References and Notes

1. There are a number of instances where toxins produced by pathogenic fungi (*2*), and to a far lesser extent by mycorrhizal fungi (*3*), are taken up and chemically modified by the host plant. However, these situations are quite different from the one reported here in that those toxins are clearly harmful to the plant, and, in fact, the outcome of the host-fungus battle strongly depends upon the chemical response of the host to the attacking microorganism (*4*).
2. J. Kuc, in *Microbial Toxins*, A. Ciegler and S. J. Ajl, Eds. (Academic Press, New York, 1972), vol. 8, p. 211; H. H. Luke and V. C. Gracen, in *ibid.*, p. 131; K. Rudolph, in *Physiological Plant Pathology*, R. Heitefuss and P. H. Williams, Eds. (Springer-Verlag, New York, 1976), p. 270.
3. E. Hacskaylo, *U.S. Dep. Agric. Misc. Publ. No. 1189*; R. Watling, *McIlvainea* **3**, 12 (1977).
4. H. Grisebach and J. Ebal, *Angew. Chem. Int. Ed. Engl.* **17**, 635 (1978); H. Schildknecht, *ibid.* **20**, 164 (1981).
5. S. M. Kupchan, D. R. Streelman, B. B. Jarvis, R. F. Dailey, Jr., A. T. Sneden, *J. Org. Chem.* **42**, 4221 (1977).
6. J. R. Bamburg and F. M. Strong, in *Microbial Toxins*, S. Kadis, A. Ciegler, S. J. Ajl, Eds. (Academic Press, New York, 1971), vol. 7, p. 207.
7. J. R. Bamburg, in *Mycotoxins and Other Fungal Related Food Problems*, J. V. Rodricks, Ed. (ACS Advances in Chemistry Series, American Chemical Society, Washington, D.C., 1976), vol. 149, p. 144; T. W. Doyle and W. T. Bradner, in *Anticancer Agents Based on Natural Product Models*, J. M. Cassidy and J. D. Douros, Eds. (Academic Press, New York, 1980), p. 43; Ch. Tamm, *Fortschr. Chem. Org. Naturst.* **31**, 63 (1974); in *Mycotoxins and Human and Animal Health*, J. V. Rodricks, C. W. Hesseltine, M. A. Mehlman, Eds. (Pathotox, Park Forest South, Ill., 1977), p. 209.
8. Ch. Tamm and W. Breitenstein, in *The Biosynthesis of Mycotoxins, A Study in Secondary Metabolism*, P. S. Steyn, Ed. (Academic Press, New York, 1980), p. 69.
9. Three separate collections of *B. megapotamica* at varying times of the year have all yielded the same result with respect to the baccharinoids. These collections, which range from 3 to 1300 kg, were made from a marsh located near Curitiba, Brazil, approximately 1000 miles south of Rio de Janeiro. In addition to the four baccharinoids reported in the literature (*5*), *B. megapotamica* contains a large number of additional closely related macrocyclic trichothecenes, including roridins D and E (B. B. Jarvis, unpublished results).
10. Baccharinoids **1** to **4** were isolated as sets of diastereoisomers, epimeric at C-13′. We recently isolated isororidin A from a culture of *Myrothecium verrucaria* (ATCC 24571), which is epimeric with roridin A, also at C-13′ (*11*). This same culture also yields roridin E (*12*) and isororidin E (*13*), which we suspect also differ in configuration only at C-13′.
11. J. O. Midiwo, thesis, University of Maryland, College Park (1981).
12. P. Traxler, W. Zürcher, Ch. Tamm, *Helv. Chim. Acta* **53**, 2071 (1970).
13. M. Matsumoto, H. Minato, K. Tori, M. Ueyama, *Tetrahedron Lett.* (1977), p. 4093.
14. R. F. Bryan, unpublished results.
15. A number of simple trichothecenes (*6*) and three recently isolated macrocyclic trichothecenes, verrucarin L and its acetate (*16*) and roridin K acetate (8α-acetoxyroridin E) (*11*), also possess a hydroxyl or ester group at C-8. However, all of these fungal metabolites are substituted at C-8 in the α configuration.
16. B. B. Jarvis, J. O. Midiwo, T. DeSilva, E. P. Mazzola, *J. Antibiot.* **34**, 120 (1981).
17. These seeds, as well as 2-year-old plants grown from seed in a greenhouse, were provided by G. M. Christenson, Economic Botany Laboratory, U.S. Department of Agriculture, Beltsville, Md.
18. We also have carefully examined several other species of *Baccharis* (*B. halimifolia*, *B. pterimoides*, and *B. sarathroides*) and find no sign of baccharinoids. Furthermore, the National Cancer Institute has screened nearly 90 species of *Baccharis* and finds, because of lack of P388 activity in vivo, that none of the species of *Baccharis* is likely to contain baccharinoids (M. Suffness, personal communication).
19. Seedlings of *B. megapotamica* (about 15 cm tall) were suspended so that only the roots were in contact with an aqueous solution of the mycotoxin. The solutions were prepared by the addition of 25 mg of mycotoxin in 2.5 ml of ethanol or 50 mg of mycotoxin in 5 ml of ethanol to 50 ml of distilled water (10 μ*M* in calcium sulfate). After a suitable time, the plant was removed, and the roots were washed with water and separated from the upper portions of the plants. The plant material was freeze-dried and extracted (40°C overnight in absolute ethanol). The crude extracts were filtered through small portions of activated silica gel and the silica was washed with 20 percent methanol in methylene chloride. The resulting dark green gums were analyzed by HPLC (methanol in methylene chloride) on a silica gel column. The roots showed only a trace of trichothecenes. Controls with plants that had stood only in the aqueous solution with and without added ethanol showed no trace of mycotoxins.
20. Roridin A (**5a**) and verrucarin A (**6a**) were isolated from a large-scale (760 liters) fermentation with *Myrothecium verrucaria* carried out under the direction of R. Geoghegan, Frederick Cancer Research Center, Frederick, Md. A number of new trichothecenes were isolated from this fermentation [B. B. Jarvis, G. Pavanasasivam, C. E. Holmlund, T. DeSilva, G. P. Stahly, E. P. Mazzola, *J. Am. Chem. Soc.* **103**, 472 (1981)].
21. W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **43**, 2923 (1978).
22. Prepared by the selenium oxidation of roridin A [B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, E. P. Mazzola, *J. Med. Chem.* **23**, 1054 (1980)].
23. G. M. Christenson, personal communication.
24. H. C. Cutler and J. H. LeFiles, *Plant Cell Physiol.* **19**, 177 (1978).
25. A. N. Kishaba, D. L. Shankland, R. W. Curtis, M. C. Wilson, *J. Econ. Entomol.* **53**, 211 (1962).
26. *Baccharis megapotamica* causes serious toxicosis in cattle in the local area that graze on this plant (*23*).
27. J. F. Anderson, unpublished data.

28. Supported by National Cancer Institute grant CA 25967. We thank G. M. Christenson for assistance in obtaining seeds and plant samples of *B. megapotamica*.

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Direct Electrical Connections Between Transient Amacrine Cells in the Catfish Retina

Abstract. *Transient amacrine cells were identified by their intracellularly recorded response to flashes of light. These cells typically respond with a transient depolarization, often followed by a steady-state response during the stimulus. When two electrodes were placed in different transient amacrine cells, current of either polarity passed through one electrode produced a steady-state voltage change that was recorded by the electrode in the nearby cell. Following identification of the physiological type, transient amacrine cells were injected with horseradish peroxidase and the tissue was processed for light and electron microscopy. Both conventional chemical synaptic junctions and gap junctions were found to connect amacrine cells.*

Gap junctions have been found in both the outer and inner plexiform layers of the retina in several species (*1*). In the inner plexiform layer, amacrine cells form electrical junctions with bipolar cells (*2*). There are only two reports of gap junctions between amacrine cells: in the cat retina, between connected type A_2 (bistratified, narrow field) cells (*2*), and in the inner plexiform layer of the rat retina (*3*). However, physiological identification of these amacrine cells remains uncertain. Jensen and DeVoe (*4*) described a physiological type of amacrine cell which is probably similar to the transient amacrine cell discussed here. On the basis of dye coupling with other cells, they suggested that this type of cell might be electrically coupled to other amacrine cells. We report a class of amacrine cell which makes electrical connections with other physiologically identified amacrine cells.

Eyecup preparations of catfish retinas were continuously superfused with moist oxygen (*5*). Microelectrodes filled with 2*M* potassium citrate with or without horseradish peroxidase (HRP; 4 percent, weight to volume) were used to record from amacrine cells. After the electrophysiological experiment the retina was processed according to the procedure described by Christensen (*6*).

In some experiments two separate microelectrodes were used to record from individual transient amacrine cells locat-

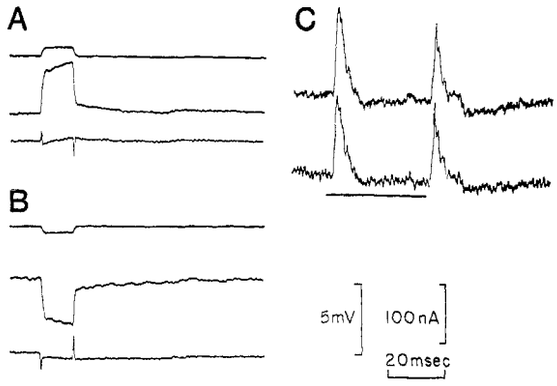

Fig. 1. (A and B) Voltage pulse applied to cell (top trace), voltage response from coupled cell (middle trace), and voltage response with electrode just outside the cell (bottom trace). (C) Intracellular response recorded with two separate electrodes, from transient amacrine cells during a flash of light. Length of bar indicates duration of light (0.5 second).

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ed near one another. Figure 1C shows the responses of the two cells during a flash of light. Both responded transiently during the turning on and off of the stimulus. There was no steady-state response. The top traces in Fig. 1, A and B, show a depolarizing and a hyperpolarizing step applied to one of these cells. The voltage response recorded from the other cell is shown in the middle traces. When the recording electrode was located just outside the cell in the extracellular space, only the capacitative transient responses were recorded (bottom traces). This suggests that these cells are electrically coupled, since there is no rectification to the passage of current. We conclude that transient amacrine cells form a space that has a low internal resistance but a high resistance with respect to the rest of the retinal space. Furthermore, displacement of the intracellular potential by a few millivolts does not bring about the cell's characteristic transient response. This suggests that the transient response is not produced by this pathway (at least not directly). On the other hand, current of similar amplitude applied to horizontal cells did cause transient responses in this type of amacrine cell.

A recording from another transient amacrine cell is shown in the inset to Fig. 2. The initial response was a rapid depolarization followed by a steady-state response. When the stimulus was turned off the phasic depolarization was repeated. This cell was injected with HRP and processed for electron microscopy. Figure 2 presents a drawing of the cell as seen in a flat-mount preparation. This cell has the same general morphology as transient amacrine cells labeled with Procion yellow (*7*).

Figure 3 shows light and electron micrographs of the cell whose drawing is presented in Fig. 2. Figure 3A is a light micrograph of a 10-μm section that was cut in the radial plane. The arrowheads indicate portions of HRP-labeled dendrites in the inner plexiform layer. Figure 3B is a low-power electron micrograph of the cell. Part of the HRP-labeled dendrite is indicated, and the double arrows show the location of a gap junction (illustrated in Fig. 3F at higher magnification). The postsynaptic element must be an unlabeled amacrine cell process because of the conventional chemical synaptic contact this process makes with another unidentified cell (circled).

Figure 3, C and D, shows serial electron micrographs of the circled area in Fig. 3B. The dendrite designated by *amc* is the same one that makes a gap junction contact with the HRP-labeled dendrite.

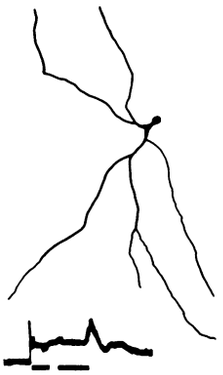

Fig. 2. Drawing of an HRP-injected transient amacrine cell. The distal retina is to the right and ganglion cell layer is to the left. Inset shows intracellular response of the cell to a flash of light. Length of the bar indicates duration of the light (1 second).

In Fig. 3C this dendrite forms a small chemical synaptic contact with an unidentified postsynaptic process. The absence of a ribbon structure at the presynaptic membrane identifies the presynaptic element as an amacrine cell process (*8*). In Fig. 3D a bipolar axon terminal forms a ribbon synapse with the amacrine cell and the unidentified process. This synaptic arrangement forms a typical dyad (*9*). Figure 3E shows a different portion of a dendrite from this same cell; the arrowheads indicate the location of a gap junction. This section was tilted in the microscope to make the membrane of the conventional chemical synapse visible. Because of the intense reaction product, synaptic vesicles are barely visible, although the synaptic membranes are apparent.

Transient amacrine cells are identified by their rapid transient response when a light is turned on and off. They may or may not respond with a maintained steady-state potential. Structural input to this class of amacrine cell from the distal retina occurs through bipolar cells. Current application indicates that horizontal cells may also form direct or indirect connections with transient amacrine cells. Transient amacrine cells make chemical or electrical synaptic junctions with each other and form chemical synapses with bipolar, amacrine, and ganglion cells (*8*).

It is difficult to determine from these results the contribution from gap junctions or chemical synapses to either the dynamic or steady-state components of the synaptic potential generated during a photic input. One might conclude that during a step of current applied to one cell, a steady voltage change would result in the other cell. This is implied by the results shown in Fig. 1, A and B.

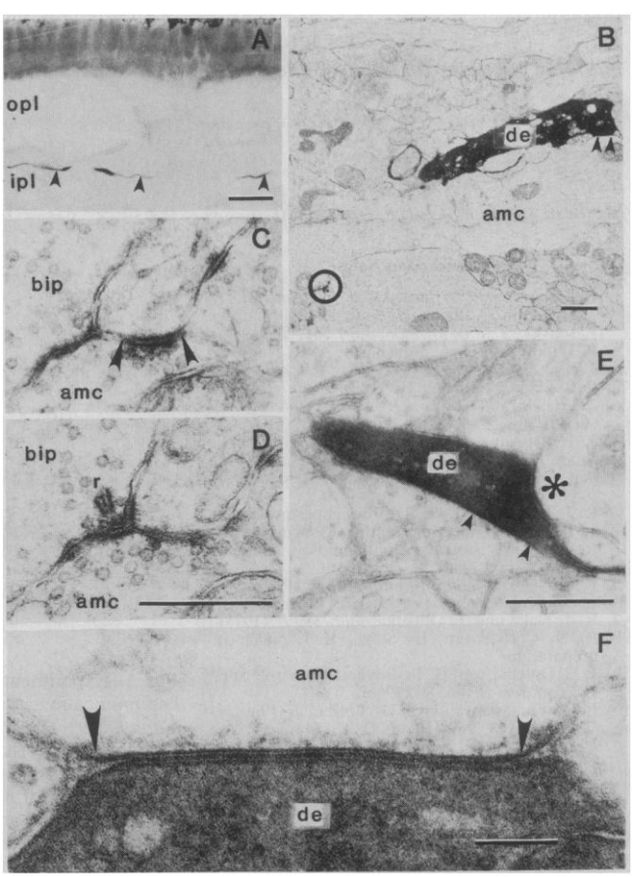

Fig. 3. (A) Light micrograph of the retina in transverse section. (B) Low-power electron micrograph of a thin section. Scale bar, 1 μm. (C and D) Serial electron micrographs showing that the postsynaptic process indicated by *amc* in (B) is an amacrine process. Scale bar, 0.5 μm. (E) Portion of an HRP-labeled dendrite from the same cell. A gap junction is indicated with arrows and a chemical synapse with an asterisk. Scale bar, 0.5 μm. (F) High-power electron micrograph showing the gap junction from the area indicated by the arrowheads in Fig. 3B. Scale bar, 0.1 μm. Abbreviations: *opl*, outer plexiform layer; *ipl*, inner plexiform layer; *bip*, bipolar cells; *de*, dendrite; *r*, ribbon synapse.

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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References and Notes

1. F. Chen and P. Witkovsky, *J. Neurocytol.* **7**, 721 (1978); H. Kolb, *ibid.* **6**, 131 (1977); A. Lasansky, *J. Physiol. (London)* **301**, 59 (1980); P. Witkovsky, D. A. Burkhardt, A. R. Nagy, *J. Comp. Neurol.* **186**, 541 (1979).
2. E. V. Famiglietti and H. Kolb, *Brain Res.* **84**, 293 (1975); H. Kolb, *J. Neurocytol.* **8**, 295 (1979).
3. J. E. Dowling, in *The Neurosciences: Fourth Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1979), p. 163.
4. R. J. Jensen and R. D. DeVoe, paper presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla. (April 1980).
5. K.-I. Naka, *J. Neurophysiol.* **40**, 26 (1977).
6. B. N. Christensen, *ibid.* **39**, 197 (1976).
7. K.-I. Naka and T. Ohtsuka, *ibid.* **38**, 72 (1975).
8. B. N. Christensen, H. Sakai, K.-I. Naka, in preparation.
9. J. E. Dowling and B. B. Boycott, *Proc. R. Soc. London Ser. B* **166**, 80 (1966).
10. E. J. Furshpan and D. D. Potter, *J. Physiol. (London)* **145**, 289 (1959); D. C. Spray, A. L. Harris, M. V. L. Bennett, *J. Gen. Physiol.* **77**, 77 (1981); A. L. Harris, D. C. Spray, M. V. L. Bennett, *ibid.*, p. 95.
11. We thank R. Polich for technical assistance. Supported by NIH grants NS-14429 and NS-13778 and by NSF grant BNS-7823600.

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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).

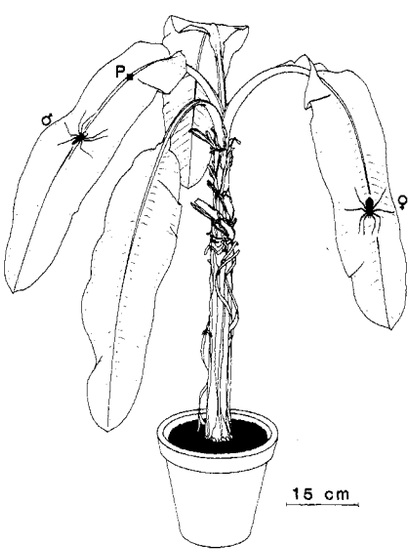

Fig. 1. Experimental setup used for studying communication through a banana plant in *Cupiennius*. The male begins courtship on a pheromone-covered leaf blade [with a vibration pickup (*P*) near the base] and then must select the correct petiole to reach a stationary female hidden on another leaf. In 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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