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Supplementary Material: Agonistic signals received by an arthropod filiform hair allude to the prevalence of near-field sound communication

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ELECTRONIC SUPPLEMENTARY MATERIAL

Agonistic signals received by an arthropod filiform hair allude to the prevalence of near-field sound communication. Roger D. Santer & Eileen A. Hebets

SUPPLEMENTARY METHODS

(i) Analysis of behaviour

Whip spider contest behaviour was filmed at low and high-speed simultaneously. We used low speed films to characterise the contest behaviour of whip spiders in terms of contest phases observed, contest duration, and ALV display duration. We viewed films on a digital videocassette recorder (DSR-11, Sony Electronics Inc, USA) and standard television screen; the videocassette recorder's counter was used for time measurements.

We used high-speed films to analyse ALV in detail. We analysed 30 consecutive ALV cycles for each animal for the typical period and amplitude of antenniform leg vibration during a display. We used 1600ms filmed sections of ALV for each animal to observe the receiver body parts beneath a signaller's vibrating antenniform leg at 10ms intervals. Both analyses were carried out using Xcitex ProAnalyst Lite software (Xcitex Inc., Cambridge, MA, USA) on a standard PC.

(ii) Stimulator design

For practical reasons, we could not measure the air movements created by whip spider ALV displays directly. However, antenniform leg movements during ALV can be considered a dipole source and, as such, reproducing these movements with an object of the same size and shape will induce the same air movements.

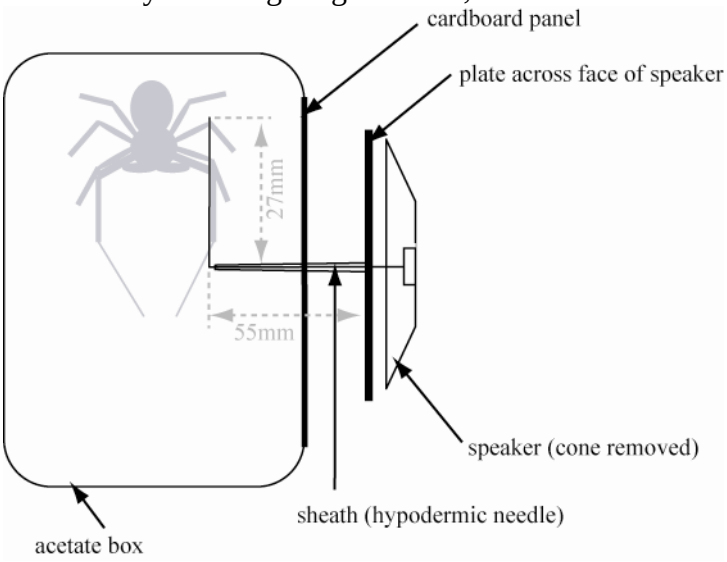
Our stimulator moved a 27mm section of 200μm diameter tungsten wire (approximately the same diameter as a whip spider antenniform leg tarsus) from side to side in the horizontal plane with the same amplitude as real ALVs. These movements were controlled using the voice coil of a mini-speaker mounted on its side during experiments. Our set up during electrophysiological experiments is shown in fig S1.

We took several precautions to ensure that stimulation was only from movements of the tungsten wire and not the rest of the stimulator. (1) We cut away most of the speaker cone to reduce air movements; (2) we shielded the entire face of the speaker using a 2mm thick sheet of cardboard that overhung the edges of the speaker by 8mm; (3) the stimulus wire protruded through this sheet, perpendicular to the voice coil, but was sheathed by a no. 16 hypodermic needle for a length of 5.5cm from the speaker shield. At its exit from the sheath, the tungsten wire was bent at 90° to leave a 27mm horizontal section that served as the stimulus in our experiments (fig S1); (4) to exclude surface-borne vibrations, experiments were conducted on a vibration isolation table (TMC, Peabody, MA, USA). The stimulator itself was held in place by a clamp secured to the perimeter enclosure of the table, preventing any vibrations from affecting the preparation itself; (5) we further shielded our preparation by enclosing it in a box. This was a free-standing cardboard wall wider than our stimulator by 6.5cm. Attached to each side of this wall were sheets of clear acetate that we bent to completely encircle the preparation, leaving a small gap for electrode wires on the side furthest from the stimulator. On top of this encircling wall we placed an extra acetate sheet to create a box. The cardboard wall had a 5mm wide hole in its centre through which the hypodermic sheath of our stimulator

protruded. The stimulator did not make contact with any part of this wall during experiments.

We carried out control experiments to confirm the effectiveness of this shielding (supplementary results).

Fig S1: Set up for simulating ALV displays. All parts of the stimulator, except the horizontally vibrating tungsten wire, were shielded from the preparation.



SUPPLEMENTARY RESULTS

(i) Typical contest behaviour

Although contests were filmed under full light conditions, contest behaviour was typical of that observed by previous authors (e.g. Fowler-Finn & Hebets 2006). Typical display behaviours were observed – pedipalp opening, antenniform leg vibration and pedipalp contact (Fowler-Finn & Hebets 2006); and contests escalated through characteristic, described phases (Weygoldt 2000; Fowler-Finn & Hebets 2006). Of 21 encounters staged, contests occurred in 19 (in 2, both competitors avoided one another without threat displays). Of these, 94.7% (18) involved an initial phase in which the opponents probed one another using their antenniform legs; 73.7% (14) escalated to a phase of ALV by one or both individuals; and 10.5% (2) escalated to full pedipalp contact. Mean contest duration, from initiation of a pedipalp threat display by one animal to retreat of the losing animal, was 191.6 ± 38.0s.

(ii) Additional data on ALV positioning relative to the receiver

Figure 2a in our article shows the mean proportion of time during an ALV display that the signaller's antenniform leg overlays particular receiver body regions. Table S2 lists the data used to produce figure 2a.

Table S2: The mean proportion of time during an ALV display (± SD) that the signaller's antenniform leg overlays particular receiver body regions. Proportions were calculated by recording antenniform leg position at 10ms intervals from 1600ms samples of ALV for each of 7 whip spiders and are illustrated in figure 2a of our article.

Receiver body part below antenniform leg	Mean proportion of ALV duration	SD
Body regions		
prosoma (cephalothorax)	0.17	0.183
opisthosoma (abdomen)	0.22	0.183
Pedipalps		
left	0.10	0.185
right	0.44	0.396
Antenniform legs		
left, proximal	0.00	0.000
left, distal	0.00	0.000
right, proximal	0.56	0.419
right, distal	0.07	0.154
Walking legs		
left leg 2, proximal	0.00	0.000
left leg 2, distal	0.00	0.000
left leg 3, proximal	<0.01	0.002
left leg 3, distal	0.00	0.000
left leg 4, proximal	0.07	0.185
left leg 4, distal	<0.01	0.002
right leg 2, proximal	0.71	0.360
right leg 2, distal	0.05	0.101
right leg 3, proximal	0.69	0.327
right leg 3, distal	0.15	0.238
right leg 4, proximal	0.29	0.169
right leg 4, distal	0.40	0.247

(iii) Electrophysiological control experiments

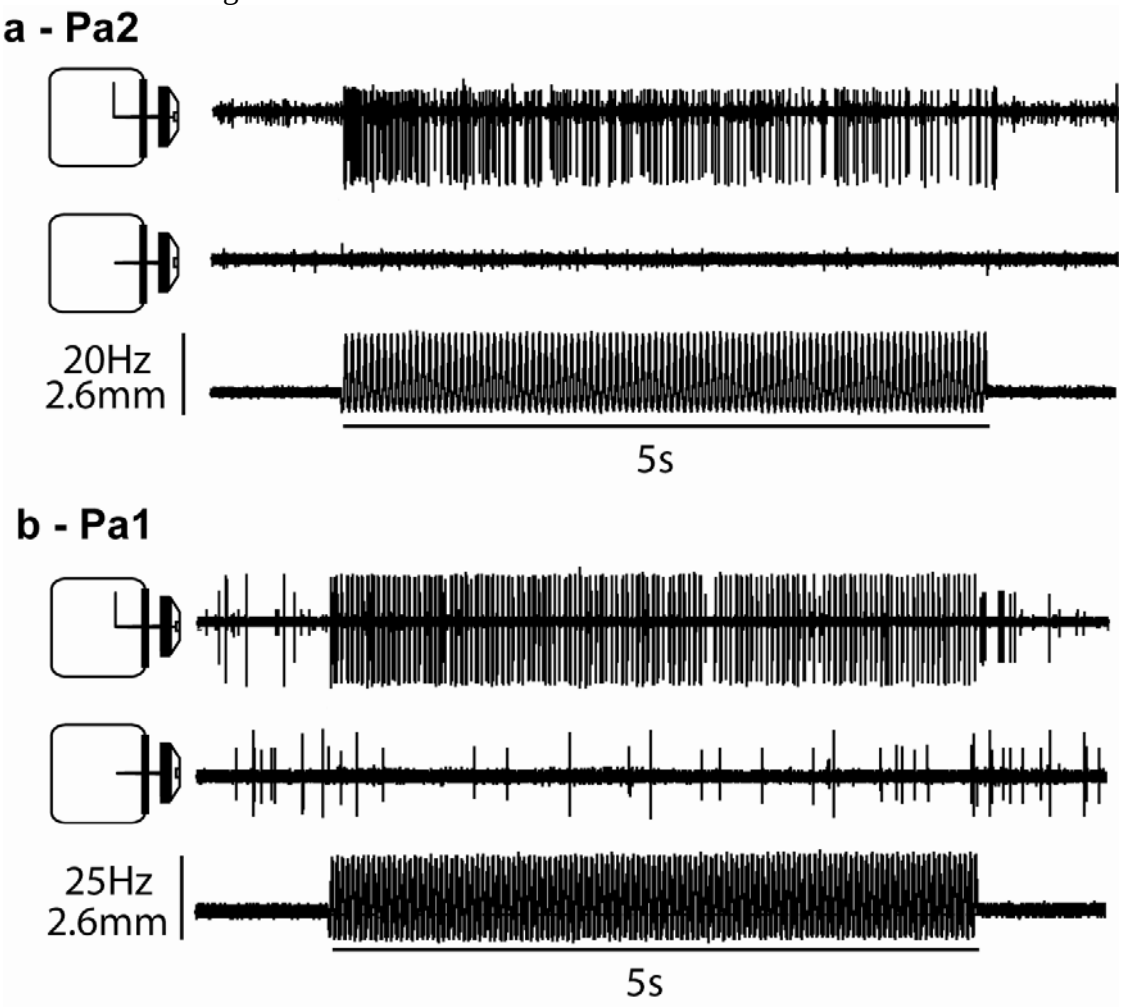
In order for air movements generated by our stimulator to reproduce those during real ALV, they must be generated only by side to side movements of the tungsten wire and not the remainder of our stimulator. We used shielding to ensure that this was the case (supplementary methods), and carried out control experiments to confirm this.

(1) We noted that moving the stimulus wire away from the trichobothrium caused a greatly reduced response. This was the case whether the stimulus wire was moved laterally away from the animal's body, or forwards, or vertically. In the latter cases, the speaker controlling wire movements was the same distance from the preparation as used during data collection. This is good evidence that air movement

stimulation comes from movements of the wire, rather than the speaker voice coil (data not shown).

(2) Cutting the tungsten wire at its 90° bend removed the horizontally-moving portion of wire that simulated ALV in our experiments. For trichobothrium Pa2, this stimulus configuration elicited no response when the stimulator was driven at any of the simulated ALV frequencies used in this study. For trichobothrium Pa1 (which showed a continuous level of background activity, even in the absence of stimulation), this stimulus configuration elicited no increase in activity from the background level (fig S3). Significantly more spikes were elicited by the stimulator with wire intact than were present as background activity during control experiments for both the large (paired t test: $T=3.82$, $df=11$, $p=0.002$) and small ($T=3.57$, $df=11$, $p=0.004$) action potentials across all stimulation frequencies. Since trichobothria are among the most sensitive air movement sensors known (Shimozawa et al. 2003), in both cases, stimulation is from horizontal movements of the tungsten wire and not the remainder of the stimulator and thus, the air movements generated should reproduce those elicited by real ALV.

Fig S3: If the horizontal tungsten wire portion is removed from the stimulator, the stimulator no longer excites trichobothrium Pa1 or 2.



(iv) Analysis of phase-locking

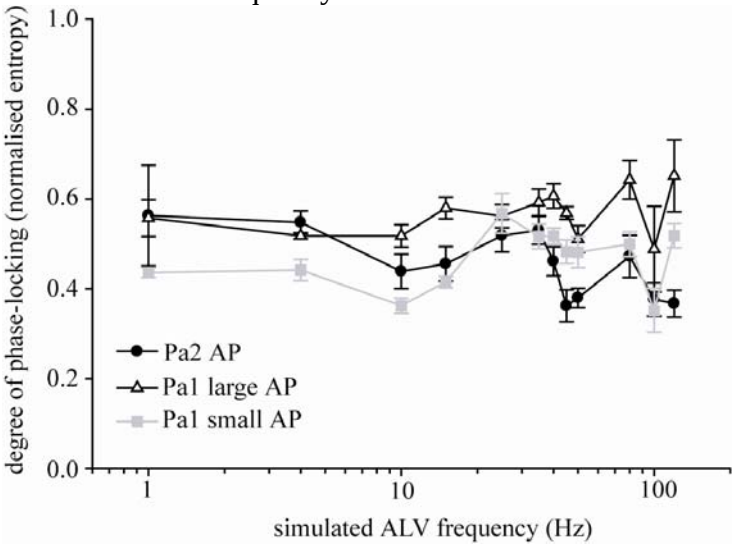
We measured the phase-locking of trichobothrium action potentials to simulated ALV using a normalised entropy index (Hurtado et al. 2004; Tort et al. 2007). For each trichobothrium response, we created phase histograms of action potential occurrences per 10° of phase bin (36 bins in total). We obtained an entropy measure (h) for these histograms (discarding those containing one action potential or less) by:

$$h = -\sum_{j=1}^{L} p_j \log p_j$$

where L is the number of bins and p_j is the probability corresponding to the j^{th} bin. We normalised this entropy measure by the maximum entropy achieved for the uniform distribution (h_{max}): $p_j = 1/L$ for all j, and $h_{max} = \log L$. Therefore, normalized entropy, $h_N = (h_{max} - h)/h_{max}$. This varies between 0 and 1, with 0 indicating a uniform distribution and 1 indicating perfect phase locking in a single bin.

We calculated mean normalized entropy across 6 whip spiders for simulated ALVs of between 1 and 120Hz, for the Pa1 large action potential, Pa1 small action potential and Pa2 action potential (fig S4). In common with a previous study, we found this measure to be conservative, with values of around 0.4 still indicating strong phase-locking (Tort et al. 2007). Phase locking seemed to decline slightly with frequency for Pa2, but since high frequency stimuli elicited very few spikes it is difficult to assess the importance of this trend. Nevertheless, we tested whether normalized entropy varied with action potential type or simulated ALV frequency using a 2-way ANOVA. The ANOVA was significant ($F=9.27$, $p<0.0001$), but there was only an effect of action potential type ($F=22.35$, $p<0.0001$) and not simulated ALV frequency ($F=0.92$, $p=0.34$). There was no interaction between action potential type and simulated ALV frequency ($F=0.42$, $p=0.65$). Therefore, the degree of phase locking varies between the three types of action potential recorded, but is not affected by simulated ALV frequency over the range tested.

Fig S4: The phase-locking of action potentials from trichobothria Pa1 and 2 with simulated ALV frequency. Plot shows mean normalised entropy ± SEM.



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