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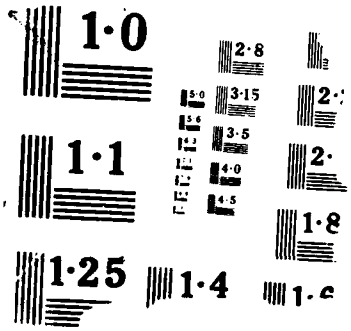
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BEHAVIORAL EFFECTS OF EXPOSURE TO THE TEMPO HIGH-POWER MICROWAVE SYSTEM

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NOTICES

This interim report was submitted by personnel of the Radiation Physics Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-01-1N.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

This report has been reviewed and is approved for publication.

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Safety standards for exposure to radiofrequency radiation must be based upon biologic consequences of exposure to such environments. Behavioral-based measures are considered to be the most sensitive indices of biological effects. Current safety guidelines are based upon average power density and may not be relevant to the high-peak power, short pulse-width microwave radiation produced by newly developed high-peak-power microwave sources. The effects of exposure to high-peak power ~~(0.5 kW/cm^2, 1.11-1.26 GHz, 85 ns)~~ radiation on reflexive responding and motor function in Fischer 344/N rats were assessed by measuring startle and general activity, and disruption of on-going performance of a rotarod task respectively. The emitter used was the TEMPO repeat pulse axially extracted vircator. ~~at the Air Force Weapons Laboratory, Kirtland Air Force Base, N. Mex.~~ Exposure to single pulses resulted in significant startle responses. Exposure to 1 pps for 10 s produced significant alterations in baseline activity and marked disruption of performance of the rotarod task. The apparently greater effect observed in the rotarod task is discussed in relation to the

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greater workload that task requires. The possibility that noise associated with the internal electronics and/or perturbations of the field by the Plexiglas apparatus may have acted as intervening variables is discussed. These data suggest that Fischer 344/N rats are behaviorally responsive to some component of the high-peak-power microwave stimulus. Experiments are currently being conducted to identify the limits of detection and the quality of the sensory/perceptual experience of exposure to the TEMPO radiation.

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BEHAVIORAL EFFECTS OF EXPOSURE TO THE TEMPO HIGH-POWER MICROWAVE SYSTEM

INTRODUCTION

Recent advances in the development of high-power microwave (HPM) sources mandates that health and safety standards be continuously reevaluated. Safety standards for exposure to radiofrequency radiation (RFR) must be based upon biologic consequences of exposure to such environments. The paucity of data on the biobehavioral effects of HPM, especially when pulsed in the very short nanosecond range, requires additional research. Current RFR safety guidelines are based upon average power density and may not be relevant to the high-power, short pulse-width radiation produced by these experimental sources.

Reviews of the RFR bioeffects literature have concluded that behavior-based measures were the most sensitive indices of biological effects (1). Alterations in behavior are frequently the first indication that a biologically significant event has occurred, and disruption of ongoing behavior is the most commonly reported effect of exposure to microwave radiation in the literature (2).

Recently, the first screening behavioral tests of the effects of HPM were conducted at the Air Force Weapons Laboratory (Kirtland AFB, N. Mex.) with the Gypsy virtual cathode oscillator (vircator) pulsed microwave emitter (3). These tests suggested that rats were sensitive to some qualitative component of the stimulus complex present during exposure to 1.62 GHz, 140 ns pulsed microwave producing power densities ranging from approximately 1 to 8 kW/cm^2.

This report describes two series of tests designed to provide data on behavioral effects of HPM in the rat, using the TEMPO emitter system. Effects on reflexive responding were assessed by measuring startle and general activity and effects on motor function were screened by measuring disruption of ongoing performance of a task requiring the rat to maintain position on a rotating rod (rotarod).

The startle response of rodents has been used extensively for the analysis of animal behavior. Much of the research has been directed at examination of habituation, the most basic form of learning (4). Since the response is a highly prepared behavior (5), that is a species-specific defense reaction to a rapid change in the environment (6), the response may be a suitable system for analysis of HPM effects on behavior. The startle response has a short, reproducible latency which occurs in virtually every rat (7). Furthermore, startle is a highly graded reflex subject to parametric variation of the eliciting stimulus, the surrounding environmental stimuli, or the general state of the animal (7, 8). Therefore, this response may prove to be a sensitive response capable of revealing detection and/or aversive thresholds to HPM.

METHODS AND MATERIALS

Animals

We used 44 experimentally naive, male, Fisher 344/N rats approximately 70 days old when received from the Inhalation Toxicology Research Institute (ITRI), Albuquerque, N. Mex. The rats were maintained in a mobile trailer located adjacent to the exposure facility. The rats were housed individually in plastic "shoebox" cages. They were handled and weighed to establish free-feeding baseline. Water and standard rat chow were available <u>ad libitum</u> throughout the experiment except during actual exposure. The rats were maintained on a 12/12 h light/dark cycle (lights on at 0600 h). The rats were randomly assigned to the following experimental treatment groups: <u>Startle Response Test</u>--Single pulse (HPM-exposed [n = 9], sham-exposed [n = 6], functional control [n = 1]), multiple pulse (HPM-exposed [n = 8], sham-exposed [n = 7]); and <u>Rotarod Motor Effects Task</u>--(HPM-exposed [n= 6], sham-exposed [n = 6]).

Radiofrequency Radiation Exposures

The TEMPO simulator with repeat pulse power source is similar to the single pulse power source Gypsy simulator that was used in the earlier experiment (3). The TEMPO simulator is an axially extracted vircator operated in this study at 1.3 GHz using a transformer based repetitive pulse power drive (Sandia National Laboratories, DOE, Albuquerque, N. Mex.). The horn and the propagated field are essentially the same as that produced by the Gypsy oscillator (9). The animal and test apparatus were located at an angle of 10° west of the antenna center line at a radius of 3 m. The microwave probe was located 10° east of the center line also at 3 m radius. The radiated pattern from the TM_{01} source is symmetric about the center line, with a single on-axis null. The radiated field is polarized with the electric field in the radial direction. Pattern shape, local power density, and frequency were measured with free-field D-dot sensors (EG&G, Inc.). An Air Force Weapons Laboratory technical report describing the simulator, its propagated field, and dosimetry is in preparation (10). Figure 1 shows the TEMPO emitter.

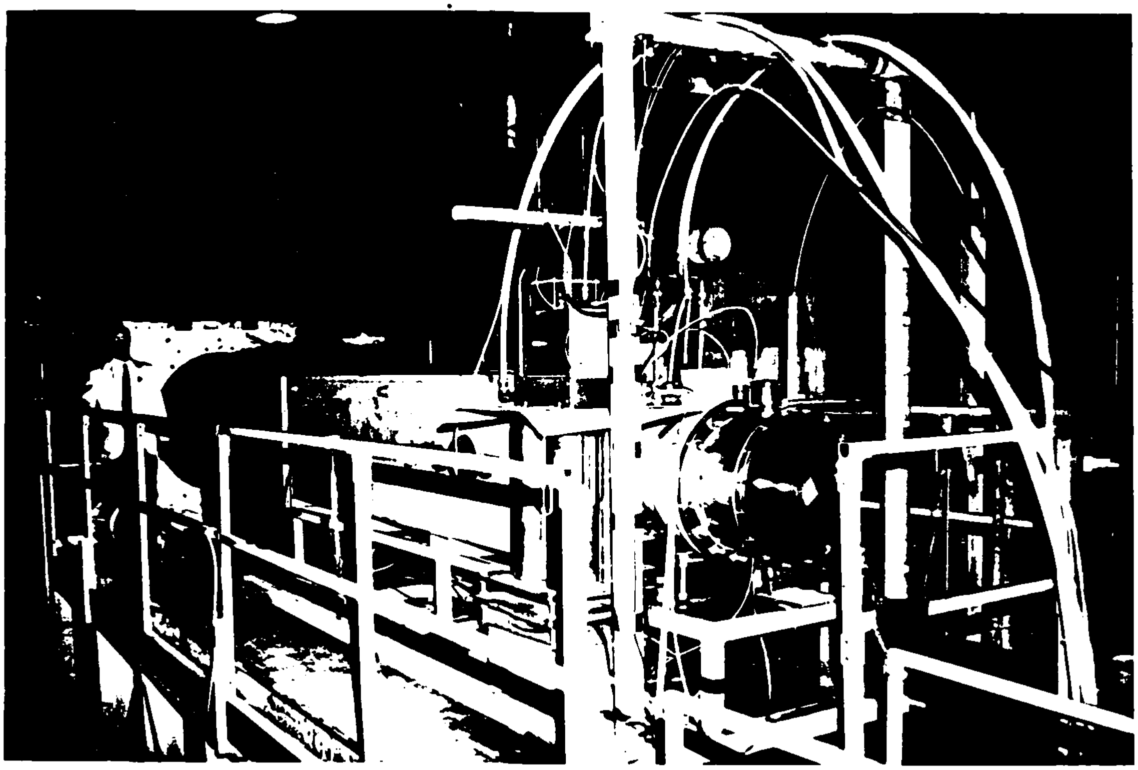

Figure 1. The *TEMPO emitter system*. Microwave radiation is emitted into an anechoic chamber on the other side of the horn antenna.

BEHAVIORAL TASKS AND RESULTS

Startle Response

Apparatus

The level of spontaneous activity and the startle response of rats to HPM was examined in a custom designed RFR compatible stabilimeter. The stabilimeter test chamber consisted of a Plexiglas cage (180 mm x 180 mm x 180 mm inside diameter). A removable floor (179 mm x 179 mm) had sections of rubber tubing (dia = 15 cm, length = 25 cm) attached diagonally across each corner with nylon screws to serve as shock absorbers. Nylon screws in each corner served for adjusting the height of the base (and floor) above the hydraulic pressure transducer, thereby providing a means for varying the maximum pressure placed on the transducer. The hydraulic pressure transducer consisted of a Tygon tube (length = 50 mm, dia = 10 mm) closed at one end and fused by way of a reduction coupling to a 10.7 m length of 4-mm diameter tubing. The tubing assembly was filled with distilled water under vacuum. The 50-mm length tube was clamped with rigid mounts at both ends and centered over a Plexiglas bar (10 mm x 10 mm x 25 mm) attached to the center of the chamber floor. The separate removable floor was seated on the shock absorbers, and a Plexiglas bar attached to the underside of the floor rested on top of the center of the Tygon tube. The chamber was equipped with a sliding lid with ventilation slots. Movements of the animal depressed the fluid-filled tube and produced a pressure differential which activated a speaker piston (located out of the field) at the end of the 4-mm diameter tubing. Movement-induced voltages from the speaker coil were amplified and recorded on a Brush (Model 220) event recorder. The startle apparatus is illustrated in Figure 2.

Figure 2. Startle apparatus.

All behavioral sessions were monitored by remote video (Hitachi, Model FP-7 camera; Sony, Model CVM-1225 monitor) and recorded with real-time date and time signal input (3-M Data Vision, Model DT-1) on a Sony (Model VO-5600) recorder with 60 m, three-quarter inch V-Matic tape (UCA).

A noise generator placed on the floor of the anechoic chamber below the stabilimeter produced 85 dB pink masking noise.

Test Procedure

Rats were placed in pint-sized plastic containers (11 cm x 12 cm x 9 cm) with ventilation holes in the cover and carried from the animal holding quarters to the anechoic chamber (approximately 30 m) and placed in the stabilimeter. Following a period of usually 2-4 min the event recorder was activated at a chart speed of 2 mm/s to collect baseline activity data. Approximately 2 s prior to exposure, chart speed was increased to 5 mm/s and remained at that speed for the duration of the test. The video recorder was turned on 60 s before the TEMPO simulator was fired. The initial series of tests were single pulse (85 ns, 1.3 GHz, 0.75 - 0.99 kW/cm^2).

Experiment 2 examined the effect of a train of these pulses. Pulse trains approximating 1 pps for 10 s were produced by manually firing the TEMPO simulator. The TEMPO horn antenna was covered with a three-quarter inch sheet of Styrofoam to attenuate the noise accompanying the firing of the TEMPO simulator. During sham exposures, a grounded sheet of aluminum foil covered the horn antenna, effectively eliminating the RFR emissions.

Design and Data Analysis

Rats were randomly assigned to either HPM-exposed or sham-exposed treatment groups. Data from the single-pulse exposures were analyzed by t-test for independent measures, Mann-Whitney U nonparametric test, and tests for differences between two proportions (11). All tests were one-tailed tests and significance levels were set at $p < 0.05$. Data from the multiple-pulse experiments were analyzed by independent or related t-tests and analysis of covariance with $p < 0.05$.

Single-Pulse Test

Functional Control. Movement-produced pen deflections on the chart recorder were measured by counting the number of 1-mm squares above zero. To obtain a relative measure of the validity of the stabilimeter, one rat was exposed to a loud hand clap. This noise produced a clearly identifiable startle response with maximum deflection of +25 mm (Fig. 3).

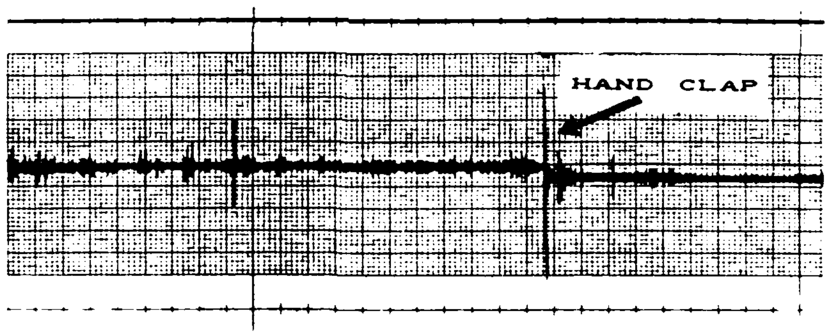

Figure 3. A loud hand clap stimulus served as a functional control for the stabilimeter apparatus producing a large spike on the chart (arrow).

Each pulse of the TEMPO simulator was accompanied by a loud sharp report from the internal electronics. Acoustic content of this noise is not known. Pulses of HPM presented without an animal in the stabilimeter failed to produce pen deflections indicating no detectable recording artifacts from equipment electrical transients.

Extraneous Variation. Several sources of possible experimenter-induced variability were identified. Because of the pilot nature of the experiments and the relatively untested state of the TEMPO (previously fired only 212 times), adjustments in operating procedures by the TEMPO technicians occurred over the course of the experiments. These adjustments resulted in variation of the adaptation period prior to exposure. Equipment modifications during the course of individual experiments introduced additional variation in the characteristics of HPM as well as associated non-RFR intervening stimulus variables.

Dosimetry. Power densities varied over the course of the experiments. During the single-pulse tests, power density ranged from 0.780 kW/cm^2 to 0.994 kW/cm^2. Power density during the multiple-pulse tests fell during the test series from 1.04 kW/cm^2 at the beginning to 0.514 kW/cm^2. Data was not available for several exposures because of instrumentation problems. Furthermore, there was approximately 58% uncertainty associated with the method of calculating power density.

This variability of the power density, and the lack of power density data, for several shots made it impossible to conduct any meaningful correlational or weighted statistics. Therefore, a conservative unweighted comparison between HPM-exposed and sham-exposed groups was done, ignoring power density.

Data Analysis. Each animal's record in both the HPM-exposed and sham-exposed groups showed a deflection concurrent with the pulse (Fig. 4).

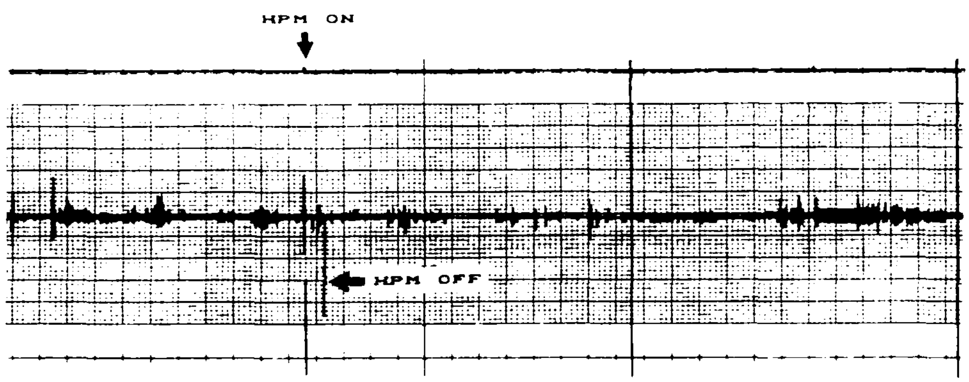

Figure 4. A representative recording of a startle response to a single HPM pulse (1.14 GHz, 85 ns, 0.828 kW/cm^2).

The deflections ranged from 2-24 mm with means of 7.33 mm in the HPM group and 1.83 mm in the sham-exposed group (Table 1). None of the rats (0/6) in the sham-exposed group exhibited responses producing deflections greater than 2 mm, suggesting that this minimal response was induced by non-RFR stimuli, possibly noise, accompanying the pulse.

TABLE 1. PEN DEFLECTIONS AFTER HPM OR SHAM EXPOSURE

HPM-Exposed		Sham-Exposed	
Rat #	amplitude (mm)	Rat #	amplitude (mm)
1	24	4	2
2	2	5	2
3	19	6	2
10	5	7	2
11	2	8	2
12	8	9	1
14	2		$\bar{x}$ = 1.83
13	2		
15	2		
	$\bar{x}$ = 7.33		

One-tailed independent measures t-test analysis of the absolute scores associated with the pulse failed to detect any significant differences ($t = 1.59$, $df = 13$) between the HPM-exposed and sham-exposed groups (Fig. 5a). A one-tailed Mann-Whitney U test was carried out because of unequal and small Ns and large variances. Again, no statistically significant differences between the groups were yielded ($U_{6,9} = 12.50$). However, many tied scores resulted from the large number of minimally responding subjects (2 mm or less deflection). Since a portion of the response, in each case, may be related to non-RFR stimuli (vide supra), a criterion was set wherein responses producing deflections greater than 2 mm were scored as startle responses. Figure 5b illustrates that the HPM-exposed group had significantly greater ($z = 1.91$, $p = 0.028$) number of rats displaying startle responses (4/9) than did the sham-exposed group (0/6).

Multiple Pulses

Recordings were analyzed by counting the number of 1-s intervals in which there was an excursion of the recording pen of 2 mm or greater above baseline. Six 10-s epochs (containing a possible maximum of 10 events in each epoch) immediately before the testing were averaged and served as a baseline value to which the exposure epoch, adjusted to a 10-s average, was compared. The exposure epochs varied from 9 s to 16 s in length. Thus, the total number of 1-s events were divided by the exposure interval length in seconds and multiplied by 10 (events/s x 10). Data were analyzed for excursions that were 2 mm or greater above baseline. Observation of cumulative and video records of the rat's response to the HPM stimulus showed that typically the rat would "freeze" at the onset of the exposure and remain relatively motionless, in a defensive/orienting posture, until the end of the train of pulses (Fig. 6).

One animal in the sham-exposed group, however, responded with an increase in activity that subsided immediately after the pulse-train ended. This rat had a very low baseline activity. Observation of the video record suggests that the increase in responses obtained on the chart record was the result of orienting responses, possibly initiated toward the sound component of the stimulus. None of the rats displayed behaviors indicative of stress immediately following either treatment.

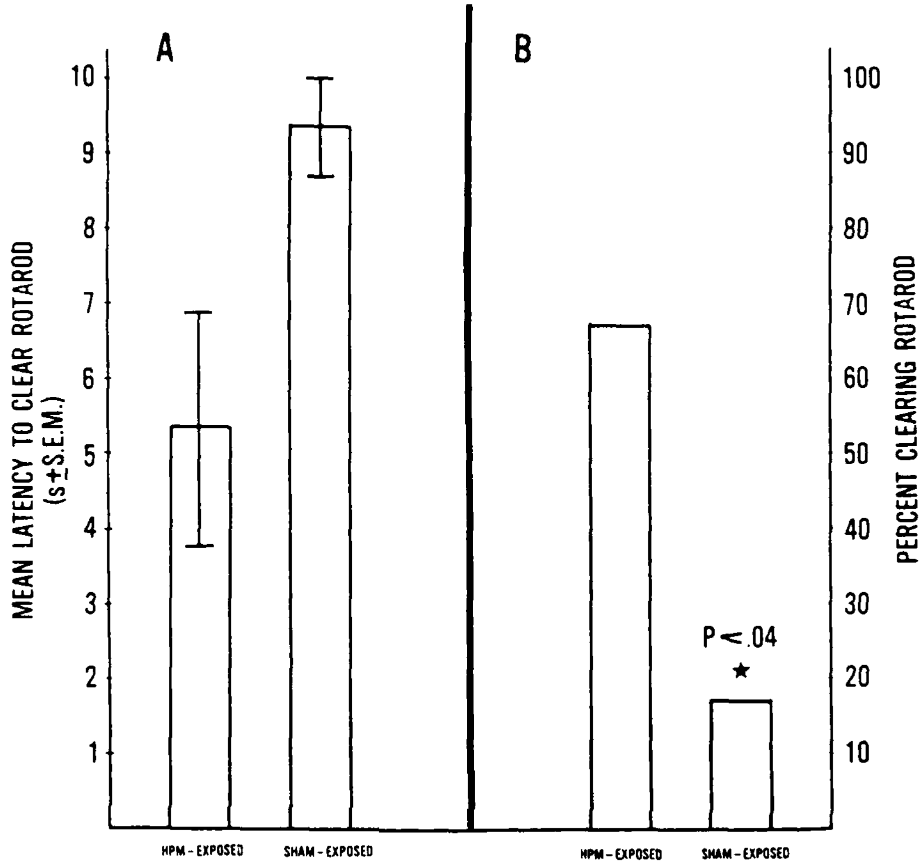

Figure 5. A. The response (measured as mm of pen deflection) of rats exposed to a single pulse of high-power microwave radiation. B. The HPM-exposed group had significantly greater number of rats (4/9) displaying startle response than did the sham-exposed group.

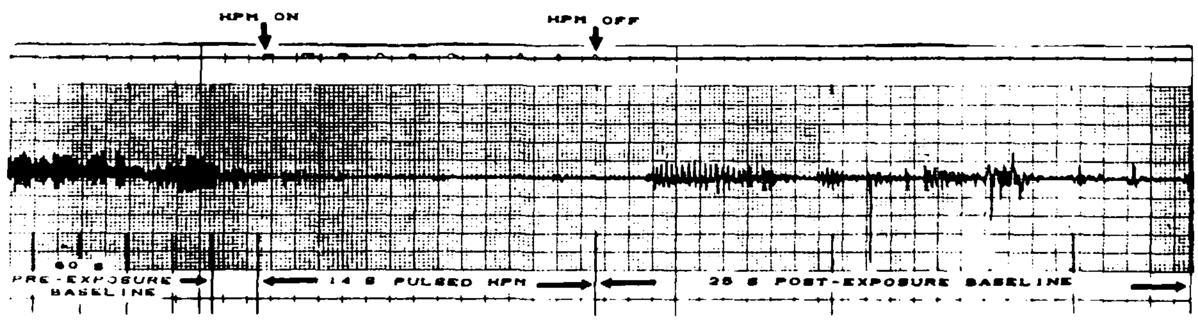

Figure 6. The event marker at the top of the strip chart indicates the train of 10 HPM pulses delivered over 14 s.

TABLE 2. RAT ACTIVITIES MEASURED IN THE STABILIMETER

	Rat#	---------events*/10 s epoch----- Pre-test Baseline						Test	Post-test	$\bar{x}$ Baseline	% Baseline	Absol. Diff.
	17	5	3	6	2	2	2	2.0	1	3.33	60	1.33
	18	3	7	9	7	7	8	4.38	8	6.83	64	2.45
H	19	10	10	10	10	10	10	.71	6	10.00	7	9.29
P	20	0	3	0	5	0	2	0	0	1.67	0	1.67
M	21	0	2	0	0	3	0	0	2	0.83	0	0.83
	30	4	7	7	6	7	2	0	1	5.50	0	5.50
							$\bar{x}$ =	1.18	3.0	4.69	21.83	3.51
	24	2	2	2	2	0	4	0	4	2.00	0	2.00
S	25	7	6	7	3	2	0	1.92	2	4.17	46	2.25
H	26	10	10	10	9	7	10	1.11	1	9.33	12	8.22
A	27	1	1	0	0	4	0	4.00	0	1.00	400	3.00
M	28	10	8	10	10	10	7	7.78	10	9.17	85	1.39
	29	10	10	10	10	10	10	7.55	7	10.00	76	2.45
							$\bar{x}$ =	3.73	4.62	5.95	103.17	3.22

*2 squares or > = event

Data Analysis. Baseline activities averaged over six 10-s epochs immediately prior to exposure (Table 2), were not significantly different between groups ($t = 0.58$, $df = 10$). Comparisons of difference scores, indicating absolute changes in activity, failed to yield significant differences between the groups ($t = 0.17$, $df = 10$). When within group absolute changes were analyzed separately, by way of t-tests for related measures, significant decreases in activity were found in both the HPM-exposed group ($t = 2.62$, $df = 5$) and sham-exposed ($t = 3.16$, $df = 5$), suggesting a non-RFR component of the stimulus was behaviorally active (Fig. 7). The noise associated with the triggering of the TEMPO device is the most likely source of this effect.

Although no differences were yielded by statistical comparisons of baseline activities, the repeated measures (pretest, posttest) design suggested that analyses of the percent of baseline activity and analysis of covariance might be appropriate. Analysis of the ratio (baseline - test/baseline) scores between the HPM-exposed and sham-exposed groups showed that significantly greater changes in activity occurred in the HPM-exposed group ($t = 2.49$, $df = 10$, two tailed). Similarly, a one-way analysis of covariance yielded a significant difference between the treatment groups ($F_{1,9} = 5.63$), indicating that exposure to HPM had significantly altered activity levels as compared to sham-exposed (Fig. 7).

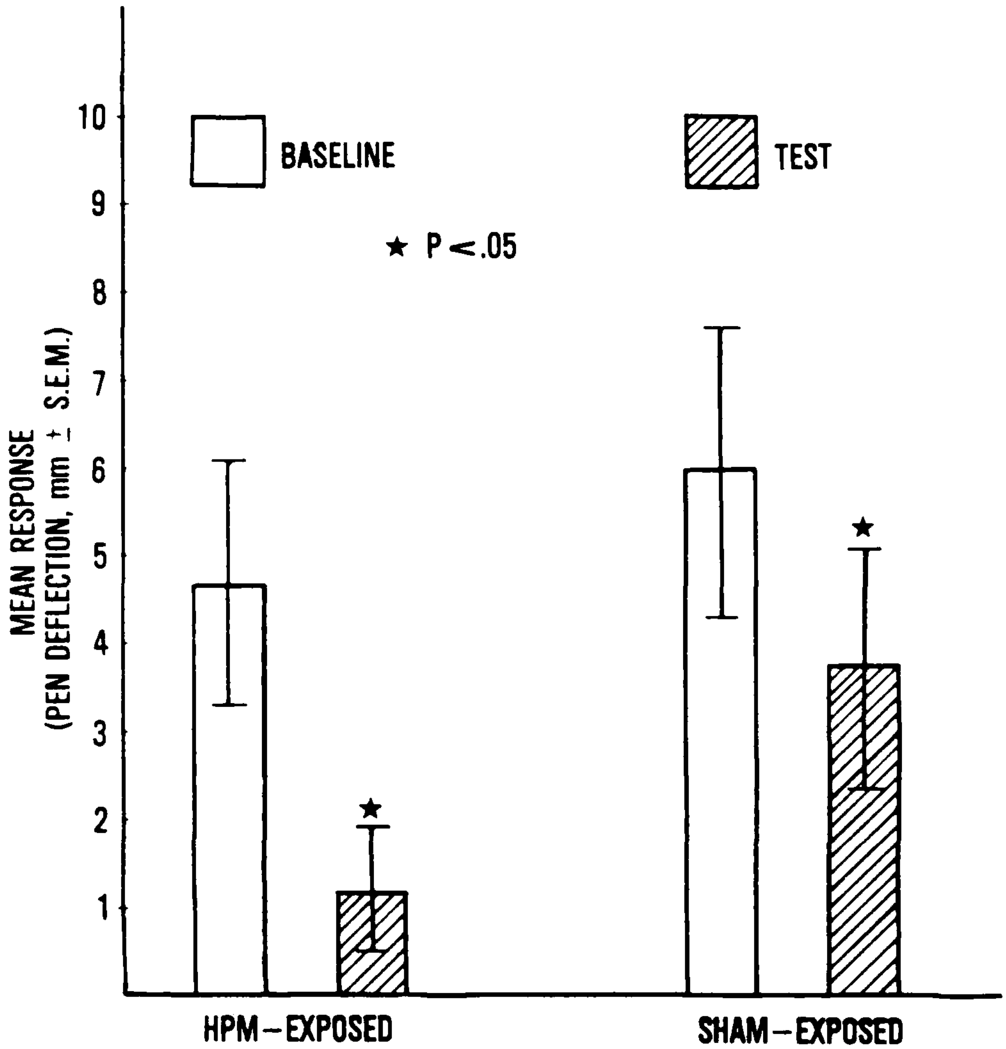

Figure 7. The effect of 10 pulses of HPM on activity. Baseline activity is averaged over six 10-s epochs immediately prior to exposure.

Rotarod Motor Effects Tasks

Apparatus and Procedure

Rats were trained to maintain a stationary position by walking on a slowly rotating (15 rpm) rod (diameter = 4 cm, width = 12 cm) over an ice water bath. The rats typically require 3 training sessions to reach criterion performance levels indicated by continued walking for 3 min without escape attempts or falling off the rotarod into the ice water bath. The rotarod axle was made of Plexiglas, and covered with a sheet of sandpaper. The axle and pulley, also made of Plexiglas, were mounted on two Plexiglas supports (width = 6 cm; height = 14 cm). The supports were mounted on a Plexiglas base (width = 15 cm; length = 29 cm). The pulley was driven by a Tygon tubing loop attached to a small electric motor which was placed well out of the electromagnetic field.

After sufficient training, the rats could easily perform the task for many minutes (12).

Dosimetry. Power density was approximately 50% that of the startle-activity test, ranging from 0.54 kW/cm^2 to 0.62 kW/cm^2. Again, power density data were not obtained for all shots in this series of tests.

Data Analysis. The effect of multiple HPM pulses on rotarod performance was dramatic. Four of 6 HPM-exposed rats exhibited marked reduction in latencies to dismount. Observation of the video records indicated that these rats displayed unmistakable escape responses; actively dismounting within 4 s of initial exposure. Only 1 of 6 sham controls failed to remain on the rotarod throughout the entire pulse train. Figure 8 illustrates the significant difference ($z = 1.79$, $p = 0.04$, one-tailed) between the proportion of HPM-exposed as compared to sham-exposed that dismounted before the fifth pulse.

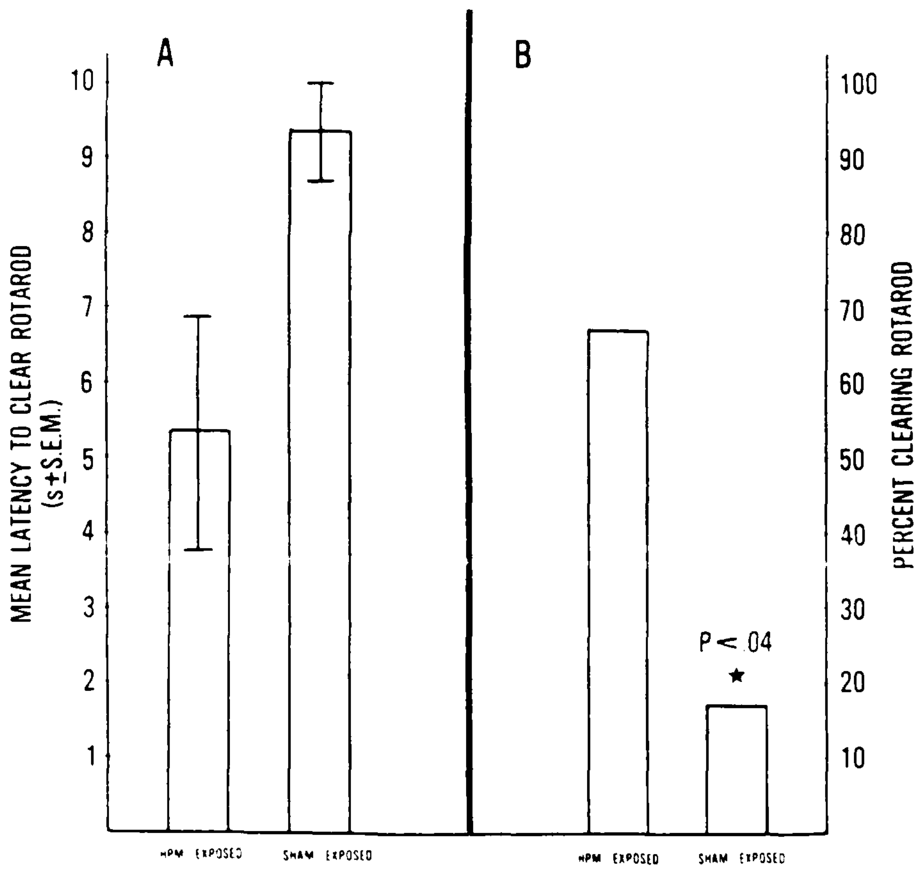

Figure 8. A. The mean latency from treatment initiation until the rat was completely clear of the rotarod. B. The proportion of rats that dismounted before the fifth pulse.

DISCUSSION

The stabilimeter detected differences in activity between the HPM-exposed and sham-exposed groups that were difficult to quantify by observation of video tape records. Although the differences were small, they were consistent enough to yield statistically significant results suggesting that some stimulus characteristic of the HPM pulse is behaviorally significant. The specific characteristic and mechanism of action of the effect cannot be determined from this data.

The results indicated that the custom-designed stabilimeter is sensitive and capable of detecting very small reflexive responses. The effect of a single pulse of HPM (0.7 - 1.0 kW/cm^2, 1.3 GHz, 85 ns) was variable and relatively small, suggesting that these HPM parameters are close to threshold for this behavioral test.

The rotarod test also proved to be a sensitive measure of the effects of pulsed HPM on performance. The magnitude of responding was markedly greater than that observed with the startle response test. Several factors may account for the differential sensitivity of the two tests. First, the rotarod test places a greater workload on the animals than the startle response test. Variables of increased attention, activity, and stress-related responses may interact with pulsed HPM to produce a greater response. Knepton et al. (13) showed that the effect of low-level RFR on behavior is influenced by the workload an animal is required to carry out. The possible rate-dependent effects (14) of low-level RFR (15, 16, 17) may also be related to the effect of workload.

The possible interactive effects related to the noise accompanying the firing of the TEMPO device are a potential source of variation that must be considered. The noise may be producing physiological and behavioral stress responses that interact with the HPM to potentiate or even attenuate the behavioral effect. Another potential source of variability between the stabilimeter and rotarod tests is the possibility that the HPM is attenuated by the stabilimeter Plexiglas cage walls. Glass rods and sheets of Lucite have been recently reported to cause major perturbations in the 1-10 GHz frequency band, if the materials are aligned in the same direction as the E field (18). There was no Plexiglas separating the rat from the horn in the rotarod task. Future experiments must take these variables into account and preferably eliminate them.

The present experiments and those of Knepton et al. (13) suggest that high-rate or complex tasks involving vigilance and/or significant motor involvement may prove to be sensitive to the effects of high-peak-power pulsed microwave radiation.

This series of tests examining the behavioral effects of concurrent exposure to high-peak-power pulsed microwaves in the 1.26 GHz to 1.11 GHz range with power densities of 1.04 kW/cm^2 to 0.514 kW/cm^2 indicates that rats of the Fisher 344/N strain respond to some component of the HPM stimulus. Finally, when the animals are engaged in a forced activity requiring significant motor involvement, they respond as if some component of the radiation is an aversive stimulus.

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