to induce primarily chemotaxis and to avoid significant cellular activation and tissue injury. Intravascular aggregation and passive sequestration of neutrophils were excluded by (i) the very rapid plasma clearance of microsphere [^{3}H]FMLP compared to the gradual accumulation of neutrophils, (ii) different microscopic locations of microspheres and neutrophils, and (iii) absence of pulmonary neutrophil accumulation (and edema) when FMLP spheres were injected without magnetic targeting. This last observation also indicates that lung injury should not occur as a side effect of targeting to nonpulmonary sites. The extravascular migration of neutrophils toward microsphere fragments indicates that chemotaxis is the principal mechanism of cell accumulation. Because FMLP can also produce smooth muscle contraction (*15*), vasospasm might have contributed to the initial intravascular phase of cell accumulation. Magnetic targeting of sufficient chemotactic peptide provides a model for acute alveolar damage (*16*) that minimizes the systemic effects of freely circulating initiators, such as cobra venom factor (*17*) and zymosan-activated serum (*18*). It also provides a rapid in vivo test for pharmaceutical agents that modulate chemotaxis (*19*). Potential therapeutic applications include restoration of local neutrophil responses in patients with specific inflammatory, infectious, neoplastic, metabolic, toxicologic, thermal (*20*), and traumatic disease processes (*21*), in which the generation of chemotaxins is blocked, chemotaxins are oxidatively degraded (*22*), or neutrophil responses are reversibly suppressed. In most local infections, sufficient chemoattractant is present; however, in life-threatening infections, such as intra-abdominal abscesses with sepsis, either receptor-specific (complement-based) or nonspecific deactivation of chemotaxis can occur (*23*). In some cases, the very high local gradients of FMLP produced by targeting may succeed in refocusing the chemotactic and microbicidal activities of the patient's neutrophils if they retain a sufficient fraction of their normal response to FMLP (*23*). However, more commonly, host cells will be almost completely unresponsive, and restoration of local inflammation may require the concomitant transfusion of fresh donor granulocytes.

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References and Notes

1. E. Schiffmann, B. A. Corcoran, S. M. Wahl, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1059 (1975); L. T. Williams, R. Snyderman, M. C. Pike, R. J. Lefkowitz, *ibid.* **74**, 1204 (1977).
2. H. J. Showell *et al.*, *J. Exp. Med.* **143**, 1154 (1976).
3. K. J. Widder, A. E. Senyei, S. D. Reich, D. F. Ranney, *Proc. Am. Assoc. Cancer. Res.* **19**, 17 (1978); K. J. Widder, A. E. Senyei, D. G. Scarpelli, *Proc. Soc. Exp. Biol. Med.* **158**, 141 (1978); K. J. Widder, A. E. Senyei, D. F. Ranney, *Advances in Pharmacology and Chemotherapy*, R. J. Schnitzer, S. Garattini, A. Goldin, G. Hawking, I. Kopin, Eds. (Academic Press, New York, 1979), vol. 16, pp. 213–271.
4. A. J. Quattrone and D. F. Ranney, *J. Anal. Toxicol.* **4**, 12 (1980); *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **158**, 141 (1980).
5. Ten milligrams of FMLP (Sigma) was dissolved in 0.25 ml of dimethyl sulfoxide and added to an aqueous slurry of 136 mg of ferrofluidic magnetite [25 percent Fe_3O_4 (weight to volume); SE-10, Ferrofluidics Corp.] containing 125 mg globulin-free human serum albumin (Sigma). This mixture was emulsified in 30 ml cottonseed oil (Sargent-Welch) and sonicated (*6*). Heat stabilization and extraction of oil were performed as reported (*6*).
6. K. J. Widder, A. E. Senyei, D. F. Ranney, *Cancer Res.* **40**, 3512 (1980).
7. Spheres were washed to remove rapidly releasable surface peptide (63.3 percent of the entrapped FMLP). Controlled release of the remaining FMLP (36.7 percent) was carried out at 10 mg spheres per milliliter of phosphate-buffered saline (*p*H 7.35, 37°C). Identical washing was performed before in vivo injection.
8. G. E. Hatch, D. E. Gardner, D. B. Menzel, *J. Exp. Med.* **147**, 182 (1978); W. D. Welch, C. W. Graham, J. Zaccari, L. D. Thrupp, *J. Reticuloendothelial Soc.* **28**, 275 (1980).
9. Luminol-enhanced chemiluminescence was performed as in (*8*), by exposing human and Sprague-Dawley rat neutrophils to 1:1000 and 1:100 dilutions, respectively, of control-release supernatants [prepared as in (*7*)]. After backcalculation for dilutions, the ratios of bioactive to total ^{3}H-labeled supernatant FMLP obtained with human and rat target cells were 1.1 ($8 \times 10^{-5}M/7 \times 10^{-5}M$) and 1.0 ($7 \times 10^{-5}M/7 \times 10^{-5}M$), respectively. Midrange responses of human and rat cells to soluble FMLP standards are $3 \times 10^{-8}M$ and $1 \times 10^{-7}M$, respectively. Chemiluminescence was used in preference to chemotaxis because of rapidity and improved quantification of small concentration differences.
10. W. C. Maloney, K. McPherson, L. Fliegelman, *J. Histochem. Cytochem.* **8**, 200 (1960).
11. R. D. Lillie, Ed., *Histopathologic Technic and Practical Histochemistry* (McGraw-Hill, New York, 1965), p. 406.
12. Thirty percent of the total microsphere FMLP injected was captured in thoracic viscera (predominantly right lung). The remainder localized principally in liver (49 percent) and spleen (8 percent). This intermediate targeting efficiency resulted from only partial inclusion of left thoracic structures within the magnetic field.
13. Prepared by hypotonic ammonium chloride lysis of blood (*8*).
14. B. C. Lee, H. van der Zee, A. B. Malik, *J. Appl. Physiol. Respirat. Environ. Exercise Physiol.* **47**, 556 (1979); R. L. Johnson, Jr., S. S. Cassidy, M. Haynes, R. L. Reynolds, W. Schulz, *ibid.* **51**, 845 (1981).
15. W. A. Marasco, J. C. Fantone, P. A. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7470 (1982).
16. T. Sachs, C. F. Moldow, P. R. Craddock, J. K. Bowers, H. S. Jacob, *J. Clin. Invest.* **61**, 1161 (1978).
17. G. O. Till, K. Johnson, R. Kunkel, P. A. Ward, *ibid.* **69**, 1126 (1982).
18. A. C. Helfin and K. L. Brigham, *ibid.* **68**, 1253 (1981).
19. N. Ackerman, S. Martinez, T. Thieme, A. Mirkovich, *J. Pharmacol. Exp. Ther.* **221**, 701 (1982).
20. G. O. Till *et al.*, *J. Trauma* **23**, 269 (1983).
21. J. I. Gallin, *Rev. Infect. Dis.* **3**, 1196 (1981); J. C. Fantone and P. A. Ward, *Am. J. Pathol.* **107**, 397 (1982).
22. S. J. Weiss, M. B. Lampert, S. J. Test, *Science* **222**, 625 (1983).
23. J. S. Solomkin, M. K. Jenkins, R. D. Nelson, D. Chenoweth, R. L. Simmons, *Surgery* **90**, 319 (1981).
24. I thank R. Mack, B. Beck, M. Glass, and D. Rodriguez for technical assistance and Wilson Engraving, Dallas, Texas, for computer enhancement of the cover photograph. Supported in part by NIH CA15673 and Eli Lilly.

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Coupling of Action Potential Activity Between Unmyelinated Fibers in the Peripheral Nerve of Monkey

Abstract. *Bidirectional coupling of action potential activity occurs between unmyelinated fibers in the normal peripheral nerve of monkey. The site of coupling is near the cutaneous nociceptive receptor associated with one of the fibers. This coupling could be due to an electrical synapse and could provide the basis for the flare associated with the axon reflex.*

Somatosensory nerve fibers have generally been assumed to have no chemical or electrical synapses with one another outside the central nervous system. However, cross talk between nerve fibers, presumably due to electrical coupling, has been observed in various forms of nerve injury (*1*). This prompted us to examine whether similar interactions between fibers might occur in normal peripheral nerves.

Monkeys (*Macaca fasicularis*, 3.5 to 6.5 kg) were anesthetized with an intravenous injection of pentobarbital (3 to 6 mg kg^{-1} $hour^{-1}$) or by inhalation of a mixture of halothane and N_2O (0.8 percent and 67 percent, respectively). The animals were paralyzed with pancuronium to eliminate artifacts caused by muscle twitching and were mechanically ventilated to maintain the end-tidal pressure of CO_2 at 32 to 40 torr. Recordings from single fibers in the peripheral nerve (superficial radial, ulnar, median, and sural) were obtained from finely teased nerve strands by the use of standard techniques (*2*). The strands were cut proximally so that only centripetally directed action potentials were recorded (Fig. 1a). Tripolar stimulating electrodes were placed on the parent nerve proximal and distal to the recording site and were insulated from surrounding tissue with plastic (*3*). The preparation was bathed in mineral oil. Action potentials recorded at the recording electrode in response to stimulation from the proximal electrode indicated coupling of ac-

tion potential activity between nerve fibers.

Coupling was observed in 33 instances. In each case the coupling was between two unmyelinated fibers (*4*). Coupling between more than two fibers was not observed. In the example shown in Fig. 1, stimulation from the proximal electrode resulted in an action potential at a latency of 280 msec. Collision techniques (Fig. 1, c to e) were used to verify that this action potential was due to coupling of action potential activity between two peripheral nerve fibers. Increasing the proximal electrode stimulation intensity to twice the threshold intensity did not result in additional action potentials. However, delivery of two suprathreshold stimuli at the proximal site (at intervals as short as 3 msec between stimuli) resulted in two coupled action potentials. In each of three fiber pairs tested, collision techniques were used to demonstrate that the action potentials could propagate in either direction across the coupling site (*5*). Therefore, coupling can occur bidirectionally.

In 15 cases, we tested for a cutaneous receptor with an action potential whose shape was the same as that observed for the coupled pair. In 13 of these cases, a receptor was identified and each was classified as a nociceptor on the basis of a monotonically increasing response to high-intensity mechanical and heat stimuli (*6*). Two types of evidence corroborated the conclusion that the cutaneous receptor was associated with the coupled action potential produced by stimulation at the proximal site. Mechanical stimulation of the receptive field (n = 13), but not the surrounding skin, resulted in an increase in the latency of the coupled action potential from proximal electrode stimulation (Fig. 2a). Electrical stimulation at the proximal site (n = 2) resulted in a subsequent decrease in response of the receptor to heat stimuli (Fig. 2b).

Intracutaneous injection of a local anesthetic (procaine) into the receptive field of seven fiber pairs tested abolished the coupled responses for about 40 minutes (Fig. 1, f to g). These results suggest that the site of coupling was at or near the receptive field. As further support of this hypothesis, the measured distance from the recording electrode to the receptive field corresponded to the distance to the coupling site computed from the collision data (*7*).

We found coupling in about 3 percent of the unmyelinated fibers tested (*8*). The true incidence of coupling is probably higher since the demonstration of coupling in this preparation depends on the chance that one of the coupled fibers occurs in the parent nerve and the other occurs in the teased strand from which the recording is obtained. It seems likely

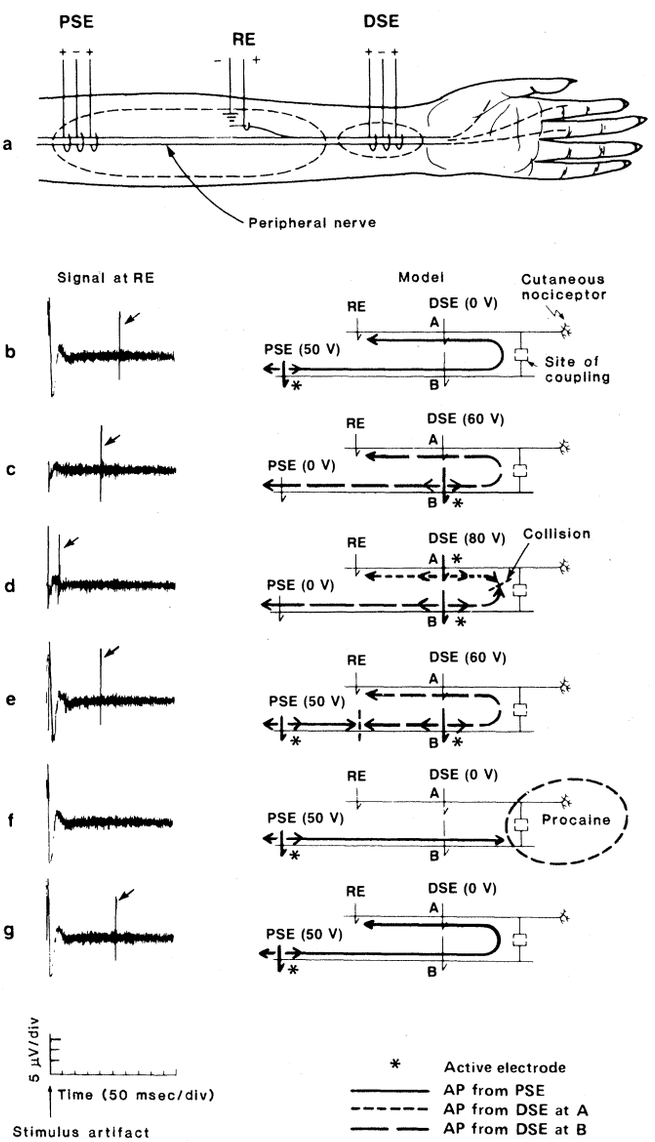

Fig. 1. Demonstration of action potential coupling between unmyelinated fibers in the normal peripheral nerve of the monkey. (a) Location of recording electrode (RE), proximal stimulating electrode (PSE), and distal stimulating electrode (DSE). All stimulus durations were 0.1 msec. (b) Electrical stimulation at the PSE resulted in an action potential (AP) at a latency of 280 msec (arrow points to the AP). On the right is the model we propose to explain the observation. The AP reached the RE via coupling between the fibers labeled A and B. (c) Stimulation at the DSE at 60 V led to stimulation of fiber B only. The same AP occurred at a latency of 220 msec as a result of coupling between the fibers. (d) When the voltage at the DSE was increased to 80 V, the same AP occurred at a latency of 46 msec, which corresponded to the directly conducted AP. As depicted in the model, the coupled AP was not observed as a result of collision. Near the threshold for this directly conducted AP, the observed latency alternated between 46 and 220 msec. Two AP's were never observed. (e) When the DSE (at 60 V) and PSE were activated simultaneously, a single AP occurred at the 220-msec latency. Near the DSE threshold for activation of fiber B, the observed latency alternated between 280 and 220 msec. Again, two AP's were never observed. (f) A cutaneous receptor was associated with this AP, which had characteristics of a nociceptor (*6*). Intracutaneous injection of procaine (2 percent, 0.05 ml) at the receptive field eliminated the coupled activity after PSE stimulation (*13*). (g) Forty minutes after this procaine injection, the coupled response returned.

that coupled fibers would tend to remain together throughout their course in the peripheral nerve, and, therefore, would not often be teased apart.

Five different mechanisms could explain the observed coupling.

1) After stimulation at the proximal site, the synchronous discharge of action potentials in multiple unmyelinated nerve fibers adjacent to the fiber from which recordings are made could artifactually alter the extracellular electric fields and thus lead to excitation. From this hypothesis, the site at which coupling occurred would more likely be along the course of the fiber in the parent nerve, where the density of fibers is greater, than at the terminal branches. This is at variance with our finding that the coupling site was at or near the cutaneous receptor.

2) Antidromic invasion of adjacent receptors could result in the release of chemicals around the receptor of the coupled fiber and thus activate this fiber. As the intensity of stimulation from the proximal (or distal) electrode is increased above threshold, more fibers would be activated, a larger release of chemicals would occur, and thus more than one action potential would be expected. However, only one action potential was observed when the intensity of the stimulus from the proximal electrode was increased by a factor of 2 above threshold. Suprathreshold stimulation from the distal electrode also resulted in one coupled action potential. One could argue that, after the first action potential, the receptor is refractory, thus preventing other action potentials from occurring. However, two suprathreshold stimuli, 3 msec apart, at the proximal site resulted in two coupled action potentials. Therefore, the refractory period is short.

3) Stimulation of sympathetic efferents at the proximal site could result in a cutaneous smooth muscle contraction that activates the nociceptors. However, coupling involving low threshold mechanoreceptors would also be expected. In addition, the observation of bidirectional coupling is not consistent with this hypothesis.

4) Peripheral nerve fibers could have a branch at the cutaneous receptor which runs back along the course of the nerve for long distances. Although there is no anatomical evidence to support this morphology, this hypothesis is consistent with the observed neurophysiological findings.

5) A bidirectional synapse (either chemical or electrical) could occur between unmyelinated nerve fibers near the cutaneous receptor. Bidirectional chemical synapses have been reported in invertebrates but not in mammals (*9*). Electrotonic coupling between cells in the nervous system has been reported both for vertebrates and invertebrates (*10*) and is thought to be associated with gap junctions. There is some evidence that gap junctions may occur in tooth pulp where coupling between myelinated fibers has been reported (*11*).

Heretofore, neuronal interactions have been presumed to occur only within the central nervous system and ganglia of the autonomic nervous system. Our finding of coupling between unmyelinated peripheral nerve fibers indicates that interactions occur in the peripheral nervous system. The function of this interaction is not clear. One noteworthy possibility is that it provides a basis for the flare response. The flare is part of the axon reflex and has been thought to result from antidromic invasion of axon branches after nociceptor activation. Although free nerve endings have been observed in the dermis and around capillaries (*12*), no axonal connection between these endings has been described. The finding of coupling involving nociceptors suggests that these two types of free nerve endings could be coupled. Another possibility is that sympathetic efferents are coupled to nociceptors. This idea is of interest in view of the occasional occurrence of certain chronic pain states, such as causalgia or reflex sympathetic dystrophy, which are marked by excessive sympathetic activity in the region affected. Further experiments will be needed to estimate better the true incidence of coupling and to determine its functional significance.

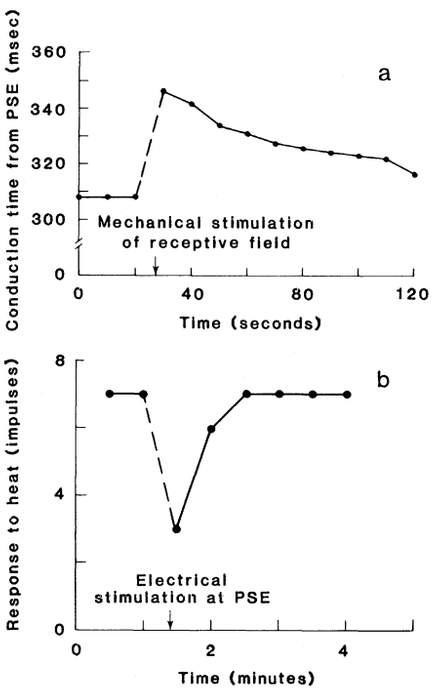

Fig. 2. Evidence that a cutaneous nociceptor is associated with the coupled fiber. (a) Proximal stimulating electrode (PSE) stimulation every 10 seconds resulted in an action potential at the recording electrode after a latency of 308 msec. The latency increased substantially after mechanical stimulation of the receptive field with a stiff nylon probe (35 g, 0.57 mm diameter) immediately preceding trial 4. The mechanical stimulation evoked 32 action potentials. (b) A laser thermal stimulator (*14*) was used to apply heat stimuli (47°C for 1 second) to the receptive field every 30 seconds. Three seconds before the heat stimulus of trial 3, the PSE was stimulated at 50 Hz for 1.5 second at a strength sufficient to elicit the coupled action potential. The response to heat was suppressed for two trials. Total response to heat per stimulus is plotted.

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References and Notes

1. Z. Seltzer and M. Devor, *Neurology* **2**, 1061 (1979); M. Rasminsky, *J. Physiol. (London)* **305**, 151 (1980); H. Blumberg and W. Janig, *Exp. Neurol.* **76**, 468 (1982); R. A. Meyer, S. N. Raja, J. N. Campbell, S. E. Mackinnon, A. L. Dellon, *Brain Res.*, in press.
2. J. N. Campbell and R. A. Meyer, *J. Neurophysiol.* **49**, 98 (1983).
3. A tripolar electrode configuration was chosen so that electrical stimulation of the nerve resulted in ortho- and antidromic action potentials propagating away from the stimulus site. This configuration also minimized artifactual current spread away from the electrodes [C. van den Honert and J. T. Mortimer, *IEEE Trans. Biomed. Eng.* **28**, 373 (1981)].
4. The mean conduction velocities for the two coupled fibers were not significantly different (for fiber A identified in the model on Fig. 1, it was 0.76 ± 0.05 m/sec; for fiber B, 0.76 ± 0.04 m/sec). In two instances not reported here, coupling was observed between two myelinated fibers. In one of these cases, the fiber had a cutaneous receptor with properties of an A fiber mechano-heat nociceptor [J. N. Campbell, R. A. Meyer, R. H. LaMotte, *J. Neurophysiol.* **42**, 1669 (1979)]. Coupling between myelinated fibers occurs infrequently in the normal peripheral nerve of cat [A. Brennan and B. Matthews, *J. Physiol. (London)* **334**, 70P (1982); S. J. W. Lisney and C. M. Pover, *J. Neurol. Sci.* **59**, 255 (1983)].
5. For bidirectional coupling to be demonstrated with our present experimental configuration, the voltage at the distal electrode needed to activate fiber A must be significantly less than that needed to activiate B (see model in Fig. 1 for location of fiber A and fiber B). Simultaneous stimulation of the distal site at a voltage sufficient to excite fiber A (but not fiber B) and of the proximal site at a voltage sufficient to elicit the coupled action potential results in a single action potential at the recording electrode with a conduction delay corresponding to the directly conducted action potential. The coupled action po-

tential from the proximal electrode is not observed because of collision. When the proximal site is stimulated after the distal site, only one action potential is recorded until the interval between the stimulations is just longer than the time necessary for the action potential initiated by the distal electrode to propagate via the coupling site to the proximal electrode. At this time interval, the action potential initiated by the distal electrode has propagated just beyond the proximal electrode and can no longer collide with the action potential activated by the proximal electrode. Thus, two action potentials are observed; the direct action potential and the coupled action potential. Since the action potential stimulated by the distal electrode propagates across the coupling site in the direction opposite that stimulated by the proximal electrode, bidirectionality of the coupling is demonstrated.

6. P. Bessou and E. R. Perl, *J. Neurophysiol.* **32**, 1025 (1962); R. E. Beitel and R. Dubner, *ibid.* **39**, 1160 (1976); R. H. LaMotte and J. N. Campbell, *ibid.* **41**, 509 (1978); R. A. Meyer and J. N. Campbell, *Brain Res.* **224**, 149 (1981); *Science* **213**, 1527 (1981).

7. The collision experiments provide data for the action potential conduction times from one electrode to another. From the distance between the electrodes, the distance from the recording electrode to the coupling site can be computed if the following three assumptions are made: (i) The conduction velocity of the coupled fibers is uniform along their length. (ii) The time required for the action potential to cross the coupling site is negligible compared to the total conduction time. (iii) The distance to the coupling site from the distal stimulating electrode is the same for the two fibers. The ratio of the computed distance to the coupling site divided by the measured distance from the recording electrode to the receptive field of the nociceptor was close to unity (0.98 ± 0.06, $n = 9$); this indicates a high degree of correspondence and thus provides further evidence that the coupling site was at or near the receptor.

8. In this series of experiments, we did not determine the total number of A and C fibers tested. However, in three experiments in the superficial radial nerve of the baboon, we determined that an average of 4.9 ± 0.5 A fibers and 5.3 ± 0.6 C fibers were present in each recorded strand ($n = 30$). Using these numbers, we estimate that in this study of 164 strands we tested 804 A fibers and 869 C fibers.

9. P. A. V. Anderson and W. E. Schwab, *J. Neurophysiol.* **50**, 671 (1983); G. McCarragher and R. Chase, *Soc. Neurosci. Abstr.* **9**, 1025 (1983).
10. For reviews, see F. E. Dudek, R. D. Andrew, B. A. MacVica, R. W. Snow, C. P. Taylor, in *Basic Mechanisms of Neuronal Hyperexcitability*, H. H. Jasper and N. M. VanGelder, Eds. (Liss, New York, 1983), p. 31; H. Korn and D. S. Faber, in *The Neurosciences Fourth Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass, 1979), p. 333, M. V. L. Bennett, in *Handbook of Physiology*, E. R. Kandel, Ed. (Williams & Wilkins, Baltimore, Md., 1977), vol. 1, p. 357.
11. B. Matthews and G. R. Holland, *Brain Res.* **98**, 354 (1975).
12. L. Kruger, E. R. Perl, M. J. Sedivec, *J. Comp. Neurol.* **198**, 137 (1981); L. Kruger *et al.*, *Soc. Neurosci. Abstr.* **9**, 777 (1983).

13. Although not shown in this figure, this procaine injection also abolished the coupled action potential at 220 msec but not the directly conducted action potential at 46 msec.

14. R. A. Meyer, R. E. Walker, V. B. Mountcastle, *IEEE Trans. Biomed. Eng.* **23**, 54 (1976).

15. We thank R. Burke and J. Aryanpur for their contributions. Supported by PHS research grant NS-14447 and teacher investigator award NS-00519 and by DOD contract N00024-83-C-5301.

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Do Frogs Communicate with Seismic Signals?

Abstract. *Male white-lipped frogs exhibit conspicuous behavioral responses to calling conspecific males that are nearby but out of view. Since the calls often are accompanied by strong seismic signals (thumps), and since the male white-lipped frog exhibits the most acute sensitivity to seismic stimuli yet observed in any animal, these animals may use seismic signals as well as auditory signals for intraspecific communication.*

White-lipped frogs (*Leptodactylus albilabris*) inhabit the Luquillo Mountains and adjacent lowlands of Puerto Rico, where males of the species often are found calling from moist ground. We observed that isolated calling males (those stationed far from calling conspecific males) often drastically alter their calling patterns in response to remote, very light footfalls, indicating acute sensitivity to substrate-borne vibrations (seismic stimuli). Therefore, we decided to study the seismic sense of the animal both physiologically and behaviorally. Knowing that the American bullfrog (*Rana catesbeiana*) derives acute seismic sensitivity from its saccule and lagena (*1*), we focused our physiological studies on the white-lipped frog's auditory-vestibular nerve. There we found acute seismic sensitivity, with individual axons exhibiting linear transfer ratios as large as 20,000 spikes per second (axon firing rate) per cm/sec^2 (substrate vibration amplitude) and 70 spikes per second per 10^{-10}m for dorsoventral sinusoidal vibration of the whole animal (*2*). Each seismic axon exhibited band-pass properties and could be characterized in part by a frequency (best vibratory frequency) approximately at the point of the passband's peak transfer ratio. We found these clustered in two groups, one ranging from 20 to 160 Hz, the other ranging from 220 to 300 Hz (Fig. 1a).

Field experiments were carried out during June of 1983 to determine the ability of the male white-lipped frog to detect seismic stimuli in its natural habitat. We placed a vertical geophone and a cardioid microphone approximately 1 m from isolated, calling frogs (*3*). This was done between 1800 and 2130 hours in remote areas of the Luquillo Mountains where there was virtually no automobile traffic or other forms of human activity. A calling, isolated white-lipped frog typically emits 40-msec chirps at a rate of approximately 4 per second. This call probably serves as an advertisement of the male's location (*4*). Using a stereophonic system with headphones to monitor and record simultaneously the microphone and geophone responses, we immediately noticed a transient geophone response (a "thump") at the onset of each chirp. Analysis of the recorded waveforms verified this observation, showing transient vertical (Rayleigh) waves with peak accelerations in the neighborhood of 2 cm/sec^2 (1 m from the frog) concomitant with each chirp (Fig. 1b). Analysis with fast fourier transforms revealed that the power in each thump was distributed over frequencies below 150 Hz, extending down to at least 10 Hz (the low-frequency corner of our geophone response) (*3*), but confined predominantly between 20 and 70 Hz (Fig. 1c). The thump spectrum thus corresponded well to the lower range of seismic sensitivity in the frog's ear (Fig. 1a). Subsequent studies of Rayleigh waves, which are conducted along substrate surfaces, in the same soils in Puerto Rico and a variety of similar soils in California have shown that the waveform and spectrum of the frog thump are typical of vertical geophone responses to impulsive seismic stimuli (for example, taps on the soil surface with a rubber mallet). The frequency range of the airborne acoustical power in the recorded chirps was distinctly higher than that of the substrate-borne thumps, being from about 1.0 to 2.3 kHz (Fig. 1d). The carrier frequency of each chirp began near 1 kHz and then increased in about 15 msec to its final level of approximately 2.3 kHz (Fig. 1e).

Among 11 calling frogs studied (at 11 different sites) five produced thumps and six did not. For 8 of the 11 frogs, we were able to identify the substrate from which they were calling. Four of these were thumpers and were found to be directly on mud; four were nonthumpers and were found to be perched either on grass or on loose, gravelly substrate. Two of these nonthumpers, observed in the act of calling, were found to have their gular pouches suspended above the ground as a result of the dense grass. The typical posture of the calling frog from muddy substrate is prone, with its gular pouch pressed against the substrate. We were unable to produce thumps by playing recorded airborne chirps through a loudspeaker positioned either above the ground or directly against it. Thus the thump appears not to be a consequence of acoustical coupling of the call itself to the ground, but possibly a consequence of the motion of the gular pouch during the explosive onset of the chirp. This conjecture is supported by the consistent concomitancy of the onset of the Rayleigh wave and the rising phase of the