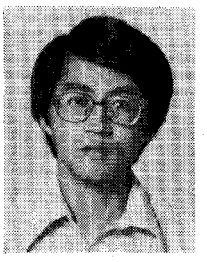
Kun-Mu Chen (SM'64–F'76) was born in Taipei, Taiwan, China, on February 3, 1933. He received the B.S.E.E. degree from the National Taiwan University, Taipei, Taiwan, in 1955, and the M.S. and Ph.D. degrees in applied physics from Harvard University, Cambridge, MA, in 1958 and 1960, respectively.

While at Harvard University, he held the C. T. Loo and the Gordon McKay Fellowships. From 1956 to 1957, he was a Teaching Assistant at the National Taiwan University, and from 1959 to 1960, he was a Research Assistant and Teaching Fellow at Harvard University. From 1960 to 1964, he was associated with the Radiation Laboratory, University of Michigan, Ann Arbor, where he was engaged in studies of electromagnetic theory and plasma. In 1962, while on leave from the University of Michigan, he was a Visiting Professor of Electronics at Chao-Tung University, Taiwan. Since 1964, he has been with Michigan State University, East Lansing, first as Associate Professor of Electrical Engineering, and since 1967, as Professor of Electrical Engineering. From 1968 to 1973, he was the Director of the Electrical Engineering program of the Department of Electrical Engineering and Systems Science. He has published numerous papers on electromagnetic radiation and scattering, plasmas, and the interaction of electromagnetic radiation with biological systems.

Dr. Chen is a fellow of the American Association for the Advancement of Science, a member of U.S. Commissions A, B, and C of the International Scientific Radio Union, Sigma Xi, Phi Kappa Phi, and Tau Beta Pi. He was the recipient of the Distinguished Faculty Award from Michigan State University, E. Lansing, in 1976. He was also the recipient of Excellent Achievement Award from Taiwanese American Foundation in 1983.



Huey-Ru Chuang (S'81) was born in Tainan, Taiwan, on August 18, 1955. He received the B.S. and M.S. degrees from the National Taiwan University in 1977 and 1981, both in electrical engineering. He worked on multibeam satellite antennas for his masters degree research. Currently, he is working toward the Ph.D. degree in electrical engineering at Michigan State University, E. Lansing, in the area of interaction of electromagnetic waves with biological bodies.

Mr. Chuang is a registered professional engineer in Taiwan.

The Absence of Significant Short-Term Electromagnetic Bioeffects in Giant Algal Cells Exposed to CW and Pulse-Modulated *X*-Band Bursts

ASHOK V. GOKHALE, KATHLEEN MONTAIGNE BRUNKARD, AND WILLIAM F. PICKARD, SENIOR MEMBER, IEEE

***Abstract*—Giant cells of the algae *Chara brauni* and *Nitella flexilis* were exposed to continuous wave and pulse-modulated bursts of *X*-band microwaves and the vacuolar potential was monitored for immediate radiation-correlated offsets. No such offsets were observed despite a resolution of approximately 5 in 10^5, and despite the wide variety of frequencies, power levels, and pulse protocols employed.**

I. INTRODUCTION

THE BIOLOGICAL EFFECTS of nonionizing electromagnetic radiation now appear to be numerous and are being studied intensively (e.g., [1]–[4]). Those which seem to arise from direct electromagnetic heating of the test preparation (i.e., *thermal* effects) are generally the best characterized and least controversial. They tend to be associated with incident fields on the order of 100 W/m^2 (10 mW/cm^2) or greater. There are, however, in addition to putatively thermal effects, many others which, because of their occurrence at low power levels or because of the form of their variation with system parameters, can not readily be explained in ordinary thermal terms. These so-called *athermal* effects tend to be less well characterized and more controversial. They have been reviewed comprehensively by Adey [5].

Two characteristics common to most thermal and athermal phenomena are 1) a prolonged period of irradiation (at least minutes) is necessary for the effect to manifest itself and 2) the ability to resolve shifts in the vari-

Manuscript received October 12, 1983; revised March 7, 1984. This work was supported by the National Science Foundation under Grant ECS-8105485 and the office of Naval Research under Contract N00014-82-K-0261.

A. V. Gokhale and W. F. Pickard are with the Department of Electrical Engineering, Washington University, Saint Louis, MO 63130.

K. M. Brunkard is with the Department of Biology, East Stroudsburg University, East Stroudsburg, PA 18301.

0018-9480/84/0800-0795$01.00 ©1984 IEEE

ables observed is small (less than 1 part in 10^2). In this laboratory, a divergent strategy has been adopted in which effects that occur early in the irradiation (within a few tens of milliseconds) are sought with very high resolution (greater than 1 part in 10^3) [6].

This paper presents an extension of these techniques into the 8.2–12.4-GHz (*X*-band) frequency range in an effort simply to detect the short term effects (if any) of low-level microwave irradiation.

II. Materials and Methods

A. Materials

All experiments were conducted on the eukaryotic green algae *Chara braunii* and *Nitella flexilis* using the giant (roughly 10 mm long and 0.3 mm in diameter) internodal cells. Cells from *Chara* cultures were normally in the electrogenic state (i.e., vacuolar resting potential more negative than −120 mV) while those from *Nitella* cultures were in the nonelectrogenic state (i.e., vacuolar resting potential less negative than −95 mV) [7].

B. Electronic Methods

These were modified but slightly from those employed previously [6], [8].

The exposure apparatus consisted of a 1.0-mm-wide microstrip fabricated on a 0.62-mm-thick sheet of ceramic whose dielectric constant was 75. The line was terminated by a microwave chip resistor to match its approximately 12.5-Ω characteristic impedance. A 2.2-mm-wide gap, perpendicular to the microstrip, formed a channel in the dielectric sheet down which an electrolyte solution at 25° C was flowed and into which the cell under test was placed with its upstream end under the microstrip. Because of the relatively good match 1) between cell and channel water, and 2) between channel water and dielectric sheet, the field should be uniform along the microstrip and the field pattern impressed upon the cell's upstream end should be that of the line's quasi-TEM mode.

The vacuolar resting potential of the cell was sensed by a glass micropipette inserted into the downstream end of the cell, and this potential was amplified and filtered as described previously [6]. Segments of the potential signal 400 ms long and phase locked to irradiation bursts were digitized and averaged for analysis. The average of 20 segments permitted a search for offsets on approximately the 3-μV level, a resolution of roughly 5 in 10^5 when compared to the vacuolar potential.

C. Irradiation Protocol

Bursts of irradiation 100 ms long were delivered to the exposure apparatus by a directional coupler and a double stub tuner. Nominal total power incident upon the fluid-filled channel was derived 1) by calibrating, frequency by frequency, the power to the tuner in terms of coupler output, 2) setting the tuner for negligible back reflection, 3) applying a theoretical correction factor for loss on the microstrip, and 4) allocating a 1.0-dB loss to the tuner. Nominal power density to the exposed end of the cell was derived by dividing this power by the cross-sectional area of the microstrip (0.62 mm^2).

TABLE I
Protocol for Pulse-Burst Irradiation at 9.09 GHz*

	Pulse Interval (μs)				
	2000	1000	500	200	100
Pulse Width (μs)	$2^{\S}$	$1^{\S}$	$0.5^{\S}$	$0.2^{\S}$	$0.1^{\S}$
	20	10	5	2	1
	200	100	50	20	10
	2000	1000	500	200	100

*For most pulse protocols, the cells were tested at both 100 and 1000 W/m^2 (power averaged over the 100-ms burst); those protocols marked with the symbol § were tested only at 100 W/m^2.

To study the effects of frequency, nine different irradiation frequencies (8.20, 8.64, 9.09, 9.58, 10.08, 10.62, 11.18, 11.78, and 12.40 GHz) were used. This logarithmic coverage of the *X*-band was chosen so that any frequency-resonant offset of $Q \lesssim 20$ would be observed. The nominal power density of each 100-ms burst was 100 W/m^2, and three different burst protocols were used: 1) continuous wave (CW); 2) 1.0-μs pulses 1 ms apart; 3) 0.1-μs pulses 0.1 ms apart.

To study the effects of pulse repetition rate, a frequency of 9.09 GHz was chosen and 100-ms bursts of the pulse structures described in Table I were employed.

To study the effects of lower power density, a frequency of 9.09 GHz was chosen and 100-ms bursts were applied at nominal power densities of 100, 50, 20, 10, 5, 2, and 1 W/m^2. At each power density, both 1) CW bursts and 2) bursts of 1.0-μs-wide pulses separated by 1.0 ms were employed.

III. Results

Each irradiation condition described above was tested on three electrogenic cells from *Chara braunii* and three nonelectrogenic cells from *Nitella flexilis*. In no instance was a significant radiation-correlated offset of the resting potential observed.

Fig. 1 shows two sample chart recordings containing summed responses of *Chara braunii* (a) and *Nitella flexilis* (b) to twenty radiation bursts. The traces reveal only background noise.

IV. Discussion

It is evident that the resting potential showed no significant response under the irradiation protocols employed. Since the resting potential is commonly believed to be a sensitive indicator of general cellular function and condition, and since sufficiently powerful bursts of *X*-band irradiation are known to have observable effects [9], one must examine a number of hypotheses as to why the protocols employed caused no observed offsets.

First, there may be no cellular effects of significance. This is possible, but, in view of the present controversy

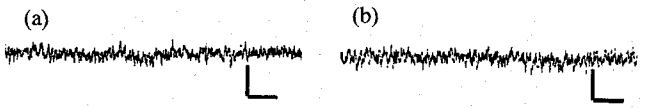


Fig. 1. (a) Electrogenic *Chara braunii* of resting potential −163 mV. Irradiation protocol: 100-ms bursts of 9.09 GHz radiation at an average nominal power density of 1000 W/m^2; each burst contained 200 5.0-μs pulses. (b) Nonelectrogenic *Nitella flexilis* of resting potential −60 mV. Irradiation protocol: 100-ms bursts of 11.18 GHz radiation at an average nominal power density of 100 W/m^2; each burst contained 1000 0.1-μs pulses. In each case, irradiation began about 60 ms after the beginning of the trace, vertical bar = 20 μV, and horizontal bar = 40 ms.

about microwave bioeffects [10], such a supposition may well be unwarranted.

Second, there may be effects, but not on the vacuolar potential. Given the sensitivity of this variable to cellular conditions, this seems unlikely.

Third, there are *X*-band effects, but with such narrow frequency resonances that they were missed by the protocols used. Millimeter-wave effects of $Q \gtrsim 1000$ are indeed known [11], [12], but such sharp resonances have not yet been reported in *X*-band.

Fourth, these protocols have effects but not ones which become manifest with the application of short, widely spaced bursts. This could well be the case. Unfortunately, the techniques employed here are less well suited to the study of long-term irradiation since the very high resolution achieved depended upon using the cell as its own control. This cannot be done over periods of many minutes because of slow millivolt-level stochastic fluctuation of the resting potential [13].

Thus, the principal importance of these data is that there appears to be no simple, rapid, obvious electrical effect of *X*-band irradiation in our preparation. The possibility of sharp resonances in power or frequency, or the existence of long-term effects, can not be ruled out. Moreover, these conclusions apply only to *X*-band and do not extend to higher (or lower) frequencies where there are theoretical reasons for expecting effects [14], [15].

Acknowledgment

The authors wish to thank K. Cox for technical assistance.

References

[1] M. A. Stuchly, "Interaction of radiofrequency and microwave radiation with living systems," *Radiat. Environm. Biophys.*, vol. 16, pp. 1–14, 1979.

[2] K. Montaigne and W. F. Pickard, "Offset of the vacuolar potential of Characean cells in response to electromagnetic radiation over the range 250 Hz–250 kHz," *Bioelectromagn.*, vol. 5, pp. 31–38, 1984.

[3] C. F. Blackman, J. A. Elder, C. M. Weil, S. G. Benane, D. C. Eichinger, and D. E. House, "Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effect of modulation frequency and field strength," *Radio Sci.*, vol. 14, no. 65, pp. 93–98, 1979.

[4] Y. H. Barsoum and W. G. Pickard, "Effects of electromagnetic radiation in the range 20–300 MHz on the vacuolar potential of Characean cells," *Bioelectromagn.*, vol. 3, pp. 193–201, 1982.

[5] W. R. Adey, "Tissue interaction with nonionizing electromagnetic fields," *Physiol. Rev.*, vol. 61, pp. 453–514, 1981.

[6] W. F. Pickard and Y. H. Barsoum, "Radiofrequency bioeffects at the membrane level: Separation of thermal and athermal contributions in the Characeae," *J. Membr. Biol.*, vol. 61, pp. 39–54, 1981.

[7] N. A. Walker, "The transport systems of charophyte and chlorophyte giant algae and their integration into modes of behavior," in *Plant Membrane Transport: Current Conceptual Issues*, R. M. Spanswick, W. J. Lucas, and J. Dainty, Ed. Amsterdam: Elsevier, 1980, pp. 287–304.

[8] Y. H. Barsoum and W. F. Pickard, "The vacuolar potential of Characean cells subjected to electromagnetic radiation in the range 200–8200 MHz," *Bioelectromagn.*, vol. 3, pp. 393–400, 1982.

[9] A. V. Gokhale, K. Montaigne, and W. F. Pickard, "Vacuolar hyperpolarizing offsets in Characean cells exposed to mono- and bichromatic CW and to squarewave modulated electromagnetic radiation in the band 200–1000 MHz," Submitted to *Bioelectromagn.*, 1983.

[10] "Experiments show microwaves can damage chromosomes," in *The Institute*, [News Supplement to *IEEE Spectrum*], vol. 7, p. 1, Aug. 1983.

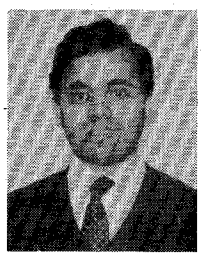
[11] W. Grundler and F. Keilmann, "Nonthermal effects of millimeter microwaves on yeast growth," *Z. Naturforsch.*, vol. 33, pp. 15–22, 1978.

[12] W. Grundler, F. Keilmann, V. Putterlik, and D. Strube, "Resonant-like dependence of yeast growth rate on microwave frequencies," *Brit. J. Cancer.*, vol. 45, pp. 206–208, 1982.

[13] R. L. Roa and W. F. Pickard, "The use of membrane electrical noise in the study of Characean electrophysiology," *J. Exp. Bot.*, vol. 27, pp. 460–472, 1976.

[14] H. Fröhlich, "The biological effects of microwaves and related questions," *Adv. Electronics and Electron Phys.*, vol. 53, pp. 85–152, 1980.

[15] W. F. Pickard and F. J. Rosenbaum, "Biological effects of microwaves at the membrane level: Two possible athermal electrophysiological mechanisms and a proposed experimental test," *Math. Biosci.*, vol. 39, pp. 235–253, 1978.

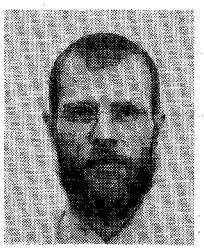


Ashok V. Gokhale was born in Amravati, India, in October, 1957, and lived in England from 1962–1982. He received the B.Sc. degree with honors (1979) and the Ph.D. degree (1982) from the University of Liverpool, England.

He is currently a Postdoctoral Research Associate at Washington University in St. Louis, MO, where he is studying the biological effects of microwaves.

Kathleen Montaigne Brunkard was born in New Haven, CT, in 1953. She received a B.S. degree in biology from Southern Connecticut State College, New Haven, an M.S. degree in biology from Syracuse University, Syracuse, NY, and in 1982, a Ph.D. degree in plant and soil sciences from the University of Massachusetts, Amherst.

After two years as a Postdoctoral Research Associate in the Department of Electrical Engineering of Washington University, St. Louis, MO, she joined East Stroudsburg University, East Stroudsburg, PA, where she is now an Assistant Professor of Biology. Her research is in the areas of plant cell electrophysiology and the environmental stress physiology of plants.



William F. Pickard received the Ph.D. degree in applied physics in 1962 from Harvard University, Cambridge, MA.

After postdoctoral experience at Harvard and at the Massachusetts Institute of Technology, Cambridge, he joined Washington University, St. Louis, MO, where he is now a Professor of Electrical Engineering. His research concerns the fundamental processes of mass and charge transport in organisms and the techniques by which these processes can be applied to the search for microwave bioeffects.