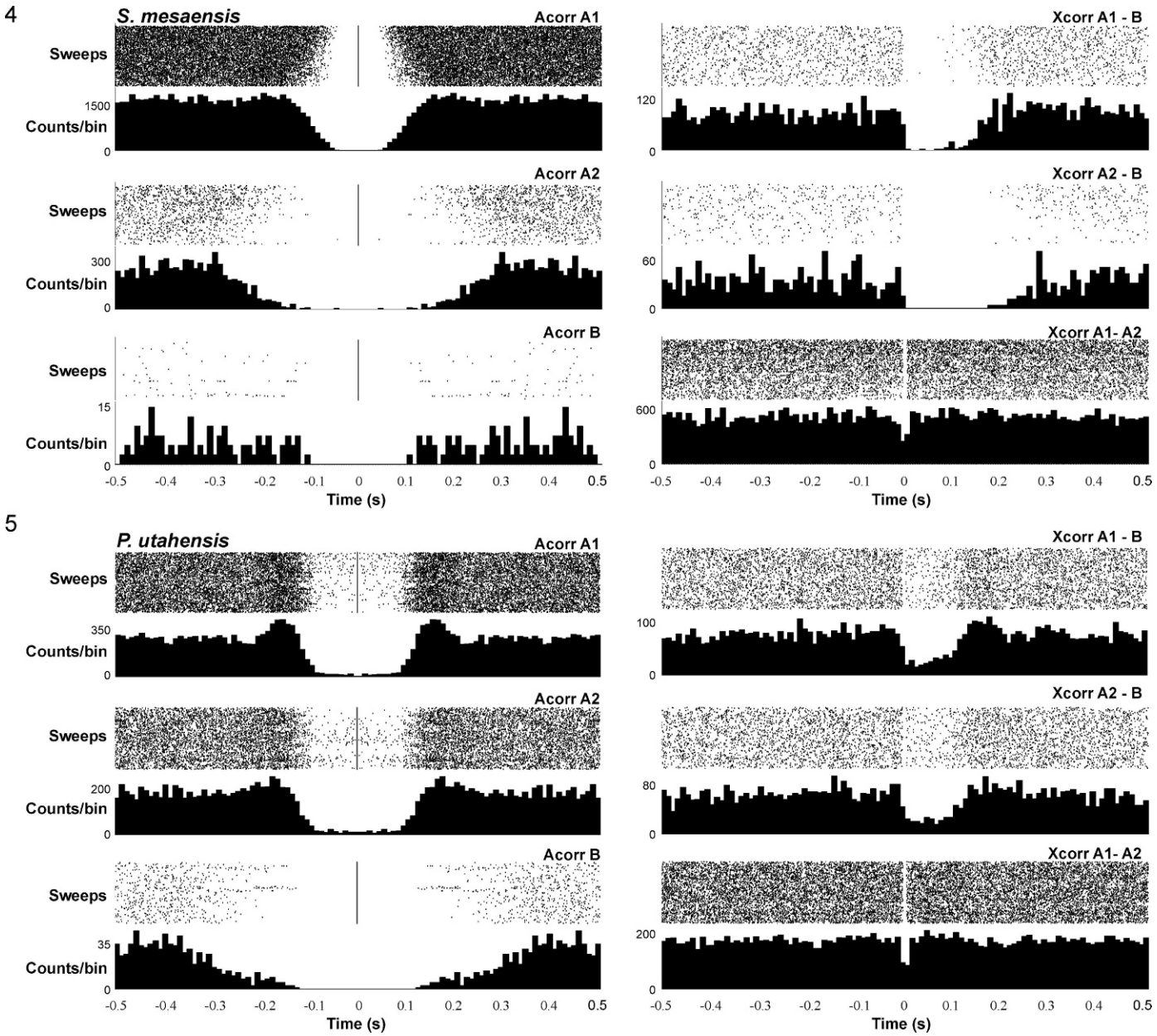
Figures 2, 3.—Electrophysiological recordings from scorpion peg sensilla. Baseline recordings from *Smeringurus mesaensis* (2) and *Paruroctonus utahensis* (3) are parsed by spike waveform analysis to separate traces classified as putative cell types A1, A2, and B; superimposed time-expanded waveforms are shown at right. The top plots show the activity of all three cells smoothed by a 30 s running average.

Finally, I compared the number of expected co-firings of putative cells to the observed number of doublets in the record. The expected number of A1/A2 doublets (i.e., the number of times that A1 and A2 fired within the same 75 point spike capture window) was calculated by first determining the average spiking frequency of each spike class (after adding in the reclassified type 0 spikes; B/A doublets were assigned to A1 or A2 based on the relative frequency of firing of A1 and A2 in the record). I multiplied the 0.004 s spike capture window (75 * 50 μs = 0.004 s) by the average spiking frequency of each class (in Hz) to determine the total time per second accounted for by each spike class. The expected number of doublets per second was then determined by adding the products of the average spiking frequency of one spike class by the time per second accounted for by the second spike class. I multiplied this number by the duration of the record to determine the expected number of A1/A2 doublets in the entire record. In a similar manner, I calculated the expected number of B/A1 or A2 doublets in the record. Finally, I compared these expected values with the actual number of reclassified doublets of each class.

RESULTS

The recordings of spike activity analyzed here had signal-to-noise ratios of about 6 to 1, well above the level required to clearly discriminate sensory spike events above background noise. The spike recognition algorithm of Spike 2 identified three distinct waveforms, which is typical for recordings from peg sensilla of *S. mesaensis* and *P. uthahensis*. The firing of these three cells (A1, A2, and B) is displayed for each of the species on separate tracings (Figs. 2, 3). In the *S. mesaensis* record, the combined spiking frequency of the three units averaged 5.8 Hz and steadily increased from the beginning to the end of the 2100 s recording (top trace of Fig. 2). In the *P. utahensis* record, the combined spiking frequency averaged 9.2 Hz and remained relatively steady from the beginning to the end of the 1200 s recording (top trace of Fig. 3).

Figures 2, 3 also show the superimposed waveforms of the three spike classes recognized by the template recognition software. In both species, the A1 and A2 waveforms were clearly distinguishable across the breadth of their patterns, and the B waveform had a characteristic inflection midway in its pattern.



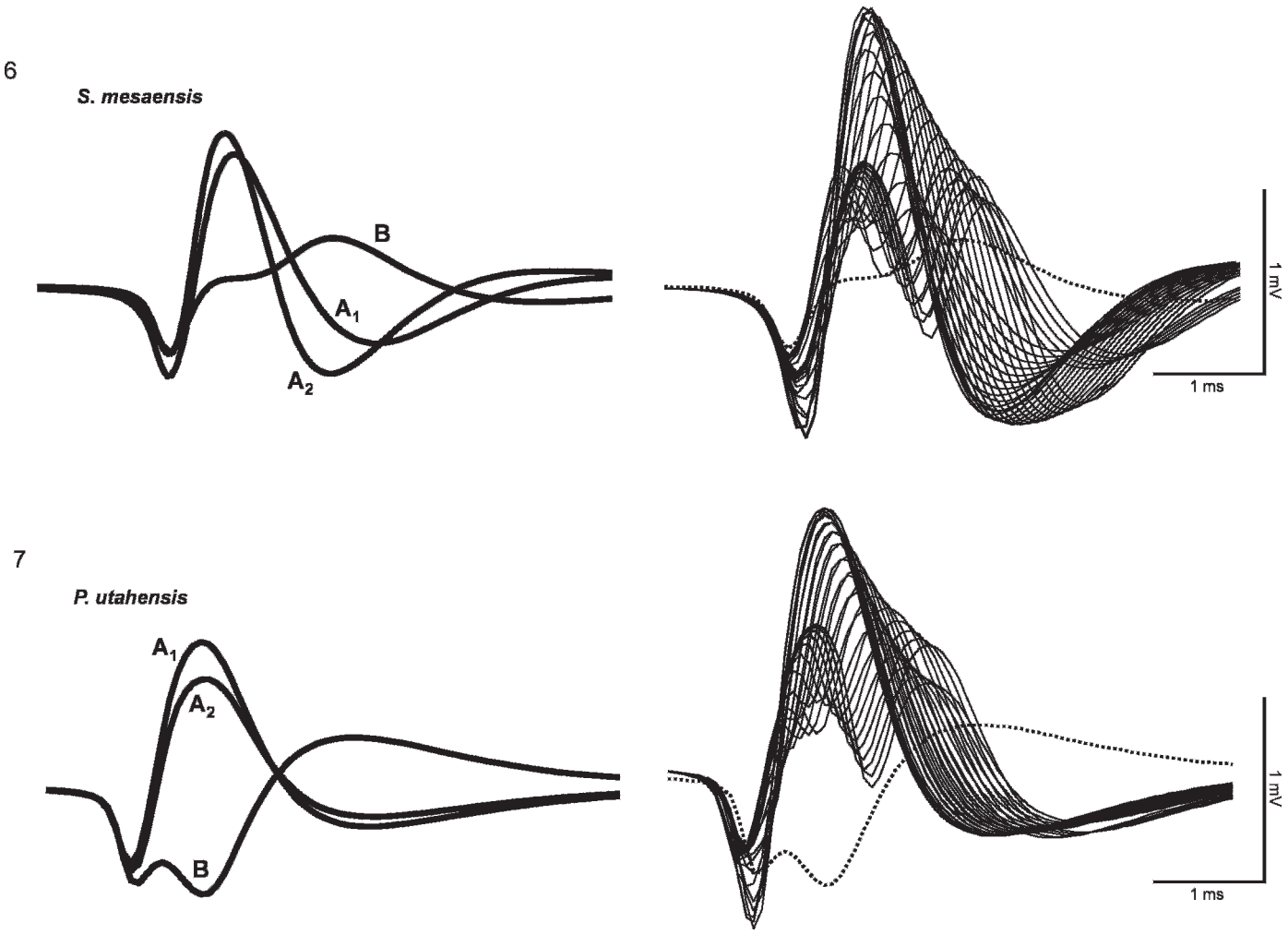
Figures 4, 5.—Correlation analysis of peg sensilla events. Auto-correlograms (right) and pair-wise cross-correlograms (left) of A1, A2, and B activities in *Smeringurus mesaensis* (4) and *Paruroctonus utahensis* (5). The raster plots at the top of each panel show the auto-activity 0.5 s before and after cell firing; the bars at bottom are sums of these tracings by 0.01 s bins.

Autocorrelograms of A1, A2, and B in *S. mesaensis* were free of contaminating events around the referenced event (Fig. 4, left) and nearly free in *P. utahensis* (Fig. 5, left). These clearings reflect the refractory period of each cell and indicate that the spike classifications were accurate. The steady increase in spiking frequency across the 2100 s *S. mesaensis* record is discernable in the raster plots at the top of the *S. mesaensis* autocorrelation panels – especially for A1. In contrast, the raster plots of *P. utahensis* autocorrelations do not show this increase, reflecting the relatively steady spiking frequency across the recording.

Cross-correlograms of A1 vs. B, A2 vs. B, and A1 vs. A2 show characteristic inhibition of A1 and A2 by B for both species (Figs. 4, 5, right). No apparent interaction exists between A1 and A2 of either species. Of note are the activities of A1 and A2 immediately prior to the firing of the B event in the top two cross-correlograms for both species. The activity is high and sustained right up to the firing of B and then drops abruptly. This is contrary to what would be expected if B were an electrical coupling of A1 and A2.

The average waveforms derived from Figs. 2, 3 are shown in Figs. 6, 7 (left) along with the results of summing the A1 and A2 waveforms in a series of time displacement calculations (right). The family of curves generated (representing a total of 1.5 ms displacement of A1 relative to A2) did not produce any curves similar to the average B waveform (dotted lines in Figs. 6, 7, right) for either species.

The left side of Figs. 8, 9 shows the unclassified waveforms that result from the near-temporal occurrence of A1 and A2



Figures 6, 7.—Summation of A1 and A2 waveforms. Right: Average waveforms for A1, A2, and B cells as calculated from individual waveform captures for *Smeringurus mesaensis* (6) and *Paruroctonus utahensis* (7). Left: Family of waveforms derived by adding the A1 and A2 waveforms successively displaced by 50 μs for a total displacement of +/− 0.75 ms (or 1.5 ms overall displacement) relative to each other. The dotted line is the average B waveform for reference.

waveforms for both species. The overlaid waveforms show a range of relative firings, similar to that generated in the calculations of Figs. 6, 7. The tracings at the top of the records indicate the time of occurrence of these A1/A2 doublets. Predictably, the frequency of occurrence of doublets in the *S. mesaensis* record directly correlates with the increase in spiking frequency of A1 and A2 as the record progresses (Fig. 2), while the frequency of A1/A2 doublets is relatively consistent across the *P. utahensis* record.

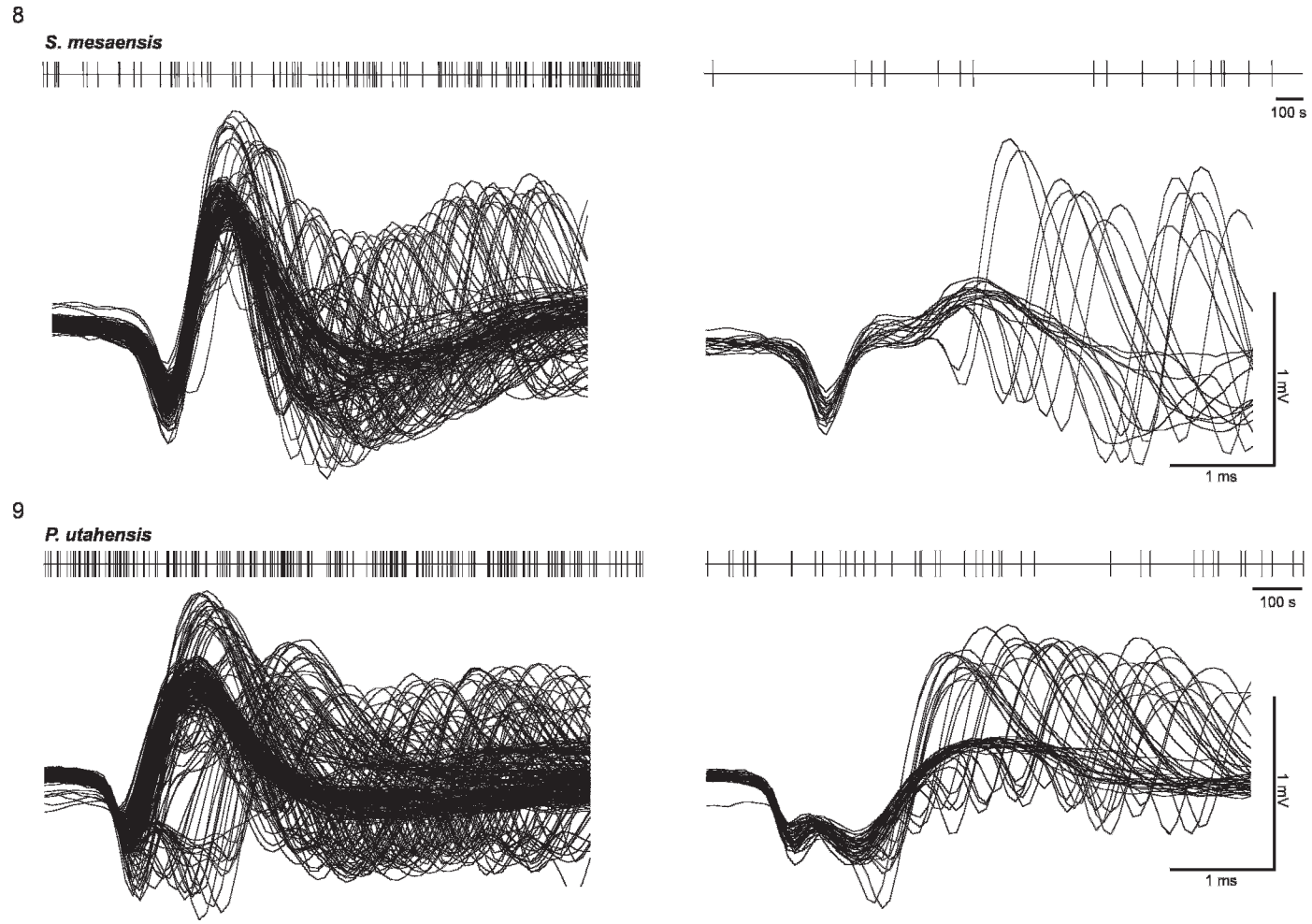
Figures 8, 9 (right side) also show the unclassified waveforms that resulted from the near-temporal occurrence of A1 or A2 waveforms after the firing of a B waveform. In all, I identified 17 of these doublets in the *S. mesaensis* record and 45 in the *P. utahensis* record. The tracing at the top of each record indicates the time of occurrence of these B/A1 or B/A2 doublets and, as before, the frequency of these doublets in the *S. mesaensis* record increases in direct relation to the increase in spiking frequency of the individual units.

I wanted to determine if the number of observed doublets in the records approximated the number predicted based on the unit firing frequencies and the duration given to each waveform captured by the analysis software. Since I know the average spiking frequency of each of the classified units, the time window for each spike (4 ms), and the duration of the records, I can estimate how many doublets ought to be captured by the software. Table 1 compares the number of doublets observed to the number predicted for both species. In the *S. mesaensis* record, I identified 103 A1/A2 doublets, while the predicted number based on unit firing frequency was 96. I counted 17 B/A doublets, which is similar to the expected number of 13. In the *P. utahensis* record, I identified 193 A1/A2 doublets, while the predicted number based on unit firing frequency was 150. I counted 45 B/A doublets, which is similar to the expected number of 48.

DISCUSSION

If the B cell observed in extracellular records from scorpion peg sensilla is actually a product of the electrical coupling of the A1 and A2 cells, then the following conditions should be met.

- The B waveform should be derivable from a direct addition or subtraction of the individual A1 and A2 waveforms. This was not supported.



Figures 8, 9.—Temporally close peg sensilla waveforms. 8. Superimposed images of firings of A1 and A2 waveforms for *Smeringurus mesaensis* (left) and firings of B spikes where A1 or A2 waveforms occurred within the same spike-sampling window (right). 9. Similar representations of event co-firings for *Paruroctonus utahensis*. The traces at the top of each panel show when these temporally close firings occurred in the records.

- Cross-correlograms of A1 or A2 vs. B should show restricted firing of A1 and A2 before and after the occurrence of B. They do not show activity immediately before B.
- There should be no contamination of the B waveform by other proximally occurring A1 or A2 waveforms because B results from the co-occurrence of A1 and A2, and the normal refractory period of the two waveforms would prevent them from occurring within the period of the B waveform. This is not the case. There were 17 identified co-occurrences of B and A1 or B and A2 waveforms in the *S. mesaensis* record and 45 in the *P. utahensis* record. Both of these numbers were in line with the predicted number of co-occurrences based on firing frequencies of the individual cells. The slight discrepancy is likely due to variations in the spiking frequency across the record and/or unclassifiable spikes.

Taken together, it appears clear that the B event is distinct and separate from the A1 and A2 events, and that the B event inhibits the activity of the A1 and A2 events. This finding is further supported by the ability to generate similar cross-correlogram patterns using a simulated neural network (Duffin 2000) involving two interacting units, one inhibiting the second (Gaffin 2002).

While the B event appears to be separate from A1 and A2, this does not preclude the possibility that it could result from the coupling of at least two other events within the peg sensillum. Alternatively, it could be a summation of two active conductances within the same cell. This is the first formal report of the spiking patterns in *P. utahensis*; however, the regular inflection of the B waveform has also been reported in another scorpion, *Hadrurus arizonensis* Ewing 1928 (Scorpiones: Iuridae), where it also inhibits the A1 and A2 events (Gaffin 2002). A different situation exists in the main three spiking units of *Centruroides vittatus* Say 1821 (Scorpiones: Buthidae). Again, three active cells are typical, but two of the cells have inflections in their waveforms. The waveform of the third cell is smoothly biphasic and does not appear to affect the two triphasic events. However, one of the triphasic events in *C. vittatus* excites the other (Gaffin 2001).

Previous interpretations of chemical synaptic interaction in scorpion peg sensilla are supported by these analyses. The interactions appear ubiquitous in the tens of thousands of pegs

Table 1.—Expected vs. observed number of doublets.

Species	Spike class	Number of events	A1/A2 doublets	B/A doublets	% A1 or A2	B/A doublets assigned	Total spikes	Record duration (s)	Spiking frequency (Hz)	Spike duration (s)	Time/s accounted for by spike type	Predicted doublets/s	(Expected number of doublets/s)* (record duration)	A1+A2
S. mesaensis	A1	8494	103	**17**	0.75	13	8610	2103.81	4.09	0.004	0.016	0.0228	48	96
	A2	2824	103	17	0.25	4	2931	2103.81	1.39	0.004	0.006	0.0228	48	
	B	643						2103.81	0.31	0.004	0.001	0.0067	**14**	
P. utahensis	A1	5045	193	**45**	0.55	25	5263	1200.00	4.39	0.004	0.018	0.0623	75	150
	A2	4047	193	45	0.45	20	4260	1200.00	3.55	0.004	0.014	0.0623	75	
	B	1511						1200.00	1.26	0.004	0.005	0.0400	**48**	

on the distal surfaces of pecten teeth. The utility of these extensive interactions is still under investigation. They may enhance information content in chemical identification (Gaffin & Brownell 1997a, 1997b). Alternatively, they may serve as a governor or brake on A1 and A2 and be related to the hypothesis that peg sensilla function as a parallel sampling system for rapid acquisition of ground-based chemical information (Gaffin & Walvoord 2004).

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