

balanced salt solution. The Maximow assembly is a slide with a sealed cylindrical compartment especially designed for repeated microscopic examination of developing cultures. The successive layers of the compartment are glass wall, air, and the culture growing on a thin cover slip held against an outermost cover slip by a film of fluid.

10. Stimulating electrodes were glass pipettes with fire-polished orifices of 5 to 20 μm . Currents of 10 to 30 μa and less than 0.1 msec in duration were supplied from an isolation unit. Conventional glass microelectrodes filled with 3M KCl, with d-c resistances of 15 to 30 megohms and tip potentials of less than 2 to 3 mv, were used for intracellular recording. Potentials were led via a chlorided wire to a high-input impedance negative capacitance amplifier and were further amplified and displayed on a Nihon-Kohden oscilloscope. An agar salt bridge contacting a chlorided silver wire connected the chamber to ground. The stimulating electrode, held by a micromanipulator, was gently applied to the surface of either spinal cord or nerve fiber. With another micromanipulator, the recording electrode was guided into a muscle cell in the vicinity of a visible nerve-muscle contact.
11. The term "excitatory junctional potential" is used to describe brief depolarizing responses

- to spinal cord or nerve stimulation identical in time course and sometimes in amplitude to spontaneous potentials. They gave rise to action potentials when sufficiently large and, when tested, were blocked by *d*-tubocurarine.
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Behavioral Sensitivity to Microwave Irradiation

Abstract. *Rats assayed by the technique of conditional suppression were able to detect the presence of 12.25-centimeter microwaves at doses of power approximating 0.5 to 6.4 milliwatts per gram. The assay, which controlled for sensitization, for pseudo and temporal conditioning, and for several possible sources of artifactual cueing, revealed that irradiation by microwaves, although lacking the saliency of an auditory stimulus, can function as a highly reliable cue. Efficiency of detection was strongly and positively related to the amount of microwave energy to which the rats were exposed.*

Nearly a decade ago, Frey (1) reported that human beings can detect pulse-modulated electromagnetic energy at wavelengths of 10 to 70 cm and at average power densities of 0.4 to 2.1 mw/cm². The sensations reported were usually auditory in character and were often described as "hissing, buzzing, and clicking sounds." Although no confirmatory studies have been published since Frey's reports, three instances of verification have been communicated to him (2), and he has referred to successful use of microwave energy as a signaling stimulus in cats (3). Two directly related studies yielded negative results. Jones (4) reported that none of 20 college students could discriminate presence from absence of 30- or 60-cm microwaves. Justesen and King (5) intermittently presented 12.25-cm microwave energy to each of six rats as a cue for obtaining sugar water, but none of the rats discriminated the cue. Since unmodulated energy was used in Jones's study, its negative findings comport with Frey's belief (2) that modulation is necessary for perception of microwaves. Modulated energy was used by Justesen and King, but the assay for perception

was based upon appetitive rather than aversive motivation and may have lacked sensitivity. Much indirect evidence of relevance to detection of microwaves has been published, particularly in the Soviet literature (6); altered thresholds to physiologically adequate stimulation have been reported as sequelae of microwave irradiation in olfactory (7), auditory (8), visual (9), and cutaneous (10) modalities. Other aftereffects include cardiovascular changes (11-13), irritability and irascibility (11), neurasthenia (13), and headache and disturbance of sleep (11). Acute responses observed during irradiation include changes of blood pressure (14), heart rate (15), and cortical and subcortical electrophysiological activity (3, 16).

Although the mechanisms responsible for chronic and acute changes are unresolved and much debated (3, 17), the evidence suggests that microwaves at densities below the safety limit of 10 mw/cm² observed in the United States (18) can affect nervous activity and could, therefore, possess stimulus properties. We report here attempts to assess in rats the efficacy and the re-

liability of modulated 12.25-cm microwaves as a warning stimulus for impending electrical shock.

Six male albino rats of common age were obtained from the Simonsen Company of Minnesota. Three randomly selected rats (R-1, R-2, and R-5) served as subjects for irradiation; control rats (R-3, R-4, and R-6) were never irradiated, but were maintained in their home cages with unrestricted access to Purina Lab Chow and water. Although irradiated rats had the same access to water, they were partially deprived of food until their individual weights fell to 75 percent of that before experimentation; a diet that led to 75 percent of normal gains in weight was thereafter instituted on the basis of data on weight gained by the control animals. Weights of R-1, R-2, and R-5 at the commencement of experimentation were, respectively, 409, 455, and 427 g.

A highly sensitive measure of conventional sensory stimulation, the technique of conditional suppression (19), was used. With this technique, a subject is reinforced after making an operant response; then reinforcement is scheduled intermittently until the response occurs frequently and consistently. Finally, a Pavlovian conditioning regime is superimposed in which a warning signal is presented from time to time, always terminating in a brief, but aversive, unconditional stimulus (US). After repeated presentations of the warning signal and the US, a subject will respond stably except when the warning signal is being presented; that is, operant behavior is conditionally suppressed. The operant response required of our rats was the tongue lick, which was detected photoelectrically and reinforced by discrete volumes (30 μl) of sugar water (dextrose, 16 g/100 ml). A radiolucent ensemble by which licks were detected and reinforced is described elsewhere (20). An aperiodic schedule of reinforcement was used during all experiments; the passage of each 2-second interval after reinforcement led to availability of another reinforcer with a probability (*P*) of .25, .125, or .0625. The *P* value was not changed during a given experiment, but was varied across experiments to maintain stable responding. The US was unavoidable electrical shock to the feet presented by a radiopaque floor-grid of aluminum rods (21). A conventional warning stimulus, with which microwave irradiation was compared for cueing efficacy, was a 525-hz tone,

and was produced by a 3-inch, 4-ohm loudspeaker (Jensen VK-300) driven by a sinusoidal current at 800 μ W of continuous power.

Because conventional (open space) methods of exposure to microwaves require immobilization of a subject in order to preserve constancy of incident energy (5, 22, 23), we irradiated our animals in the closed space of a multimodal exposure cavity, a modified Tappan R3L microwave oven (5, 22). Microwaves (at 2450 ± 50 Mhz) were generated by a QK707-A magnetron and were doubly modulated at 60 and 12 hz. The exposure cavity was fitted with a Plexiglas conditioning chamber (internal dimensions, 26 by 37 by 24 cm) and with the radiolucent ensemble by which licks were detected and reinforced (20). Dosimetry was accomplished by measuring available microwave power (22) within the chamber by water calorimetry (24), then dividing obtained wattage values by mean weight of the three rats to yield average doses within ± 15 percent of 6.4, 4.8, 2.4, and 1.2 mw/g. The smallest dose was not based upon calorimetry but was estimated to be $500 \pm 90 \mu$ W/g on the

basis of the level of focusing current used to control and monitor the output power in the exposure cavity was accomplished by applying 5 kv of 60-hz a-c voltage to the anode of the magnetron.

The exposure cavity was cooled and sounds transmitted to the conditioning chamber were masked by fans that provided a continuous flow of air from an external, thermostatically controlled source. Temperatures within the operant chamber were maintained at $24^\circ \pm 2^\circ$ C; relative humidity was between 20 and 40 percent. One-minute periods of tonal stimulation or of microwave irradiation and 0.5-second periods of electrical shock (averaging 790 μ a root-mean-square) were programmed for automatic presentation by a punched-tape control system. The number of licks that occurred during 60-second control ("safe") periods, and during ensuing 60-second periods of warning stimulation (which usually terminated in shock), were tallied by digital counters and cumulative recorders. A rat's discriminative efficiency (that is, the

degree to which an animal's operant responding was suppressed during warning stimulation) was quantified by the formula $[(S - W)/S] \times 100$ (where S is the number of responses made during safe periods and W is the number made during periods of warning stimulation). Each of the three rats was tested in a total of 14 experiments based upon 62 2-hour sessions. The 62 sessions were interspersed with another 87 sessions (without irradiation) in which baselines of operant responding were established or reestablished, all 149 sessions being conducted within 6 months. Each session was 120 minutes long, and the minimum interval between sessions was 22 hours. During each session conditioning stimuli were presented eight times, and the length of intervals between presentations was made random to control for temporal conditioning. Each of the 14 experiments comprised a set of two or more contiguous sessions (Fig. 1).

None of the rats exhibited signs of spurious (unconditional) suppression when the tonal stimulus was presented without shock to the feet (session set 1); but during sessions of the second set,

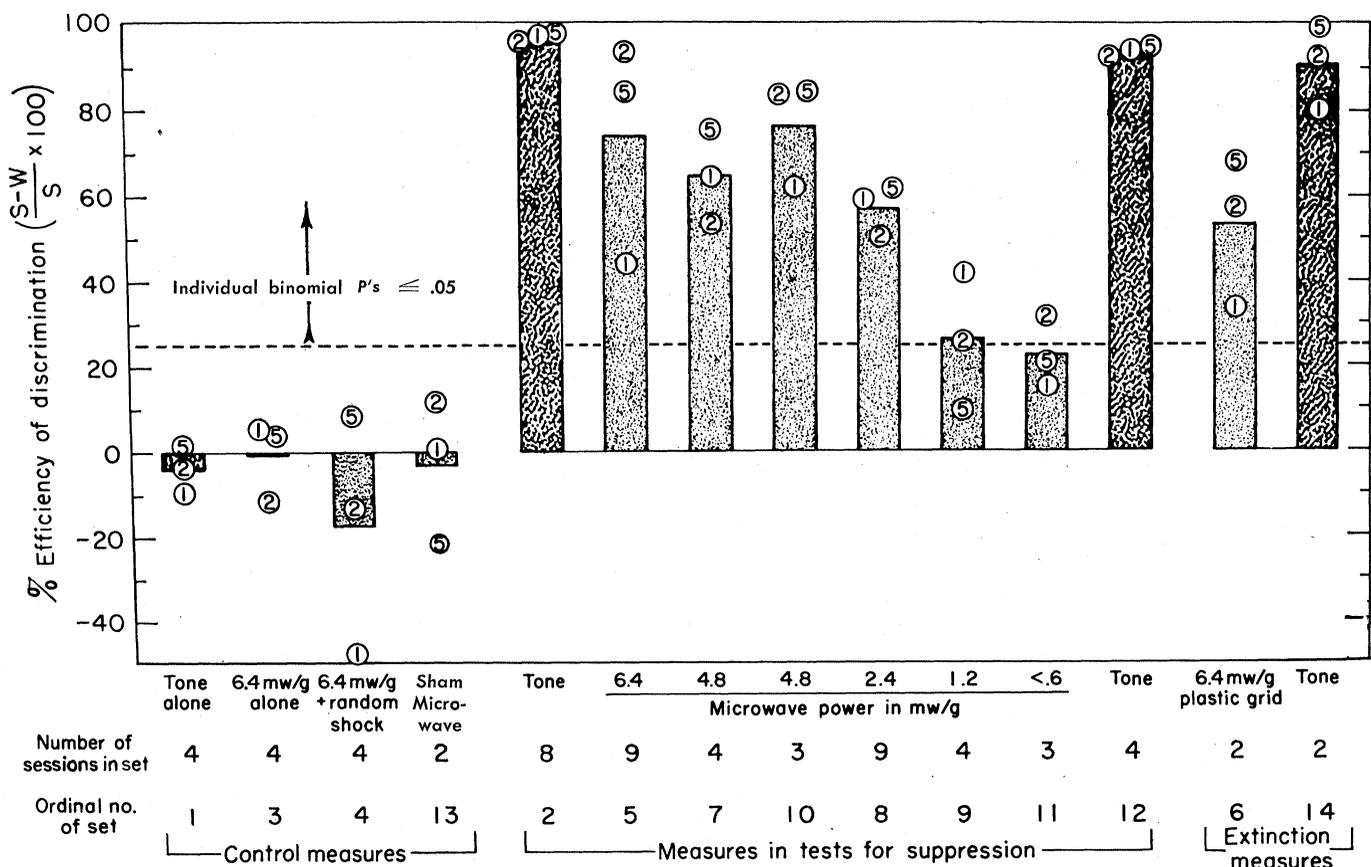


Fig. 1. Mean efficiencies of rats discriminating a 525-hz tone or microwave irradiation presented as a warning stimulus that usually preceded unavoidable footshock. Means of individual subjects (rats 1, 2, and 5) are shown by encircled numbers; overall means are illustrated by vertical bars. A total of 14 experiments was ordered across time in sets of two or more contiguous sessions as indicated in the two rows of entries at the bottom of the figure. Conditions are explained in text.

when shock did follow the tone, suppression of responding was quickly conditioned. When periods of microwave irradiation were presented alone (session set 3) or made random in time with respect to an equivalent number of shocks (session set 4), neither spurious suppression nor aversive sensitization, respectively, was observed. During the fourth set, one rat, R-1, responded reliably more often during periods of irradiation than it did during control periods ($P < .01$); since shocks never coincided with irradiation R-1 apparently learned that it was "safe" to respond when irradiated. During sessions of the fifth set, shock was presented at the termination of each period of irradiation, and the rats were again irradiated at 6.4 mw/g. All rats suppressed reliably, R-1 less efficiently than the others. During sessions of the sixth set, a conditioning chamber formed entirely of Plexiglas was inserted into the exposure cavity. Only microwaves were presented. This arrangement permitted assessment of resistance to extinction (which was relatively high in all animals), but was primarily used to control for (and was found to eliminate) the possibility that the suppression observed during the prior set of sessions was produced by demodulated microwave energy that could have been wave-trapped by the aluminum shock-grid and could have led to artifactual electrical stimulation of the footpad. Periods of microwave irradiation terminating in shock were programmed during several subsequent sessions (sets 7 through 11), but at lower doses. During the 12th set of sessions the 525-hz tone again preceded shock; efficiency of discrimination was almost as high for all three rats as during the second set. In order to test for spurious auditory or vibratory cueing by relays and switches controlling presentations of microwave energy, neither irradiation nor shock was presented during the 13th set of sessions. The source of current to the anode of the magnetron was interrupted, preventing generation of microwave energy, but all control relays and switches were operated as in the fifth set. Spurious suppression was not observed. During the 14th and final set of sessions, resistance to extinction to the tonal stimulus was measured. All three rats continued to suppress during tonal stimulation in spite of the absence of shock.

Detection of microwaves was generally less efficient than detection of the

tonal cue, but was highly reliable at all but the two lowest doses: binomial P values ranged between 10^{-7} at 6.4 mw/g for the best performing rat to $\sim .10$ at 1.2 mw/g for the worst performing (26). Even at the lowest dose, one of the rats discriminated reliably (R-2, $P < .001$). The appearance of a strong relation between dose and response was tested by plotting mean efficiency of discrimination against average dose as presented during session sets 5, 7, 8, 9, 10, and 11. The product-moment r is .95 (at 4 d.f., $P < .01$).

Under the conditions described, the albino rat can either detect microwave energy or is sensitive to some concomitant of irradiation. Because of the strong dose-response relation, an artifactual cue would have varied in intensity with the amount of available power. X-irradiation is a possibility and is not only generated by high-voltage, thermionic devices such as the magnetron, it has also been demonstrated to function as a signaling stimulus (27). However, a metallic wall of the exposure cavity and the 1-cm-thick Plexiglas sheet from which the conditioning chamber was fabricated separated the rats from the magnetron; x-rays at the low photon energies developed in a magnetron would have little probability of penetrating the metallic wall and the Plexiglas sheet. Another possible cue derives from the small portion of microwave energy that is absorbed by the metallic walls of a multimodal cavity. The loudspeaker used to present the tonal cue was located on the external surface of the cavity and could have trapped demodulated microwave energy and translated it into an audible vibration of the speaker-cone. Even though our experiments controlled for this possibility (compare session sets 2 and 3), we took the additional precaution of removing the loudspeaker from the apparatus during most of the tests for microwave cueing.

The only unargued fate of absorbed microwave energy is thermalization. However, rats exposed to 6.4 mw/g and lesser doses of irradiation for 60-second periods have never exhibited reliable elevations of whole-body temperature in our laboratories, even when measured by expanded-scale electronic thermometers with a resolution of 0.05°C . Some investigators (28) would conclude that nonthermal effects are implicated; others (29) would argue with some cogency that the rats were simply more sensitive to a weak thermal stimulus than were our thermometers.

Whatever the mechanism of detection (30) we offer our data as evidence that confirms and extends the generality of Frey's findings: mammals are sensitive to something that inheres in or accompanies illumination by microwaves at low levels of available power.

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 24. A volume of distilled water approximating the gram weight of a mature rat is placed in a thick-walled vessel of foamed polystyrene at the ambient temperature of the exposure cavity. The water (which is continuously agitated or stirred) is then irradiated and the average available power is obtained by the formula $w = T_A V / kt$, where w is the average available (thermalized) power in watts, T_A is the temperature increment in degrees Celsius, V is the volume of water in milliliters (weight in grams), k is Joule's conversion factor (0.239), and t is the duration of irradiation in seconds. Temperatures are measured either by expanded-scale spirit thermometers or by electronic thermometers with thermistor sensors.
 25. Since the unit-mass (watts per gram) dose is an estimate of absorbed energy, and the conventional unit-surface (watts per square centimeter) dose is a planar index of field density, the two doses cannot be precisely equated. Maximum limiting values of the unit-surface dose can be approximated (5) and for the unit-mass doses as given are approximately 20, 15, 7.5, 3.75, and < 2 mw/cm².
 26. Individual binomial probabilities were derived as follows: (i) The number of responses generated by an animal during an S interval was compared to the number generated during the succeeding W interval; (ii) if the former number was higher, an instance of cueing was noted; if equal or lower, an instance of no cueing was noted; and (iii) frequencies of positive and negative instances were cumulated across a total set of sessions and evaluated for reliability by use of the binomial theorem.
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 28. Frey (2) has discussed the practice by some investigators of assuming that irradiation which does not lead to measurable thermalization is ipso facto "nonthermalizing." Such an operational fiat may lead one to overlook the possibility of nonlinear heating of tissues of a biological preparation or of relative insensitivity of thermal measurements.
 29. See the discussion by L. D. Sher (5, p. 192).
 30. The only creature suspected of possessing a specialized sensory-motor apparatus capable of receiving and transmitting microwaves is the corn earworm; see the *J. Microwave Power* 5, 149 (1970).
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Bispectrum Analysis of Electroencephalogram Signals during Waking and Sleeping

Abstract. *The degree of interaction of component waves making up a single electroencephalogram trace was strongly correlated with alpha activity, lead placement, and state of consciousness. Significant quadratic coupling of the waves was found only for awake subjects with high alpha activity. For these subjects about 50 percent of beta activity can be attributed to harmonic coupling with the alpha peak. During sleep, the degree of interaction was of borderline significance and did not follow a consistent pattern with respect to subject, frequency, state, or lead.*

The statistical description of the electrical characteristics of the brain has been greatly advanced by applying the method of time series analysis to electroencephalogram (EEG) records (1, 2). With one exception (3) the analysis methods have dealt with only second moment statistical quantities—that is, the spectrum and the cross spectrum. As might be expected, the spectrum approach has raised some important questions: for instance, "Is there any coupling (interaction) between the various wave components that make up the EEG trace?" This report supplies a partial answer to this question.

Ordinary spectrum analysis gives no information on the degree of coupling between the (idealized) component waves that make up an EEG record. However, by computing and analyzing

the bispectrum (third movement) of the EEG activity, it is possible to discern interacting components. If the bispectrum of the record is nil, we can infer that none of the component waves are related or coupled to each other. Hence the EEG can be consid-

Table 1. Bicoherence values at the frequency coordinates (10 hz, 10 hz). Values above 0.02 are significant at the 95 percent confidence level.

Subject	Lead area		
	F ₃	O ₁	C ₃
1	0.00	0.01	0.01
2*	.03	.02	.04
3*	.09	.22	.08
4	.00	.00	.00
5	.02	.06	.01
6	.01	.01	.00
7*	.09	.13	.09
8*	.14	.12	.22

* Subject with high alpha activity.

ered as a linear superposition of statistically independent component waves. A significant bispectrum, on the other hand, indicates a quadratic coupling (or phase locking) among various of the component waves. In such a situation the bispectrum provides a quantitative measure of the cross coupling. For simplicity, we shall identify this measure by the term "bicoherence," which can be considered as merely a suitably normalized version of the bispectrum. The bicoherence has an expected value of zero for a signal that represents a purely random process. Significant deviations from zero, although small, occur for different spectrum components whose phase and amplitude are quadratically related. A good description of the bispectrum and its properties can be found elsewhere (4).

Values of bicoherence that were significant at the 95 percent level were found in the awake alpha region for our subjects with high alpha activity (see Table 1). In the awake state, then, the nonzero bispectra appear to be highly correlated with high alpha intensity in the power spectrum. Though significant bicoherence values were found in all leads (electrode sites), their magnitude was dependent on lead placement. The largest bicoherence (R^2) values occurred in the left central (C₃) and left occipital (O₁) leads. Only one of the subjects with low alpha activity had a significant alpha R^2 value, and this was in the O₁ lead.

The waking bispectra, where significant, were characterized by sharp peaks that indicated interactions within narrow frequency bands. Of the observed significant bicoherences, 50 percent occurred in the (10 hz, 10 hz) frequency space. This essentially means that waves with a frequency of 10 hz have associated with them a phase-locked first harmonic component of frequency 20 hz. Of the other significant bicoherences, 20 percent fell in the (10 hz, 20 hz) region, 10 percent in the (10 hz, 30 hz) area, and 20 percent at approximately the (7 hz, 2 hz) region. These results indicate that for subjects with high alpha activity, their alpha activity is composed not only of a pure sine wave of approximately 10 hz but also of its second harmonic (20 hz) and, in some instances, its third harmonic (30 hz). To a much smaller degree, energy in the alpha band appears to be due to interactions between frequencies of approximately 7 hz and 2 hz.