Nevertheless, a photic input did not produce the steady-state depolarization in either of these cells (Fig. 1C). Increasing the resistance of the gap junctions would be a mechanism for turning off current flowing through them. This can be done if the channels are voltage-dependent. Such channels have been described for gap junctions in other preparations (*10*). However, the results shown in Fig. 1, A and B, suggest that these junctions do not rectify, at least not on a time scale of about 10 msec, since equal and opposite current steps in one cell produce equal and opposite voltage changes in the other. Perhaps these channels require a longer time to develop voltage-dependent inactivation.

In other central neurons where gap junctions couple cells synaptically there is a definite direction to the flow of electrical information between pre- and postsynaptic cells. In such examples there is mismatching of impedance when current is applied to an element of relatively higher input impedance. In the case of amacrine cells, it is difficult to consider one cell as presynaptic to another because they appear to be reciprocally interconnected. Since the synaptic contacts are between dendrites of approximately equal diameter, such impedance mismatching probably does not occur and is unlikely to contribute to changes in the size of the postsynaptic potential.

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Vibratory Communication Through Living Plants by a Tropical Wandering Spider

Abstract. *Female* Cupiennius salei *pheromone on banana and* Agave *plants elicits patterned oscillations by the male. Resulting pulse trains of vibrations through the leaf average 76 hertz. The brief vibratory response by the otherwise immobile female hidden up to at least 1 meter away on another leaf guides the male across the plant to her location. Reciprocal signaling continues in the presence of random noise that masks the male's airborne sounds.*

Cupiennius salei Keyserling (Ctenidae) is a nocturnal wandering spider (body length, 27 to 40 mm; weight of female, 2.8 to 4.6 g) that lives on banana (*Musa sapientum*) and *Agave* (*1, 2*). Although the male is known to signal mechanically, only the airborne sounds of palpal drumming have been noticed (*2*). We report the existence of low-frequency vibrations, produced in both sexes by oscillations (abdomen and legs), whose airborne components are much less audible to the human ear than the higher frequency (> 125 Hz) percussive ones. We found that the low-frequency signals of *Cupiennius* are transmitted over surprisingly large distances through the plants and are important in bringing the sexes together (*3*).

Pots containing *Musa* and *Agave* plants were placed in sand-filled bins to reduce the ambient vibrations of the laboratory. After restricting a female *Cupiennius* to one leaf blade for 1 to 3 days with a plastic bag, we removed both the bag and the female. A male placed on this leaf usually began courtship in response to the pheromone secreted by the female (*4*). In later experiments a visually hidden female was present on another leaf of the plant (with or without an enclosing bag) before and during the time that the male was on the plant (Fig. 1).

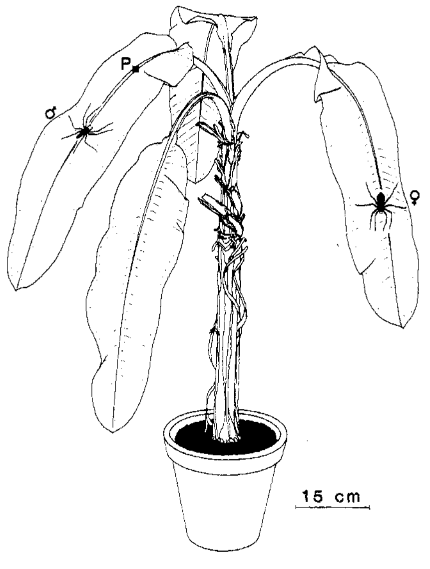

Fig. 1. Experimental setup used for studying communication through a banana plant in *Cupiennius*. The male begins courtship on a pheromone-covered leaf blade [with a vibration pickup (*P*) near the base] and then must select the correct petiole to reach a stationary female hidden on another leaf. In some trials a plastic bag enclosed the female's leaf blade. Mean length of leaf blades is 62 cm.

The sequence of behaviors that brought the male to the female included (i) pheromone deposition by the female; (ii) vibration production by the male on the pheromone-laden leaf; (iii) vibration production by the female on another leaf; and (iv) reciprocal signaling as the male departed from the pheromone-laden leaf and selected the correct leaf to reach the female. The attractiveness of the female's vibratory response was shown by the male's resistance to our occasional attempts to block his departure from the pheromone-rich leaf.

The use of the female's vibratory signal by the male for orientation was revealed by an experiment based on the banana plant's structure. After climbing down the petiole to the region where all the petioles diverge radially from the "stem," the male had to choose the correct path from among five other petioles in the first five tests and from four petioles in the second five tests. The male signaled repeatedly while remaining at the central point, his outstretched legs touching several petioles. In seven of the ten tests males correctly selected the petiole of the leaf on which the hidden female rested (*5*). A substrate signal may be superior to airborne sound in facilitating such a choice because of the probably discontinuous increase in strength when the correct petiole is contacted (*6*).

During courtship the male's legs oscillated rapidly while remaining in contact with the leaf. Such behavior is similar to that recently described for the sparassid spider *Heteropoda venatoria* (*7*). Early in courtship the mean interval between bouts of signaling was 34 ± 11.9 seconds ($N = 15$), as timed from the end of one bout to the end of the next. (The sharply defined terminal pulses were better for this measurement than the less regular initial pulses.) After the first few responses by a receptive female, the inter-

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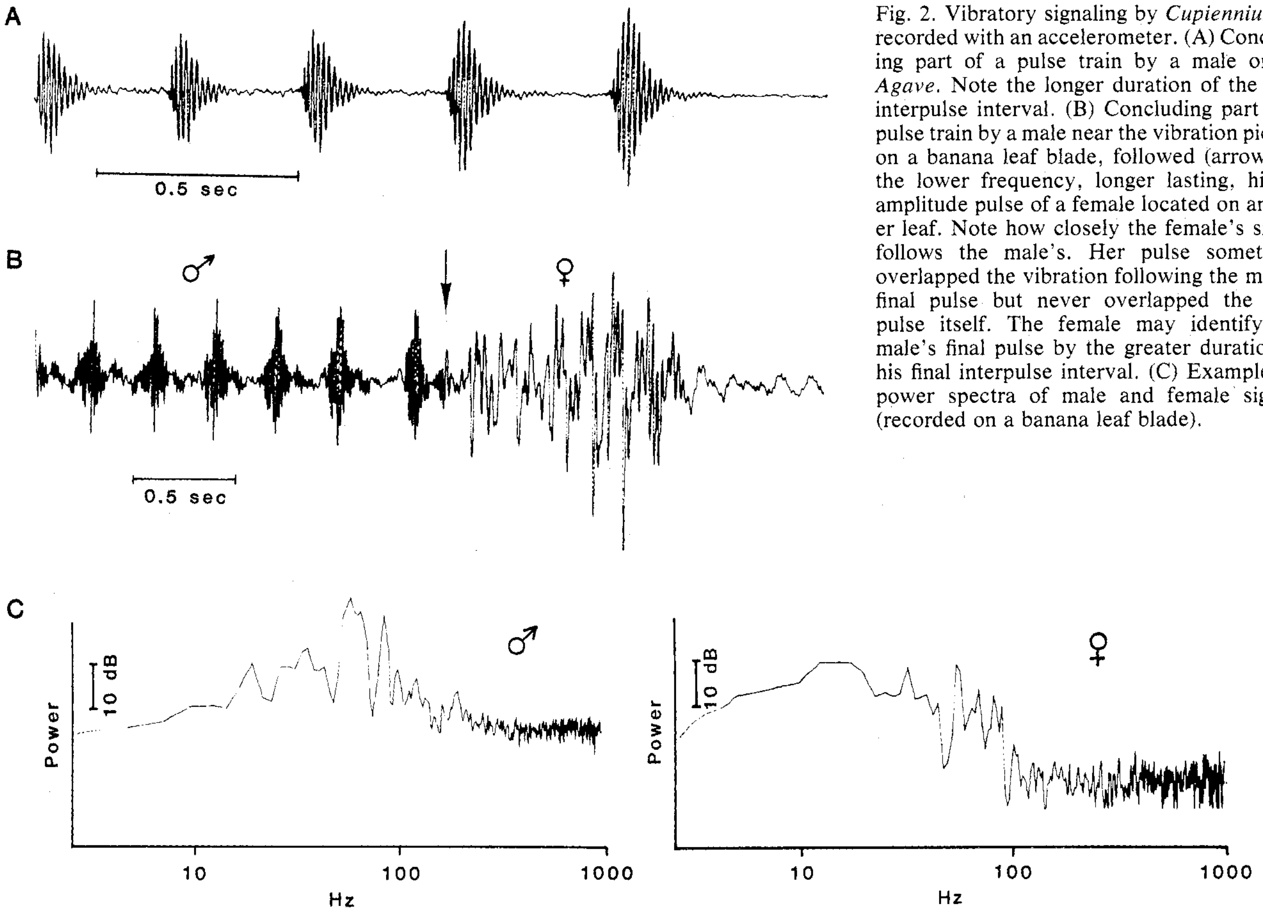

Fig. 2. Vibratory signaling by *Cupiennius*, as recorded with an accelerometer. (A) Concluding part of a pulse train by a male on an *Agave*. Note the longer duration of the final interpulse interval. (B) Concluding part of a pulse train by a male near the vibration pickup on a banana leaf blade, followed (arrow) by the lower frequency, longer lasting, higher amplitude pulse of a female located on another leaf. Note how closely the female's signal follows the male's. Her pulse sometimes overlapped the vibration following the male's final pulse but never overlapped the final pulse itself. The female may identify the male's final pulse by the greater duration of his final interpulse interval. (C) Examples of power spectra of male and female signals (recorded on a banana leaf blade).

val between bouts of signaling by the male decreased to 22 ± 3.4 seconds (N = 30; t = 4.98, $P < .001$). Males alone on the plant never attained such high rates of calling but instead gradually decreased their signaling activity. Each of the male's signaling bouts concluded with an approximately 4-second pulse train in which the final eight to ten pulses were relatively constant in amplitude. The peak-to-peak interpulse interval averaged 0.36 ± 0.047 second (N = 50); vibration frequency, 76 ± 6.6 Hz (N = 20) (Fig. 2) (*8*). Lower amplitude, higher frequency vibrations from about 150 Hz to 1 KHz, caused by palpal percussion, usually preceded or accompanied many pulses of each train, except for the last few.

Receptive females remained immobile throughout the courtship except for a brief response after about one of every three (35 ± 11 percent) of the male's signaling bouts. Without lifting her tarsi from the substrate, the female responded by trembling her legs for 0.41 ± 0.44 second (N = 20). This typically produced a single irregular pulse of 49 ± 21.5 Hz (N = 7) (Fig. 2, B and C) (*9, 10*). Sometimes this signal included higher frequency percussive components that resulted from one to several (1.9 ± 0.47, N = 14) abdominal drumbeats against the leaf. Her response was limited to a narrow time frame beginning 0.89 ± 0.53 second (N = 34) after the end of the male's final pulse. The female occasionally responded in less time than the male's interpulse interval (Fig. 2B); however, she never interrupted his pulses. The longer interval before the male's final pulse (Fig. 2) may provide the cue that the following pulse is the terminal one (*11*); playbacks of altered signals are needed to test this hypothesis. The precise timing (and possibly the power spectrum) of the female's signal probably yields adequate information to overcome noise in the channel resulting from irregularly timed phenomena, such as the leaves hitting together in a breeze.

Masking experiments showed that airborne sound is not needed for courtship communication in this species. The amplified output of a random-noise generator (General Radio model 1390B; bandwidth 50 Hz to > 10 KHz) (*12*) was used to drive a speaker to yield sound pressure levels (SPL's) that began at 71 dB and which we increased at intervals during the courtship to a maximum of 86 dB (measured at the location of the responding female). This range exceeded the level of the male's airborne sound, which had a peak amplitude of only 58 ± 3.1 dB SPL (N = 24) 2 to 3 cm from his body. Furthermore, the aerial distance separating the sexes during these experiments was 50 to 70 cm. Reciprocal signaling between the sexes continued in most cases (*13*).

It is clear that *Cupiennius* uses substrate-borne vibrations to bring the sexes together; the airborne sounds audible to human ears are not essential for communication. The slit sense organs on the spider's appendages, known to be involved in the detection of substrate vibrations generated by prey (*14*), are probably also used to receive intraspecific communicatory vibrations. While the maximum distance over which the *Cupiennius* system functions must still be determined, our findings demonstrate that reciprocal signaling can occur between individuals at least 1 m apart. Such long-distance communication through a substrate is remarkable in a spider that does not live in webs. It is remarkable even among arthropods in general (*14, 15*).

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3. Vibrations were transduced by an accelerometer pickup (B & K Instruments type 4339) attached to the midrib (*Musa*) or middle (*Agave*) of the leaf. From the preamplifier (B & K 2625), the signal was fed to a measuring amplifier (B & K 2606) whose output was monitored with headphones and an oscilloscope (Tektronix D10) and recorded on a frequency-modulated tape recorder (SE Laboratories Eight-Four) at 19 cm/sec, along with whispered protocol on a separate channel. In other trials we recorded airborne sounds; a tripod-mounted condenser microphone (B & K 4133) and preamplifier (B & K 2619) provided the signal to the measuring amplifier. Analysis and graphing of the signals involved use of a Mini-Ubiquitous FFT computing spectrum analyzer (Nicolet Scientific Corp., model 446), an X-Y recorder (Hewlett-Packard 7045A), and a pen recorder (Gould Brush 2200). Nine male and 12 female *C. salei* from a stock of laboratory-bred spiders were used. We conducted most observations between 0800 and 1100 hours and used low levels of illumination except during filming. Behavior was filmed at 100 to 200 frames per second with a Cine-8 Super-8 camera (Visual Instrumentation, model SP-1). Microscopic examination revealed no organs specialized for sound production on any part of the spider.
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8. The mean vibration frequency was determined from the predominant peak in the power spectra. This value was corroborated by counting the number of wave peaks per unit time within each pulse on the pen recordings.
9. Since the female's pheromone is probably species-specific, there may have been less selective pressure on her to evolve a vibratory signal that is as regular as the male's.
10. The female's signal included waves at least four times greater in amplitude than those of a vigorously courting male, as recorded during the period just prior to copulation. (At that time, both spiders are on the female's leaf and roughly equidistant from the vibration pickup, the latter being on the leaf used for the male's introduction.) This amplitude difference is evident in Fig. 2B, in which an actively signaling male was recorded while still on the leaf used for introduction and only 15 cm from the pickup, while the female was on another leaf and 76 cm from the pickup (measured through the plant). Even at that distance the waves of her signal caused acceleratory amplitudes greater than those of the male's signal waves.
11. We measured the durations of the five peak-to-peak intervals separating the final six pulses of the male courtship bouts to examine this point. The last interval (0.41 ± 0.073 second, $N = 15$) was significantly longer ($t = 6.15$, $P < .001$) than the preceding intervals (0.34 ± 0.022 second, $N = 60$). That this was not due simply to a trend of increasing intervals was indicated by the lack of a significant difference ($t = 0.91$) between the first interval (0.34 ± 0.025 second, $N = 15$) and the fourth (0.35 ± 0.023 second, $N = 15$).
12. The system yielded a fairly flat response down to about 100 Hz and fell only 5 dB at 70 Hz—not rolling off until about 50 Hz (acoustically measured at the loudspeaker).
13. The high levels of airborne masking noise induced resonant vibrations in the banana plant. Such added noise in the substrate reduced the maximum distance over which the spiders' signals could be transmitted effectively. At the highest level of airborne noise (86 dB SPL), one female responded to a male courting 0.5 m away on another leaf but did not respond when the male was placed at a greater distance until the airborne noise was reduced back to 80 dB SPL. The distance limitations imposed by various levels of induced substrate noise require future study.
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Choline Stimulates Nicotinic Receptors on Adrenal Medullary Chromaffin Cells to Induce Catecholamine Secretion

Abstract. *Choline stimulated secretion of catecholamines from primary dissociated cultures of bovine adrenal medullary chromaffin cells by interacting with nicotinic receptors. Secretion was readily detected at a choline concentration of 1 millimole per liter and was maximal at 3 to 10 millimoles per liter; it was completely calcium-dependent. Further analysis suggested that choline acts as a partial nicotinic agonist.*

Reduced function of cholinergic nerves in the central nervous system (CNS) is suspected of playing a role in a variety of neurological disorders including tardive dyskinesias (*1*), Alzheimer's disease (*2*), and memory loss in old age (*3*). A potential approach to the treatment of these diseases is to increase cholinergic function. This can be accomplished by administering choline or lecithin (from which choline is derived) (*4*). Acetylcholine is synthesized from choline by the action of choline acetyltransferase in cholinergic nerves. Preliminary trials involving this strategy have met with some success (*5*), but the actual mechanisms of action are poorly understood. In addition to being a precursor for acetylcholine, choline may have direct effects on acetylcholine receptors. Recently, Krnjević and Reinhardt (*6*) reported that choline has a muscarinic effect on CNS neurons. We now report that choline interacts directly with nicotinic receptors on chromaffin cells from bovine adrenal medulla inducing catecholamine secretion.

Chromaffin cells from bovine adrenal medulla were dissociated and maintained as monolayers in 16-mm-diameter plastic wells (Costar, Cambridge, Massachusetts) for up to 21 days (*7, 8*). Cell density was 450,000 cells per well and catecholamine content was 9 to 30 nmole per well, depending on the preparation. Secretion experiments were performed at 25°C in physiological salt solution (PSS) containing 142 m*M* NaCl, 5.6 m*M* KCl, 3.6 m*M* $NaHCO_3$, 2.2 m*M* $CaCl_2$, 15 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid (*p*H 7.4), 5.6 m*M* glucose, and 0.57 m*M* ascorbic acid, as described in the legend to Fig. 1.

Choline (3 m*M*) induced detectable secretion of catecholamine after 30 seconds, and secretion continued for approximately 5 minutes (Fig. 1A). The time course of choline-induced secretion was virtually identical to that of carbachol-induced secretion (*9*). Secretion was observed at 1 m*M* choline and was maximal at 3 to 10 m*M* (Fig. 1B). At higher concentrations, secretion decreased. Because 1 m*M* and 3 m*M* choline stimulated secretion in solutions in which NaCl concentrations were maintained at 142 m*M*, the secretion induced by choline was not caused by a reduction in the NaCl concentration, as has been suggested (*10*).

Secretion induced by choline was completely Ca^{2+}-dependent. The percentage of total catecholamine released into the medium after a 15-minute incubation in Ca^{2+}-free PSS containing 0.5 m*M* $MgCl_2$ was 2.2 ± 0.6 and 2.6 ± 0.2 percent in the presence and absence of 3 m*M* choline, respectively (three wells per group). In contrast, the percentage of total catecholamine in the medium after a 15-minute incubation in PSS containing 2.2 m*M* Ca^{2+} and 0.5 m*M* $MgCl_2$ was 9.7 ± 0.8 and 3.0 ± 0.2 percent in the presence and absence of 3 m*M* choline (three wells per group; $P < .01$, Student's *t*-test). Secretion induced by the physiological agonist acetylcholine or by carbachol or nicotine is also Ca^{2+}-dependent and occurs by exocytosis (*7, 9, 11*). The similar dependence of the effects of choline on Ca^{2+} suggests that choline also causes exocytosis.

Catecholamine secretion by these cells is induced by stimulation of nicotinic but not muscarinic receptors. Secretion is induced by the nicotinic agonists nicotine and 1,1-dimethyl-4-phenylpiperazinium and the mixed (nicotinic and musca-