

# NEURAL FUNCTION AND BEHAVIOR: DEFINING THE RELATIONSHIP

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Over the years, this laboratory has studied radio-frequency (rf) energy effects, such as evoked potentials in the brains of cats, conditioned responses in cats and monkeys, frog heart arrhythmia, and hearing phenomena in cats and humans. A few years ago, one of us (A.H.F.) discussed the possible mechanisms by which rf energy could affect biologic systems in general and the nervous system in particular.<sup>1</sup> The possibilities considered and evaluated included rf energy-induced transmembrane potential changes, conformational changes in membranes, field-induced forces at the neural junction, microthermal effects, and also possible rf effects on tissue thought to have electret characteristics or piezoelectric properties. The solid-state characteristics of the nervous system were regarded within the context of possible rf energy-induced effects, and these possibilities were evaluated. Other possibilities were also contemplated. The evaluation was concluded with the observation that, "The question is not whether there is a possible mechanism, but rather which of numerous possible mechanisms. . . ."

In the work reported here, we made a first approach to answering the question and began to define the relationship between neural function and behavior. We have used essentially the same rf energy parameters in experiments that involved avoidance behavior and brain tissue permeability change as indexed with a fluorescent dye. We found an association between changes in behavior and in brain permeability. We have also found a difference in effectiveness of pulsed and continuous energy on behavior and brain permeability. We wish to make quite clear that we have not established a causal relationship, only an association.

## BEHAVIOR EXPERIMENT

In the behavior experiment, we compared pulsed illumination, continuous wave (cw) illumination, and sham illumination effects on rats in a shuttle box. The shuttle box was constructed of white polystyrene, divided in half by a low hurdle. One half was shielded from the rf energy. The dependent variable was the amount of time spent in the rf-shielded half as compared to the unshielded half of the box. The box balanced as a see-saw on a 2 cm high wedge that permitted limited travel. At each end was a microswitch that registered animal location on an Esterline Angus event recorder. The rf energy source was a Microdot model 411A power oscillator that was connected by an RG-8 cable to a model 11-1.1 Scientific Atlanta coax to wave guide adaptor and standard gain horn antenna. The antenna emitted energy into an rf anechoic chamber that was constructed of FR 340 absorber, which minimized spurious reflections and created essentially free field conditions. Prior to each day's testing, all equipment was turned on, tested, and adjusted. The power rheostat on the rf source was turned up to emit rf energy into the anechoic chamber if the animal inside was a member of the pulsed or cw groups. If the animal in the chamber was from the sham-illuminated group, the power rheostat on the rf source was not

turned up, and no energy was emitted into the anechoic chamber. Temperature readings were taken several times a day inside the shuttle box; they neither deviated from the normal room temperatures found elsewhere in the laboratory nor did they deviate in association with an rf condition.

Each of 18 female Sprague-Dawley rats was randomly assigned to either pulsed, cw, or sham exposure groups. There were four exposure sessions for each animal, and each session lasted 30 min. The order of exposure and the side shielded from rf energy during each session were randomized to control for side preference and diurnal effects. The carrier frequency was 1.2 GHz. Each 0.5-msec pulse had a 1- $\mu$ sec rise time and then an immediate exponential decay to less than 1/3 pulse amplitude at pulse-off. The pulse repetition rate was 1000 pps. The pulsed energy group was exposed to a peak power level of 2.1 mW/cm<sup>2</sup> and an average power density of 0.2 mW/cm<sup>2</sup>. The cw group was illuminated with an average power density of 2.4 mW/cm<sup>2</sup>. The sham-illuminated group was exposed to 0 mW/cm<sup>2</sup>. Peak power was estimated by measurement of oscilloscope wave forms and verified by extrapolation from measurements of average power. The power densities selected were intended to be close to the lowest perceptible, as estimated from data previously gathered by us with this technique. The power density was measured before and after each session in the box with a half wave dipole connected via RG-58 coaxial cable to a Hewlett-Packard 477B thermistor mount and a Hewlett-Packard 430C power meter. Because of the well-known difficulties in rf field measurement, we consider all measurements to be order of magnitude.<sup>1</sup>

In FIGURE 1, we compare the results obtained over days from the three groups. There were no significant differences among groups the first 2 days, as indicated by the Mann-Whitney *U* test. This is logical inasmuch as the energy density was intended to be minimally perceptible. The differences among groups were significant

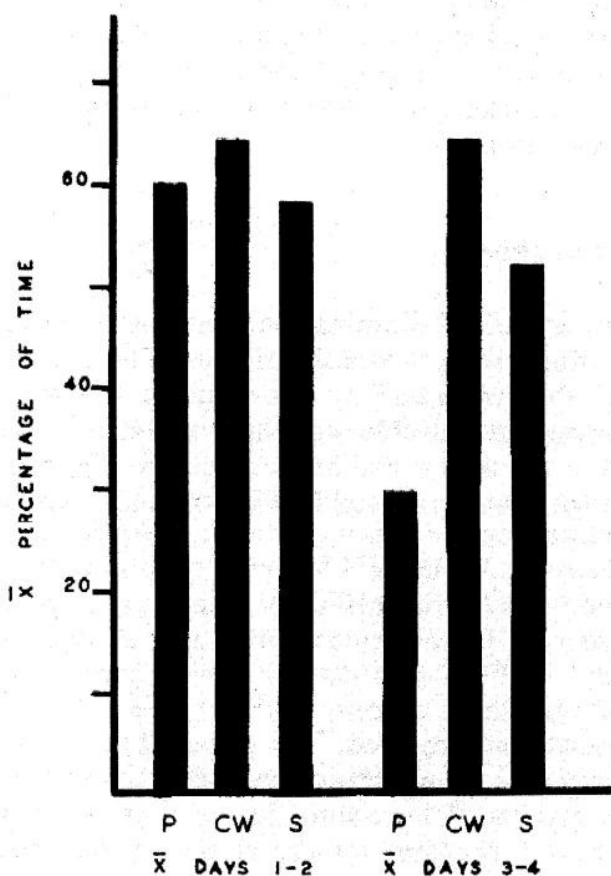


FIGURE 1. Mean percentage of time spent on rf-illuminated or sham-illuminated side of shuttle box by the pulsed energy exposure group (p), continuous wave energy exposure group (cw), and sham-illuminated group (s).

the last 2 days. In the last 2 days, the pulse-illuminated group spent 30% of their time in the unshielded half of the box; the sham-illuminated group spent 52% of their time in the unshielded half of the box, and the cw-illuminated group spent 64% of their time in the unshielded half of the box. The difference between the pulsed group and the sham group was significant ( $U = 4$ ,  $p = 0.013$ ), and the difference between the pulsed group and the cw group was also significant ( $U = 4$ ,  $p = 0.013$ ). However, the difference between the cw group and the sham group was not significant ( $U = 13$ ,  $p > 0.05$ ).

As may be seen, we have here a behavioral technique that differentiated between pulsed and cw exposure and that provides the potential for relating behavioral change to a brain change. We shall now show a brain permeability change that results from exposure to rf energy with the same parameters that yielded the behavioral modification.

### BRAIN EXPERIMENT

Several fluorescent dyes bind to serum protein when injected into the bloodstream. These have been used to study brain barriers and have been found to be quite useful. One of these dyes is sodium fluorescein. Although not necessarily the best dye for our purpose, sodium fluorescein was chosen because it has been widely used and provides a substantial data base. The procedure was to illuminate Sprague-Dawley rats with rf energy. Immediately after illumination, the rats were injected intravenously with the dye, which was allowed several minutes to equilibrate in the circulation. The animals were then exsanguinated, and the brains were perfused with saline, sectioned, and the sections examined for fluorescence under ultraviolet light. Fluorescence indicated alteration in permeability of brain tissue barriers. Through the use of this technique, we have shown permeability changes in the brains of mammals illuminated with rf energy.

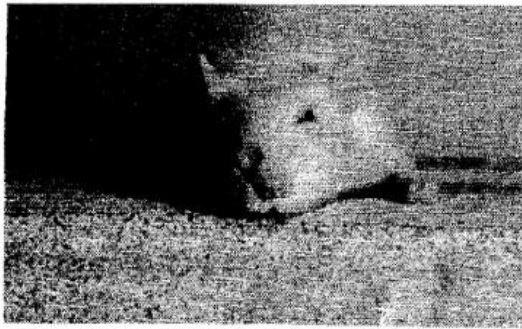
A factorial design was used with the same three rf exposure conditions employed in the behavior experiment. FIGURE 2 illustrates the five head positions used, as viewed from the energy source. During actual or simulated exposure, the animals lay on a block of Eccosorb FR 340 energy absorber within an rf anechoic chamber.

The analytic procedure followed was designed as a blind evaluation of the brain sections obtained. The subjects were 90 male Sprague-Dawley rats that weighed approximately 225 g. Six animals were used in each cell of the design and were randomly assigned to the various experimental conditions. Experimental conditions were distributed over time to avoid confounding by time-locked effects on any particular day of experimentation.

Three experimenters were involved in this study. The experimenters' procedures and work loads were arranged such that two of them evaluated the brain sections for degree and extent of fluorescence blindly, without knowledge of the rf exposure condition. One experimenter handled the energy exposure and extraction of the brain from the skull; a second experimenter, who did not know the exposure condition, sectioned the brain and evaluated the sections; and the third experimenter, who also did not know the exposure conditions, evaluated the sections.

The first experimenter injected sodium pentobarbital into an animal (50 mg/kg, ip). The unconscious animal was laid on a block of energy absorber in the rf anechoic chamber. It was then exposed to actual or simulated rf illumination, as required by the experimental design. After a 30-min illumination, the experimenter removed the animal from the chamber. A small skin incision was made over the femoral vein, and 0.15 ml of 4% sodium fluorescein was injected into the vein. To verify that the





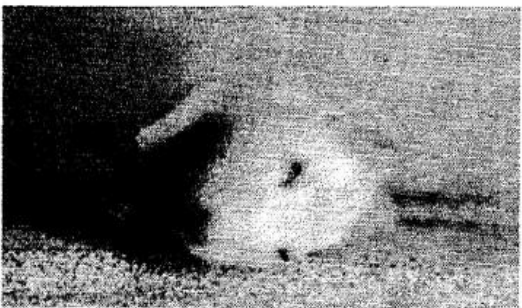
**I**



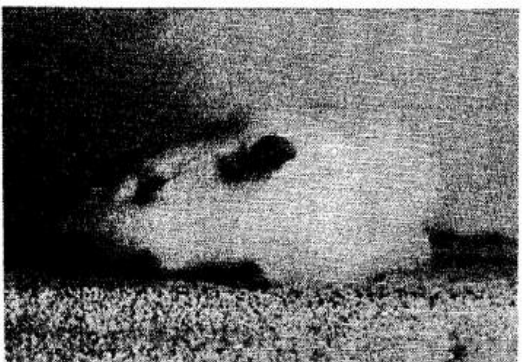
**II**



**III**



**IV**



**V**

**FIGURE 2.** Head positions of animals used in the brain permeability experiment as viewed from the energy source. The pictures are close-up for clarity; the animals were in far-field during exposure.

dye was injected properly, the paws, ears, nose, mouth, and tail were inspected under ultraviolet light to ascertain fluorescence. Approximately 5 min after injection, the animal was exsanguinated, and the brain was removed and embedded in a 10% gelatin solution. The mold of gelatin was coded and stored in a refrigerator for 20 min.

A second experimenter removed the mold from the refrigerator and placed the gelatin block that contained the brain on the stage of a freezing microtome. After the gelatin block was frozen by irrigation with CO<sub>2</sub>, coronal sections were cut at a thickness of 625  $\mu$ m. Serial sections were placed on a set of coded slides, four brain sections to a slide. Approximately 28 serial sections were obtained from each brain. The experimenter then dark adapted and viewed the serial sections under uv light. Which sections fluoresced and the location and intensity of the fluorescence were recorded. The intensity was scaled on a four-point scale. A third experimenter normally viewed the sections under uv light in the darkened room and, with rare exception, concurred with the second experimenter's assessments. On several occasions, the first experimenter also included saline-injected instead of dye-injected control animals as blanks, without the knowledge of the other experimenters. The brain sections of these animals never fluoresced. The blanks are not counted in the 90 animals in the design.

TABLE I lists the mean fluorescence intensity of brain sections obtained from the three exposure conditions for each of the five head positions. TABLE I also gives the median number of sections that showed fluorescence under various exposure conditions and head positions. In general, the fluorescence was seen at the diencephalon level of the brain, although on some occasions it was also seen in the mes- and

TABLE I  
EFFECTS OF RF ENERGY ON BRAIN FLUORESCENCE

Head Positions	Intensity of Fluorescence (mean)		
	Pulsed (A)	cw (B)	Sham (C)
I	1.5	0.8	0.2
II	1.8	0.7	0
III	2.0	1.0	0.2
IV	2.2	0.7	0
V	0.2	0	0
A vs B vs C p < 0.001			A vs B p < 0.001
A vs C p < 0.001			A (I, II, III, IV) vs (V) p < 0.001
B vs C p < 0.005			B (I, II, III, IV) vs (V) p < 0.005
Head Positions	Number of Sections that Fluoresced (median)		
	Pulsed (D)	cw (E)	Sham (F)*
I	2.5	0.5	0
II	9.5	1.5	0
III	7.5	3.5	0
IV	1.5	0.5	0
V	0	0	0
D vs E vs F p < 0.001			D vs E p < 0.005
D vs F p < 0.001			D (I, II, III, IV) vs (V) p < 0.001
E vs F p < 0.005			E (I, II, III, IV) vs (V) p < 0.005

\*Although the median number of sections that fluoresced was zero, some sections did fluoresce slightly. This is reflected by the non zero means in column C.

metencephalon. Fluorescence was particularly conspicuous in the vicinity of the lateral ventricles and often near the third ventricle. Note should be taken that ventricular contents are not well fixed in frozen sections.

By use of the Kruskal-Wallis analysis of variance, it was found that the effects in the pulsed condition (A and D) were significantly different from the effects in the cw condition (B and E) and that both were significantly different from the effects in the control condition (C and F). The significance in each case was beyond the 0.005 level. It was found that in both pulsed and cw conditions (both in intensity and extent of fluorescence) animals illuminated in head position V differed significantly in the effects shown from animals illuminated in the other head positions.

The results of the brain permeability experiment indicate that illumination of a small mammal with low-power rf energy affects the brain barriers. It appears that pulsed energy is significantly more effective than cw energy. Because illumination of the animals was not confined exclusively to the brain, one cannot conclude with assurance that the effect observed in the brain was direct. Conceivably, it could have been secondary, a result of some mechanism initiated by the energy illuminating another part of the body. Conversely, the apparent influence of head position in determining the effects of the energy could possibly be explained by the facial bones concentrating energy in the brain.

We believe that the conclusions drawn should be rather limited at this stage of our knowledge. It appears that rf electromagnetic energy affects brain permeability and behavior and that pulsed energy is more effective than cw energy in affecting said brain permeability and behavior. These results indicate that there is an association between behavioral modification and brain permeability change when similar rf energy parameters are employed.

#### REFERENCE

1. FREY, A. H. 1971. Biological function is influenced by low power modulated rf energy. *IEEE Trans. Microwave Theory Techn.* MTT-19(2):153-164.