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BEHAVIORAL EFFECTS OF 1300 MHZ **DTIC** FILE COPY
HIGH-PEAK-POWER-MICROWAVE
PULSED IRRADIATION

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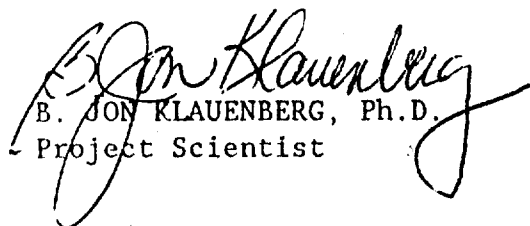
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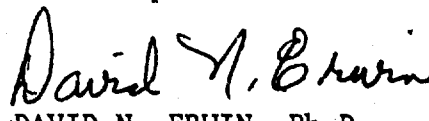
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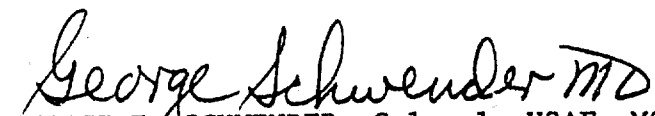
The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.


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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Results of behavioral and physiological studies on the effects of high-peak-power microwaves (HPPM) are reported. Rats were typically irradiated for 10 min using the following HPPM characteristics: 1300 MHz, 10 MW forward power, 5 and 10 pulses per second (pps), 1.8 kW/cm ² peak-power density with 1, 5, or 10 us pulse widths. Average-power densities were 9, 45 and 90 mW/cm ² at 5 pps and 18, 90 and 180 mW/cm ² at 10 pps (average colonic specific absorption rate (SAR) = 1.8, 6.5, 13.1 W/kg and 3.6, 13.1, and 26.2 W/kg, respectively). Results indicated the following: (1) Irradiation under 10 us 10 pps protocol (SAR = 26.2 W/kg) reduced locomotor activity. (2) Response rates under a variable-interval (VI) schedule declined after irradiation protocols yielding SARs greater than 13.1 W/kg. (3) All reductions in locomotor activity or VI responding were accompanied by increased colonic temperature. (4) HPPM irradiation, under the pulse width conditions tested, did not disrupt discrete-trial avoidance or escape responding. (5) A single 10-min exposure under the 10 us 10 pps protocol caused deficits in memory processing as measured using a					
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19. ABSTRACT (Continued)

passive-avoidance task. (6) A single 10-min exposure under the 10 μ s/10 pps protocol caused rats to form an aversion to the site of irradiation. (7) Similar place aversions occurred following 1- or 5-min irradiation at a peak-power density of 9.0 kW/cm² (12 μ s pulse width, 1 pps). The increase in avoidance responding after the 1-min irradiation occurred in the absence of any rise in colonic temperature. The results of these experiments establish a preliminary threshold of HPPM irradiation sufficient to consistently produce behavioral effects. The results suggest that changes in pulse width and pulse-repetition frequency are probably secondary to power density in contributing to the observed effects; that is, behavioral changes were directly correlated with increased colonic temperature and thus directly related to SAR.

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TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION.	1
MATERIALS AND METHODS	2
Subjects/Housing	2
Temperature Measurement.	2
Behavioral Observation	2
Holding and Irradiation Cages.	3
Computer Control Systems	3
Statistical Methods.	3
High-Peak-Power-Microwave Irradiation System and Dosimetry	3
BEHAVIORAL AND PHYSIOLOGICAL EXPERIMENTS.	5
Locomotor Activity Experiment.	5
Subjects and Environmental Conditions	6
Behavioral Apparatus.	6
High-Peak-Power-Microwave Testing Procedure	6
Variable-Interval Behavior	7
Subjects and Environmental Conditions	7
Behavioral Apparatus.	7
Training Schedule	8
High-Peak-Power-Microwave Testing Procedure	8
Two-way Discrete-Trial Avoidance Behavior.	9
Subjects and Environmental Conditions	9
Behavioral Apparatus.	9
Training Schedule	10
High-Peak-Power-Microwave Testing Procedure	10
Passive-Avoidance Memory Testing	10
Subjects and Environmental Conditions	11
Behavioral Apparatus.	11
High-Peak-Power-Microwave Testing Procedure	11
Passive-Place-Avoidance Testing.	12
Behavioral Apparatus.	12
Subjects and Environmental Conditions: Experiment 1	12
High-Peak-Power-Microwave Testing Procedure: Experiment 1	12
Subjects and Environmental Conditions: Experiment 2	13
High-Peak-Power-Microwave Testing Procedure: Experiment 2	13
RESULTS	13
Locomotor Activity	13
Variable-Interval Behavior	16
Discrete-Trial Avoidance Behavior.	20
Passive-Avoidance Memory Testing	21
Passive-Place-Avoidance: Experiment 1.	22
Passive-Place-Avoidance: Experiment 2.	22

	<u>Page</u>
DISCUSSION.....	24
Locomotor Activity.....	24
Variable-Interval Behavior.....	25
Discrete-Trial Avoidance Behavior.....	26
Passive-Avoidance Memory Testing.....	26
Passive-Place Avoidance Studies.....	26
GENERAL DISCUSSION.....	27
ACKNOWLEDGMENTS.....	28
REFERENCES.....	29
APPENDIX - TABLES 1 AND 2.....	33

List of Figures

<u>Fig.</u> <u>No.</u>		
1.	Cumulative Temperature Increase by Region During Dosimetry Testing...	5
2.	Locomotor Activity Study - Number of Full Crosses.....	14
3.	Locomotor Activity Study - Number of Left Crosses.....	15
4.	Locomotor Activity Study - Change in Temperature Postirradiation.....	15
5.	Locomotor Activity Study - Postirradiation Colonic Temperature.....	17
6.	Variable-Interval Study - Number of Responses.....	17
7.	Variable-Interval Study - Number of Reinforcements.....	18
8.	Variable-Interval Study - Response/Reinforcement Ratio.....	18
9.	Variable-Interval Study - Change in Temperature Postirradiation.....	19
10.	Variable-Interval Study - Postbehavioral Testing Change in Temperature.....	20
11.	Discrete-Trial Avoidance Study - Change in Temperature Postirradiation.....	21
12.	Passive-Avoidance Memory Test - Traverse Latency.....	22
13.	Passive-Place Avoidance Experiment 1 - Traverse Latency.....	23
14.	Passive-Place Avoidance Experiment 2 - Traverse Latency.....	23

BEHAVIORAL EFFECTS OF 1300 MHZ HIGH-PEAK-POWER-MICROWAVE PULSED IRRADIATION

INTRODUCTION

Research on possible biological interactions with high-power microwave (HPM) irradiation (i.e., $>1 \text{ W/cm}^2$) is a relatively new endeavor, dictated by the development of new high-power generation systems (1). While some biological effects previously have been attributed to high-peak-power exposure conditions (2), reports of behavioral and physiological effects of HPM irradiation have seen only limited distribution (3,4). Recent reports indicate possible disruption of behavioral performance and the induction of a startle response by very short duration (85 ns) pulsed HPM irradiation (5,6). The first series of experiments examining possible behavioral, physiological and biochemical effects of HPM has been conducted using a 1300 MHz klystron source at the High-Power Microwave Laboratory of Los Alamos National Laboratory (LANL, 7). Those experiments were designed to evaluate behavioral and physiological interactions across a range of power density and specific absorption rate (SAR) values, from nonthermal (undetected) to clearly thermal. Several of the experiments were conducted using repeated testing under different irradiation protocols to establish possible thresholds for HPM effects [i.e., forward power and pulse-repetition frequency (PRF) were held constant while the pulse width was increased from $1 \mu\text{s}$ to $5 \mu\text{s}$ to $10 \mu\text{s}$ on different test days]. In those experiments, significant suppression of variable-interval (VI) responding and locomotor activity was noted at a threshold power density of 90 mW/cm^2 (peak-power density = 1.8 kW/cm^2 , SAR = 13.1 W/kg).

Several factors may contribute to behavioral effects induced by exposure to HPM irradiation. The present experiments were designed first to replicate the conditions of the original experiments (7), and then to extend the results by testing an additional PRF of 10 pps and higher peak powers. The additional PRF was added both to increase power density and to determine possible effects of PRF on behavioral measures. Specifically, the use of both $10 \mu\text{s}/5\text{-pps}$ and $5 \mu\text{s}/10\text{-pps}$ irradiation protocols permitted comparison of PRF values at equivalent power densities. The PRF at biologically relevant frequencies (5-15 pps) may be an important determinant of HPM-induced effects given the apparent role of pulse modulation in radiofrequency (RF), extremely low frequency electric field (8,9), and magnetic field (10) biological effects. Higher peak-power testing was conducted to verify and extend possible thresholds for behavioral effects noted in our original experiments (7).

In both the original and present experiments, the primary behavioral endpoints of interest were performance, memory processing, and the aversive nature of HPM irradiation. Performance was evaluated following 10 min of irradiation with HPM using 3 different paradigms reflecting spontaneously emitted (locomotor activity), appetitively motivated (VI behavior) and aversively motivated (2-way discrete-trial avoidance behavior) tasks. Paradigms with different motivational properties were used to maximize the possibility of detecting centrally mediated behavioral changes.

The possible effects of HPM irradiation on memory processing were assessed with a passive-place-avoidance paradigm where footshock served as an

aversive stimulus. This test uses the formation of place aversions by rats (11 for review); that is, rats will avoid any physical location that has previously been paired with negative consequences, such as poisoning or footshock. A variety of environmental and pharmacological interventions effectively disrupt memory processing (retrograde amnesia) when presented following a training trial; the earlier the intervention, the greater the degree of processing disruption (12-16). In a series of related experiments, HPM irradiation replaced footshock as a stimulus in order to evaluate possible aversive properties of HPM irradiation.

MATERIALS AND METHODS

Subjects/Housing

For all experiments, adult naive male Long-Evans rats were purchased from Charles River Breeding Laboratories (Wilmington, MA). After a 2-week quarantine period they were housed in suspended 46 cm x 24 cm x 15 cm polycarbonate cages with wood-chip bedding. Rats were single or double housed depending on the experiment. *Ad libitum* access to water and Teklad 4% rat diet was provided, with the exception of the VI testing experiment, where access to food was necessarily restricted. Animals were initially maintained at the Health Research Laboratory of the Life Sciences Division. Prior to HPM testing, the animals were transported in a temperature-controlled vehicle to a Portable Test Facility [PTF (7)] located adjacent to the 1300 MHz HPM source at the LANL Accelerator Technology Division. Ages and body weights of rats as well as environmental conditions for individual experiments are presented at the beginning of each experiment described later.

Temperature Measurement

A digital telethermometer (Bailey Instruments, Clifton, NJ, Model BAT-8) with RET-2 probe was used to record colonic temperatures. Colonic temperature was determined by lubricating the probe with mineral oil, inserting it 5 cm beyond the anal sphincter, and recording the temperature value 7 s after insertion. The telethermometer was calibrated against a National Institute of Standards and Technology traceable quartz-thermometer in a temperature-controlled oil bath.

A fluoroptic 4-channel thermometry system (Luxtron Instruments, Inc., Mountain View, CA; Model 750) with ceramic tip silica-fiber probes was used to determine anechoic chamber and ambient laboratory temperatures. Probes penetrated the anechoic chamber through a 1.3 cm brass pass-through. The system and probes were calibrated in oil daily against the Bailey BAT-8 digital telethermometer.

Behavioral Observation

Animal behavior in the anechoic chamber was continuously monitored during all experiments with a Hitachi CCTV video camera (Model HV-62U) and an RCA TC1918 monitor. The camera was enclosed in a copper Faraday cage (16.5 cm w x 24.1 cm d x 8.9 cm h) located in one corner of the anechoic chamber adjacent to the standard gain horn antenna.

Holding and Irradiation Cages

During training and irradiation, rats were placed in Plexiglas holding cages (interior measurements 8.6 cm w x 10.8 cm h x 19.7 cm l) with ventilated sliding lids. The cage floor consisted of 0.64 cm diameter Plexiglas rods, 1.6 cm apart, perpendicular to the long axis of the cage. The cage was supported by a Plexiglas stand, the height (98.2 cm) controlled such that the midpoint of a 350 g rat would be located on the midline of the horn antenna at a distance of 1.3 m. The holding cage was modeled after that used by Toler and Bonasera (personal communication), and forced the animals to remain parallel to the long axis of the cage but provided sufficient space to minimize the stress associated with restraint (17,18). Six holding cages were used on a rotating basis for all HPM- or sham (SH)-irradiation sessions in the anechoic chamber. During the training phase of all experiments requiring repeated testing, rats were acclimated to the holding cages for a minimum of 3 days before HPM testing.

Computer Control Systems

All behavioral test systems were controlled by a MICRO/PDP 11/73 (Digital Equipment Corporation, Westminister, MA) using a LAB-LINC interface. Schedule contingencies were programmed and behavioral events recorded using a SKED-11 operating system (State Systems, Kalamazoo, MI; 19). Specifications of the specific behavioral test apparatus used are provided in the section describing each experiment.

Statistical Methods

Single factor interactions were analyzed with a 1-way analysis of variance (ANOVA) (20) with *a posteriori* contrasts by Duncan's and Scheffe's multiple range tests (21,22) or Student's t-tests. A nonparametric analysis of the number of animals scoring avoidance failures in aversion paradigms was conducted with the Test for Significant Differences Between Two Proportions (23). Multiple factor analysis was conducted using an n-way analysis of variance and covariance program (24). Repeated measures were analyzed using the method of Winer (20). For both 1-way and repeated measures analyses of variance, missing data were omitted from the analysis if their values on any dependent variable were missing. The degrees of freedom (df) reported for each statistical test were automatically adjusted to reflect missing data. All variance values in this paper are the standard error of the mean (SEM).

High-Peak-Power-Microwave Irradiation System and Dosimetry

The HPM source used for these experiments was a 1300 MHz RF power station originally constructed as prototype for the Pion Generator for Medical Irradiations (PIGMI) accelerator project intended for cancer therapy (1). A complete technical description of the 1300 MHz source used in these experiments, as well as calibration, field mapping, and dosimetry details, has been published elsewhere (7) and will only be summarized here.

The system consisted of a high-voltage power supply, a line-type modulator with klystron, and the system control racks. The modulator converted direct current (DC) high-voltage (30 kV) from the power supply to pulsed voltage (200 kV) suitable for driving the klystron. The klystron

microwave tube was a Litton Model L-3661. Ranges of output parameters of the system were:

Carrier frequency	1300 MHz
Forward power	17 MW
Pulse width	0.1-12 μ s
Pulse-repetition frequency	0-12 pps

For the majority of the behavioral experiments described below the following operating parameters were used:

DC high voltage	22 kV
Forward power	10 MW
Peak-power density	1.8 kW/cm ²
Pulse-repetition rate	1, 5, and 10 pps

During biological testing, the RF pulse width was varied independently of the high voltage settings. Pulse widths of 1, 5, and 10 μ s at PRFs of 5 and 10 pps were used for the majority of the behavioral experiments. Waveforms recorded during testing at each of these pulse widths consistently approximated a square wave. Average-power densities at these pulse widths (1, 5, 10 μ s) were 9, 45, and 90 mW/cm² at 5 pps and 18, 90, and 180 mW/cm² at 10 pps at the location of the animal. For one passive-place avoidance experiment, peak-power density was increased to 9.0 kW/cm² by increasing forward power to 17 MW and placing the animals in the extreme near HPM field, 20 cm from the center of the horn antenna. For this experiment, 12- μ s pulses were presented at a PRF of 1 pps.

Microwave output was transmitted to the anechoic chamber in the PTF through a WR650 waveguide, that was pressurized with sulfur hexafluoride (SF₆) to 5 psi for increased power handling capability. Forward and reflected power were monitored with 2 directional waveguide couplers mounted at the output of the klystron. A 3d waveguide directional coupler was mounted near the horn antenna as a 2d forward power monitor. Forward power was attenuated by <0.1 dB between the 1st and 3d directional couplers. The transmitting antenna was a standard gain (15.8 dB at 1300 MHz) horn (NARDA Model 646). Rats were irradiated in the H orientation, 1.3 m from the midline of the antenna.

Animal dosimetry (7) was conducted with rat carcasses irradiated in the H orientation. These conditions coincide with the highest power used for the behavioral tests. Environmental and power conditions for the dosimetry tests were as follows:

Room temperature	23.3 $\pm$ 0.2°C
Relative humidity	48%
Forward power	17 MW
Pulse width	10 μ s
Pulse-repetition frequency	10 pps
Peak-power density	1.8 kW/cm ²
Carrier frequency	1300 MHz

A series of 26 tests with rat carcasses ranging in weight from 262 g to 458 g was conducted. Carcasses were equilibrated to room temperature and placed in the Plexiglas holders normally used for behavioral testing. A

Luxtron Instruments fluoroptic 4-channel thermometry system with ceramic tip silica fiber probes was used to determine ambient anechoic chamber temperature and temperature increases in 3 anatomical locations. One probe was inserted 5 cm into the colon. A 2d probe was inserted 1 cm past the calvaria parallel to the long axis of the brain at point 0.5 cm below the occipital ridge. A 3d probe was inserted under the skin on the midline of the dorsal surface of the rat at a point equidistant between the base on the neck and the base of the tail. Temperature measurements were recorded at 15-s intervals for a 9-min equilibration period, a 5-min irradiation period and a 5-min postirradiation period. The SAR calculations (25,26) were based on temperature rise during the 1st min of irradiation, assuring that calculations were based on a linear temperature rise before significant heat dissipation from the carcasses. Mean colonic, brain, and skin SAR values from the carcass tests were extrapolated for 5 pps and 1, 5, and 10 μ s pulse width exposures. The approximate average and peak SARs (7) produced by each of these irradiation protocols tested is listed in Table 1 (Appendix). The rate of heating and subsequent cooling during dosimetry testing is shown in Figure 1. As demonstrated in the figure, regional temperatures remain elevated for several minutes following the cessation of irradiation. Clearly, a strong thermoregulatory response by the intact animal would be required to dissipate heat deposited at this rate.

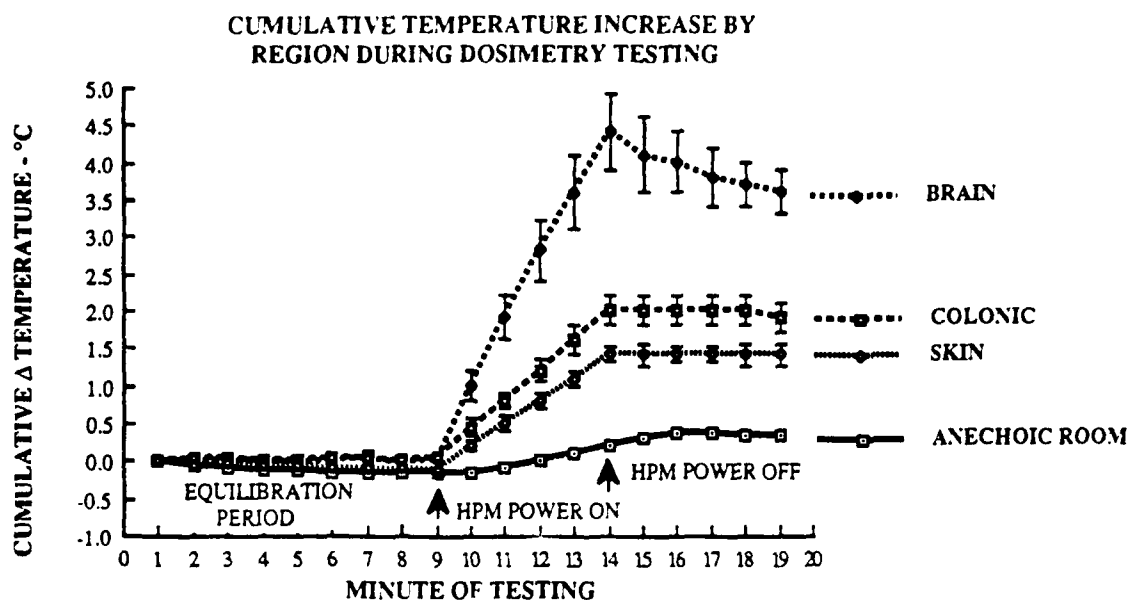


Figure 1. Mean ($\pm$ SEM) temperature ($^{\circ}$ C) in different anatomical regions during dosimetry testing. After a 9-min equilibration period, carcasses were HPM irradiated for 5 min.

BEHAVIORAL AND PHYSIOLOGICAL EXPERIMENTS

Locomotor Activity Experiment

In a previous experiment (7), suppression of locomotor activity was noted following irradiation under a 10 μ s/5 pps/10-min protocol, while no significant effects were seen following 1 and 5 μ s/5 pps/10-min exposures. In the present experiment, the locomotor activity of rats was assessed following 10 min of HPM or SH irradiation under 1, 5, and 10 μ s pulse widths

at 5 pps and 10 μ s pulse widths at 10 pps. An initial activity assessment was conducted under SH-irradiation conditions to establish a baseline activity rate and a second baseline test was included on an intermediate day to assess possible cumulative effects of HPM irradiation.

During our previous locomotor activity experiment (7), the 1, 5, and 10 μ s pulse width protocols were tested consecutively. Since a significant suppression of locomotor activity was noted only under the highest (and final) pulse width condition, the possibility exists that our original results might be attributable to the effect of the testing sequence. That is, with repeated testing, locomotor activity normally declines and the HPM effect may have been cumulative, leading to this decline. Therefore in the present experiment, the testing sequence for the first 3 test days was reversed from that of the initial series, and an additional baseline testing day was added before testing under 10 pps conditions.

Subjects and Environmental Conditions

N = 18 (9 HPM/9 SH)
Age = 89 days on 1st day of testing
Housing = Double (paired in cages - 1 HPM/1 SH)
Mean HPM group body weight = 317.6 ± 5.5 g
Mean SH group body weight = 316.2 ± 5.8 g
Mean PTF room temperature = $21.1 \pm 0.1^\circ\text{C}$
Mean PTF anechoic chamber temperature = $21.2 \pm 0.1^\circ\text{C}$
Mean PTF relative humidity = $28.5 \pm 0.2\%$

Behavioral Apparatus

Locomotor activity testing was conducted in 47 cm l x 22.5 cm w x 15.2 cm h polycarbonate cages with metal grid floors contained in sound attenuating chambers (LaFayette Instruments Model 80015). The cages were placed in photocell mounting brackets so that the photobeams (Coulbourn Instruments, Lehigh Valley, PA, photodetectors and photocell assemblies, Models S23-01 and T22-01) transected the width of the cage 14 cm from each end of the cage. Repetitive breaks of the left and right photobeams were recorded, and a locomotor activity full cross was scored each time a rat broke the 2 photobeams in sequence.

High-Peak-Power-Microwave Testing Procedure

For 1 week before HPM or SH irradiation, each rat's body weight and colonic temperature were recorded and 2 equal groups (N=9 ea) were assigned on the basis of these measures. On each test day, rats were weighed and a preirradiation colonic temperature was recorded. The rats were then placed in polycarbonate training cages. Alternating conditions, they were individually HPM or SH irradiated for 10 min in the anechoic chamber of the PTF, according to the following schedule:

<u>HPM Test Day</u>	<u>Pulse Width/PRF</u>
1 (pretest)	No HPM
3	10 μ s/5 pps
5	5 μ s/5 pps
7	1 μ s/5 pps
9 (retest)	No HPM
11	5 μ s/10 pps
13	10 μ s/10 pps

Animals were rested 1 day between each test day to allow recovery from possible HPM effects. Immediately after HPM or SH irradiation, a postirradiation colonic temperature was recorded, and animals were then placed in the locomotor activity system for a 30-min test session. A postbehavioral testing colonic temperature was recorded immediately following locomotor activity testing.

Variable-Interval Behavior

Food-deprived rats were trained for 46 consecutive days to press a lever for food pellet reinforcement, initially on a fixed-ratio schedule and ultimately on a VI 10 s (VI-10) schedule. Rats trained on the VI schedule demonstrated a high rate of responding and a very stable rate of reinforcement. Rats were tested following HPM or SH irradiation under 1, 5, and 10 μ s pulse-width conditions at 5 and 10 pps with intermediate nonHPM test sessions to determine if any shift from baseline performance had occurred. In an earlier experiment using VI behavior (7), significant suppression of responding on this task was noted only following irradiation under the 10 μ s/5 pps condition (power density = 90 mW/cm², SAR = 13.1 W/kg). Evaluation of responding by 5-min intervals indicated that significant suppression occurred only during the first 10 min following HPM irradiation. In the present experiment, response and reinforcement rates and interval data measures were again recorded and an evaluation of the response to reinforcement ratio was added to the test paradigm.

Subjects and Environmental Conditions

N = 18 (9 HPM/9 SH)
 Age = 61 days on 1st day of training
 Housing = Single
 Mean HPM group body weight = 282.9 $\pm$ 6.2 g
 Mean SH group body weight = 282. $\pm$ 6.9 g
 Mean HRL room temperature = 19.0 $\pm$ 0.3°C
 Mean HRL relative humidity = 27.1 $\pm$ 0.1%
 Mean PTF room temperature = 20.9 $\pm$ 0.3°C
 Mean PTF anechoic chamber temperature = 21.9 $\pm$ 0.1°C
 Mean PTF relative humidity = 33.9 $\pm$ 0.4%

Behavioral Apparatus

Six operant chambers (Coulbourn Instruments Model E10-10) were enclosed in Coulbourn, Model #7, isolation cubicles (40.6 cm d x 45.7 cm h x 55.9 cm w) equipped with ventilation fans, baffled air-intake and -exhaust systems. Each chamber was equipped with 2 levers mounted 3 cm from the side walls, and 3 cm

above the grid floor. Pressure on the right lever with a downward force equivalent to 15 g (0.15 N) delivered a pellet reinforcement (Bio-Serv, Inc., dustless precision pellets for rodents, 45 mg, product #0021; Coulbourn pellet feeder, Model E14-12) to a central delivery magazine.

Training Schedule

Animals were placed on restricted diets for 2 weeks before training. The animals were handled for 20 min daily for 3 days before the onset of training to habituate them to handling. During VI training, animals were allowed *ad libitum* access to water, but were restricted to an average of 13 g of Teklad 4% rat diet per day following testing (adjusted for individual body weights). The food supplement was gradually diminished to an average of 5 g/day as the number of reinforcements received during VI training increased. By the 1st day of VI training, the animals had been reduced to 78.5% of the body weight of littermate cohorts used in the shuttle-avoidance experiment. The HPM- and SH-irradiated groups were assigned on the basis of equivalent body weights and preirradiation colonic temperatures before the onset of training.

On Day 1 of training, rats were trained under an alternative fixed-ratio 1-response, fixed-time 1-min schedule. Each response on the right lever was reinforced and reinforcement also was provided after each minute during which no responding occurred. Responses on the left lever were not recorded and had no programmed consequence. On Days 2 to 14 the rats were placed under a fixed-ratio-1 schedule. On Day 15, a VI-10 schedule was initiated where responses were reinforced, on average, every 10 s (range = 3-30 s). Animals were trained in 3 groups of 6, with testing order rotated for each session. Initial training for this experiment was conducted in the Health Research Laboratory (HRL) before animal transport to the PTF on Day 39. Beginning on Day 39, animals were placed in holding cages in the PTF anechoic chamber for 10 min each day before each training session to habituate them to eventual irradiation conditions. Colonic temperatures were recorded before and following this habituation procedure. Daily VI training continued for 8 additional days at the PTF.

High-Peak-Power-Microwave Testing Procedures

Six HPM tests occurred on alternating days from Days 47 to 57. All animals received SH irradiation on Days 48, 50, 52, 54, 56, and posttest Day 58, continuing their daily test routine on the VI-10 reinforcement schedule. Before HPM or SH irradiation, each animal's weight and preirradiation colonic temperature were recorded. Postirradiation colonic temperature was recorded after 10 min of HPM or SH irradiation. Postbehavioral testing colonic temperature was recorded immediately following the 30-min VI-10 session. Animals were irradiated individually, alternating HPM and SH groups, and rotating starting times as in training. The run order of the various pulse widths and pulse-repetition frequencies was randomized except that the highest power condition (10 μ s/10 pps) was intentionally tested last to avoid residual effects of what was anticipated to be a highly thermal irradiation protocol. The order in which the different HPM protocols were tested was as follows:

<u>Test Day</u>	<u>Pulse Width/PRF</u>
1 (Day 47)	5 μ s/5 pps
2 (Day 48)	No HPM
3 (Day 49)	1 μ s/10 pps
4 (Day 50)	No HPM
5 (Day 51)	10 μ s/5 pps
6 (Day 52)	No HPM
7 (Day 53)	1 μ s/5 pps
8 (Day 54)	No HPM
9 (Day 55)	5 μ s/10 pps
10 (Day 56)	No HPM
11 (Day 57)	10 μ s/10 pps
12 (Day 58)	No HPM

Two-Way Discrete-Trial Avoidance Behavior

Rats were trained to avoid or escape an aversive electric footshock. Performance on this aversively motivated task is typically well learned and was not disrupted by any irradiation protocol tested during an earlier experiment (Hjeresen *et al.*, 7). However, in that experiment there was some evidence of an increase in the number of full shocks received following irradiation under the 10 μ s/5 pps protocol (power density = 90 mW/cm², SAR = 13.1 W/kg). In a further refinement of this test paradigm, the 30-min test session following HPM or SH irradiation was analyzed by 5-min intervals to determine if there were transitory effects of HPM irradiation on avoidance behavior.

Subjects and Environmental Conditions

N = 17 (9 HPM/8 SH)
 Age = 61 days on 1st day of training
 Housing = single
 Mean HPM group body weight = 243.1 $\pm$ 5.8 g
 Mean SH group body weight = 241.1 $\pm$ 5.7 g
 Mean HRL room temperature = 19.2 $\pm$ 0.3°C
 Mean HRL relative humidity = 27.0 $\pm$ 1.1%
 Mean PTF room temperature = 22.1 $\pm$ 0.1°C
 Mean PTF anechoic chamber temperature = 21.8 $\pm$ 0.1°C
 Mean PTF relative humidity = 32.3 $\pm$ 0.4%

Behavioral Apparatus

Six Model E10-16 Coulbourn discrete-trial avoidance chambers were enclosed in Coulbourn isolation cubicles (40.6 cm d x 45.7 cm h x 55.9 cm w) with ventilation fans, baffled air-intake and -exhaust systems. The toggle floor grid of each chamber was connected to a grid-floor shocker (Coulbourn Model E13-08). A central aluminum divider allowed access between sides through a 6.4 x 7.6 cm door. Each side of the chamber was illuminated by a Coulbourn house light module (Model E11-01), and a 2.8 kHz warning tone was emitted by a Sonalert tone module (Coulbourn Model E12-02).

Training Schedule

Before HPM testing, animals received 24 training sessions on alternate days (7 days/week), with 30 trials per session. Animals were trained in 3 groups of 6, with the test order rotated each day. After the training session on Day 20, animals were transported to the PTF. Beginning on Day 21, animals were put in holding cages and placed in an anechoic chamber at the PTF facility for 10 min before testing each day, to acclimate them to eventual irradiation conditions. The daily training procedure was as follows: after a variable interval from the start of each trial (VI-45 s) a 15 s tone was initiated. After 10 s, if the rat had not traversed to the opposite side of the chamber, a scrambled footshock (0.9 mA, 5-s duration) was delivered, while the tone continued. A traverse terminated both tone and shock. A traverse before shock onset was scored as an avoidance response. A traverse after shock onset but before its cessation was scored as an escape response. Failure to traverse during the 5-s shock was scored as a full shock. Traverses recorded between tone/shock periods were recorded as intertrial-interval responses.

High-Peak-Power-Microwave Testing Procedure

During HPM testing, discrete-trial avoidance testing continued under the same procedures as during training, with 10 min of HPM or SH irradiation immediately preceding testing. Each rat's body weight and preirradiation colonic temperature were recorded immediately before testing. Postirradiation colonic temperature was recorded after irradiation, and postbehavioral testing colonic temperature was recorded following the 30-trial avoidance session. Animals were individually irradiated, alternating SH and HPM irradiation, rotating starting times as in training. As during training, HPM-irradiation tests were conducted on alternate days, with no testing or training conducted on intervening days. The schedule was as follows:

<u>Test Day</u>	<u>Pulse Width/PRF</u>
1	5 μ s/5 pps
3	1 μ s/10 pps
5	10 μ s/5 pps
7	1 μ s/5 pps
9	5 μ s/10 pps
11	10 μ s/10 pps
13 (post test)	No HPM

Passive-Avoidance Memory Testing

The effects of HPM on memory processing were assessed with this paradigm, which takes advantage of the natural negative phototropism of the rat; that is, under free choice conditions, rats will select the darker of two otherwise identical chambers. The paradigm also takes advantage of the fact that memory processing is susceptible to disruption for a period of up to 4 h after an event (16). A variety of environmental and pharmacological interventions effectively disrupt memory processing (retrograde amnesia) when presented following a training trial; the earlier the intervention, the greater the degree of processing disruption (12-16,24,28).

In the present experiment, rats were HPM or SH irradiated immediately following footshock. A previous passive-avoidance memory test (7) indicated the possibility of an effect on this measure following very brief (16 s) irradiation under the 10 μ s/5 pps protocol (power density = 90 mW/cm, SAR = 13.1 W/kg).

Subjects and Environmental Conditions

N = 18 (9 HPM/9 SH)
Age = 106 days on 1st day of training
Housing = double
Mean HPM group body weight = 396.2 ± 8.9 g
Mean SH group body weight = 402.0 ± 9.6 g
Mean PTF room temperature = $21.6 \pm 0.1^\circ\text{C}$
Mean PTF anechoic chamber temperature = $23.4 \pm 0.3^\circ\text{C}$
Mean PTF relative humidity = $25.9 \pm 0.5\%$

Behavioral Apparatus

One Model E10-16 Coulbourn 2-compartment avoidance chamber, enclosed in a Coulbourn isolation cubicle (40.6 cm d x 45.7 cm h x 55.9 cm w) with ventilation fan, baffled air-intake and -exhaust system, was used. The exterior of 1 side of the cage was darkened by black fabric, while the other side was illuminated with 2 Coulbourn house light modules (Model E11-01). A central aluminum divider with door opening (6.4 cm w x 7.6 cm h) was modified to accommodate a remotely operated aluminum guillotine door. Coulbourn photodetector and photocell assemblies (Models S23-01 and T22-01) were arranged such that the photobeam would be broken when the door was fully raised, initiating timing procedures. Scrambled footshocks were generated by a Coulbourn grid-floor shocker (Model E13-08).

High-Peak-Power-Microwave Testing Procedure

On the day before testing, rats were assigned to equivalent HPM- and SH-irradiation groups on the basis of mean body weights and colonic temperatures from the previous 3 days. On Day 1, alternating HPM- and SH-irradiation groups, each animal was weighed and a prebehavioral testing colonic temperature was recorded. Rats were immediately placed in the lighted side of the 2-compartment avoidance chamber with the guillotine door closed. After 30 s, the door was opened, allowing access to the darkened side of the cage. Time between door opening and entry into the darkened side (latency) was recorded. One second after entry into the darkened side, a 0.9 mA scrambled footshock was administered until the rat returned to the lighted side of the cage. The animal was then removed from the apparatus and a preirradiation colonic temperature was recorded. Rats were then HPM or SH irradiated under the 10 μ s/10 pps min protocol. Postirradiation colonic temperature was then recorded, and the animal was returned to his home cage.

After 24 h, in the same order as they were tested on Day 1, animals again were placed in the lighted side of the 2-compartment avoidance chamber. After 30 s the guillotine door opened, allowing up to 120 s access to the darkened side. Latency to reenter the darkened side was recorded, but no shock was administered after entry.

Passive-Place-Avoidance Testing

The aversive properties of HPM were assessed with a passive-place avoidance paradigm conducted under 2 different irradiation protocols. In a previous passive-place-avoidance experiment (7), HPM irradiation under a 10 μ s/5 pps/10 m protocol did not result in statistically significant aversion. In experiment 1, rats were HPM or SH irradiated (10 μ s/10 pps/10 min protocol) immediately upon entry into the darkened side of a 2-compartment light/dark avoidance chamber. The following day, rats were again placed in the lighted side of the 2-compartment avoidance chamber and their latency to enter the darkened compartment was determined. In this task, a long return latency is interpreted as an aversion to the HPM irradiation. In Experiment 2, the test procedures were identical to those of Experiment 1, but higher peak-power densities were used for shorter irradiation durations (1 min and 5 min).

Behavioral apparatus

A dimensionally correct replica of a Coulbourn 2-compartment avoidance chamber (Model E10-16) was constructed of 0.95 cm Plexiglas. One side of the avoidance chamber was darkened by black fabric, while the other side remained illuminated by the ceiling light of the anechoic chamber. A center dividing wall with a 6.4 cm w x 7.6 cm h door opening was equipped with 2 pivoting doors that were manually operated from outside the anechoic chamber via nonconducting cables attached to retaining pins. Release of the 1st door allowed access to the darkened side of the chamber while release of the 2d door prevented return to the lighted side. A fiberoptic detector, triggered by a traverse of the toggle floor grid, initiated computer timing. The trigger was connected to a Coulbourn photodetector (Model S23-01) by fiberoptic cable. Behavior was visually monitored by means of a video camera and traverse latency was confirmed with manual timers.

Subjects and Environmental Conditions - Experiment 1

N = 18 (9 HPM/9 SH)
Age = 96 days on 1st day of training
Housing = double
Mean HPM group body weight = 366.7 ± 9.4 g
Mean SH group body weight = 363.8 ± 9.4 g
Mean PTF room temperature = $20.2 \pm 0.1^{\circ}\text{C}$
Mean PTF anechoic chamber temperature = $21.3 \pm 0.2^{\circ}\text{C}$
Mean PTF relative humidity = $27.3 \pm 0.3\%$

High-Peak-Power-Microwave Testing Procedure - Experiment 1

One day before testing, the animals were assigned to equivalent HPM- or SH-irradiation groups on the basis of mean body weights and colonic temperatures from the previous 3 days. On Day 1, alternating SH and HPM groups, each rat was weighed and a preirradiation colonic temperature was recorded. Rats were then taken to the anechoic chamber and placed in the lighted side of the avoidance chamber facing the door. After 30 s the door was opened, allowing up to 120 s access to the darkened side of the chamber. Upon entry into the darkened side, HPM or SH irradiation was administered under the 10 μ s/10 pps/10 min protocol. The duration between door opening

and entry into the darkened side was recorded (latency). The animal was then removed from the chamber, and postirradiation colonic temperature was recorded. On the following day, rats were placed in the lighted side of the avoidance chamber, inside the anechoic chamber. After 30 s the door opened, allowing access to the darkened side. Latency to enter the darkened side was recorded, but no irradiation was administered.

Subjects and Environmental Conditions - Experiment 2

N = 18 (12 HPM/6 SH)
Age = 122 days on 1st day of training
Housing = double
Mean HPM 1 min group body weight = 411.7 ± 13.5 g (N=6)
Mean HPM 5 min group body weight = 414.7 ± 14.3 g (N=6)
Mean combined SH group body weight = 404.5 ± 9.8 g (N=6)
PTF room temperature = $22.5 \pm 0.1^\circ\text{C}$
PTF anechoic chamber temperature = $23.7 \pm 0.1^\circ\text{C}$
PTF relative humidity = $30.5 \pm 0.5\%$

High-Peak-Power-Microwave Testing Procedure - Experiment 2

One day before testing, the animals were divided into experimental and control groups [HPM 1 min (N=6) and 5 min (N=6), SH 1 min (N=3) and 5 min (N=3)] on the basis of mean body weights and colonic temperatures. On Day 1, alternating SH and HPM groups, each rat was weighed, its colonic temperature was recorded, and it was immediately placed inside the anechoic chamber in the lighted side of the discrete-trial avoidance chamber facing the drop door. Testing procedures on Days 1 and 2 were the same as described for Experiment 1. However, in this experiment the following HPM conditions were used:

Forward power	17 MW
Pulse width	12 μs
Pulse-repetition frequency	1 pps
Irradiation duration	1 or 5 min
Peak-power density	9 kW/cm^2
Average-power density	108 mW/cm^2
Distance from antenna	20 cm

Note that the 20-cm distance from the antenna represents extreme near-field conditions relative to the 1.3-m distance used for all of the previous behavioral experiments. Given the uncertain nature of field uniformity in this region and the lack of comparable conditions during dosimetry measurements, no estimate of SAR can be provided for these conditions.

RESULTS

Locomotor Activity

The HPM-irradiated animals made significantly fewer full crosses of the apparatus (Fig. 2) when tested following irradiation only under the 10 $\mu\text{s}/10\text{pps}$ protocol [$F(1,16) = 6.7$, $p < 0.05$]. There were no irradiation condition (HPM or SH) or irradiation interactions on any of the activity

measurements when the data were analyzed with a repeated measures ANOVA, indicating that the effects of HPM irradiation were not dose dependent within the range of characteristics tested. This result suggests that the 10 μ s/10 pps protocol represented threshold conditions in the present experiment.

Similarly, HPM irradiation caused a significant suppression of activity on the right side of the activity system only under the 10 μ s/10 pps irradiation condition [$F(1,16) = 7.8, p < 0.05$]. A similar suppression of locomotor activity was noted on the left side of the activity system under the same conditions [$F(1,16) = 6.6, p < 0.05$]. On the Retest day, the HPM group was significantly more active on the left side than the SH group (Fig. 3) [$F(1,16) = 4.8, p < 0.05$], although neither group was irradiated on that day. Note that the activity of the HPM group on both right and left, as well as full crosses, on the Retest day was comparable to that seen in the same group on the Pretest day while activity among the SH-irradiated rats had declined.

There were no differences between HPM- and SH-irradiated groups in body weight. Despite having established HPM- and SH irradiation groups on the basis of equivalent body weight and colonic temperature, a repeated measures ANOVA indicates that SH groups had significantly higher preirradiation colonic temperature compared to HPM groups [$F(1,16) = 40.8, p < 0.001$]. There was no interaction between test group (HPM or SH) and day of testing, suggesting that there was no additive effect of HPM irradiation over the different protocols tested.

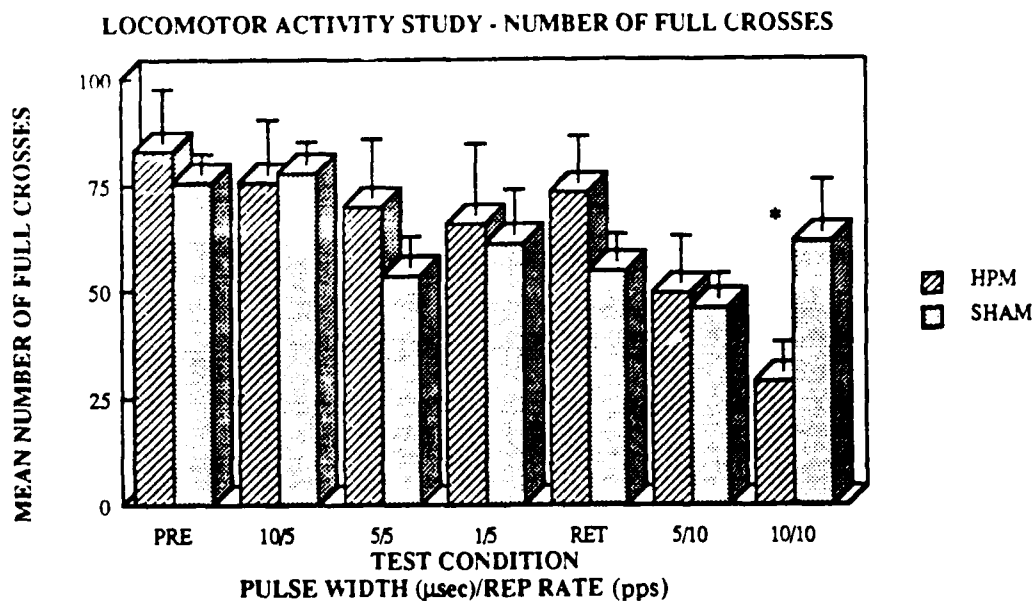


Figure 2. Mean ($\pm$ SEM) number of full locomotor activity crosses for groups of HPM- and sham-irradiated rats. Test conditions on the abscissa describe test treatments on different days of the experiment. PRE = Baseline test before the start of the experiment; RET = Retest day to reestablish baseline performance. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects.

* = $p < 0.05$.

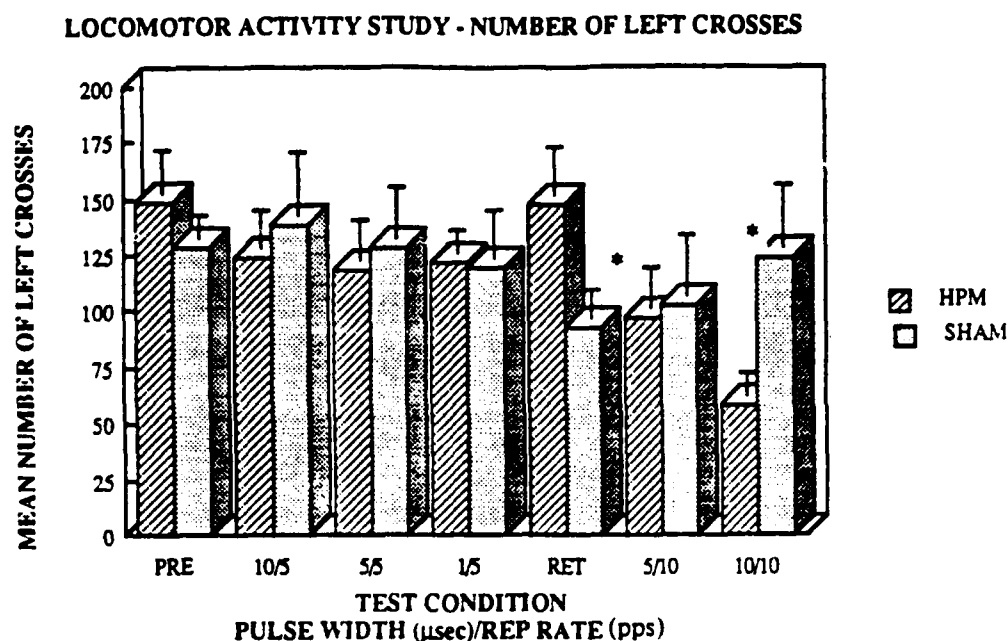


Figure 3. Mean ($\pm$ SEM) number of photobeam breaks on the left side of the apparatus for groups of HPM- and sham-irradiated rats. Test conditions on the abscissa describe test treatments on different days of the experiment. PRE = Baseline test before the start of the experiment; RET = Retest day to reestablish baseline performance. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.005$.

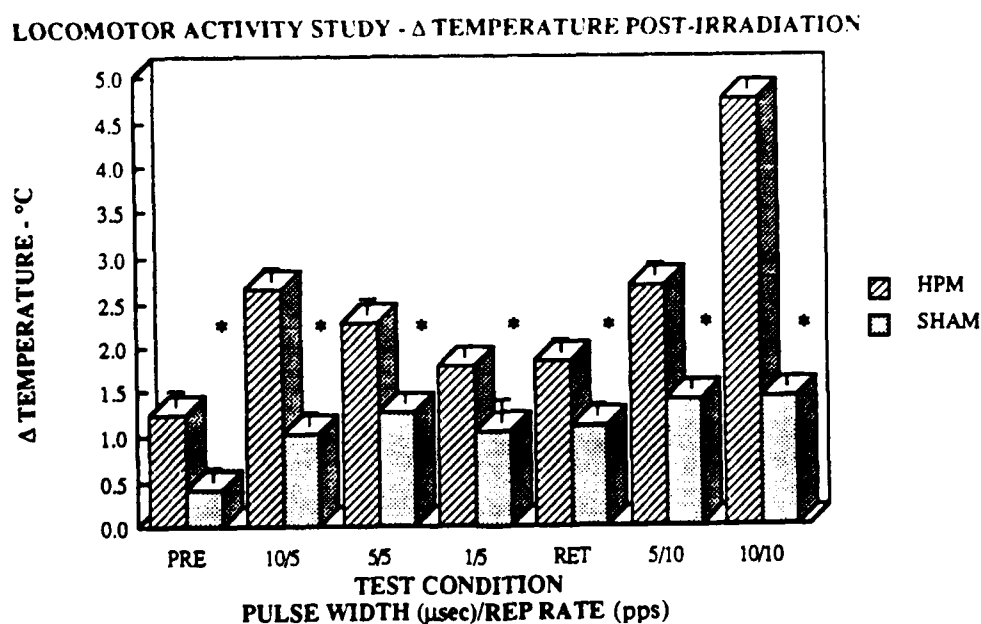


Figure 4. Mean ($\pm$ SEM) colonic temperature ($^{\circ}$ C) following HPM or sham-irradiation in locomotor activity experiment. Test conditions on the abscissa describe test treatments on different days of the experiment. PRE = Baseline test before the start of the experiment, RET = Retest day to reestablish baseline performance, and numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$.

Due to abnormally high preirradiation colonic temperature of rats in the SH group, analyses of the change in temperature values (preirradiation to postirradiation change) were deemed appropriate (Fig. 4). Rats in the HPM group had significantly greater increases in colonic temperature on each day of testing compared to SH-irradiated rats [$F(1,16) = 175.6$, $p < 0.0001$] and there was a dose-dependent relationship if only the HPM-irradiation days are considered [$F(4,64) = 32.2$, $p < 0.001$].

Absolute postirradiation temperature (Fig. 5) indicates that the highest colonic temperatures were directly correlated with the irradiation protocols yielding the greatest SARs.

Variable-Interval Behavior

There were no significant differences between the groups in rate of acquisition of the VI task. Rates of responding and reinforcement for the 2 groups during the FR-1 and VI-10 phases of training were nearly identical. Data from one animal in the HPM group were lost on the day of the 10 μ s/10pps test due to a power failure and the degrees of freedom for the various statistical tests reflect this loss.

The HPM-irradiated rats had lower response rates (Fig. 6) than SH [$F(1,15) = 5.4$, $p < 0.05$]. A significant suppression of response rate was noted following irradiation under the 10 μ s/10 pps protocol [$F(1,15) = 30.0$, $p < 0.01$]. Further, there was a significant interaction between irradiation condition (HPM or SH) and the irradiation protocol tested [$F(5,75) = 5.3$, $p < 0.01$] indicating a power-density-dependent decline in responding under the VI schedule.

While there was no overall effect of irradiation condition (HPM or SH) on reinforcements received (Fig. 7), there was an interaction between irradiation condition (HPM or SH) and the irradiation protocol tested [$F(5,75) = 5.5$, $p < 0.001$], indicating that irradiation protocols yielding higher power densities caused a decline in reinforcement rate. The HPM-irradiated animals received significantly fewer reinforcements than SH-irradiated controls only following irradiation under the 10 μ s/10 pps protocol [$F(1,15) = 10.0$, $p < 0.01$].

The HPM animals had a lower response/reinforcement ratio (Fig. 8) on HPM test days than SH, [$F(1,15) = 5.5$, $p < 0.05$]. Further, there was a significant irradiation condition (HPM or SH) by irradiation protocol interaction [$F(5,75) = 11.2$, $p < 0.001$] indicating a power-density-dependent decline in response/reinforcement ratio. The greater effect of HPM on response rate compared to reinforcement rate is reflected in decreased response to reinforcement ratios among HPM-irradiated rats following irradiation under the 5 μ s/10 pps [$F(1,16) = 5.2$, $p < 0.05$] and 10 μ s/10 pps [$F(1,15) = 26.3$, $p < 0.01$] protocols.

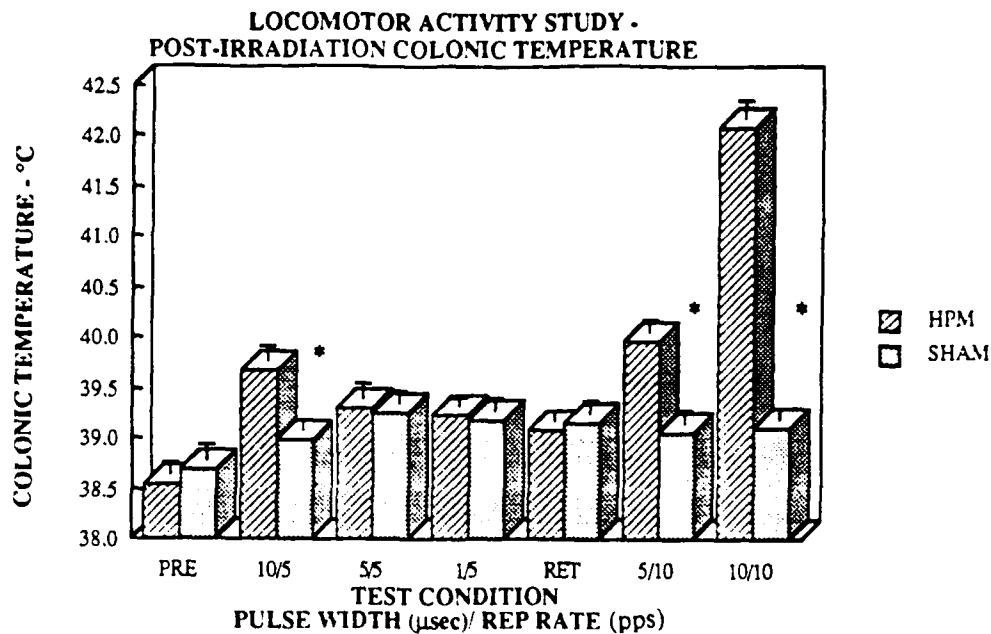


Figure 5. Mean ($\pm$ SEM) colonic temperature ($^{\circ}$ C) of groups of rats used in locomotor activity experiment following HPM- or sham-irradiation. Test conditions on the abscissa describe test treatments on different days of the experiment. PRE = Baseline test before the start of the experiment; RET = Retest day to reestablish baseline performance. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$.

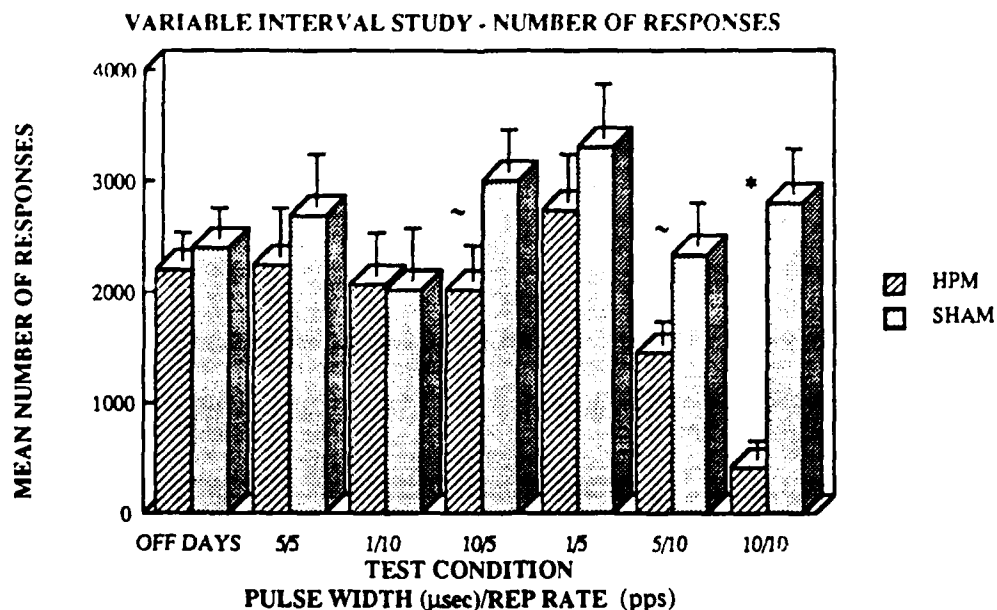


Figure 6. Mean ($\pm$ SEM) number of lever press responses on VI schedule for groups of HPM- and sham-irradiated rats. Test conditions on the abscissa describe test treatments on different days of the experiment. OFF DAYS = Combined data from the day preceding the onset of HPM testing and the days between each HPM test. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$, ~ = $0.05 < p < 0.06$.

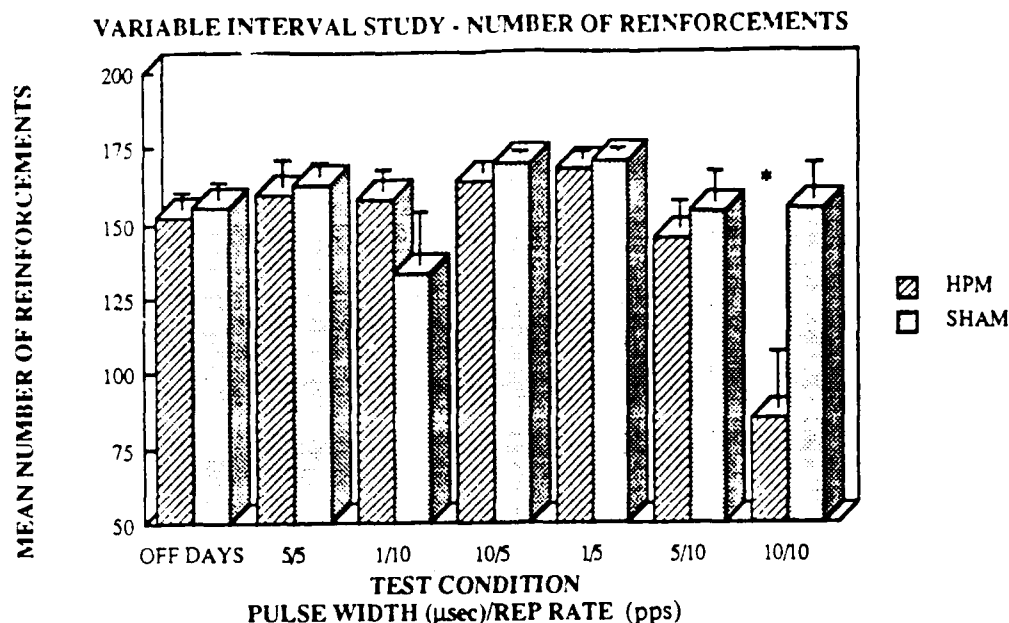


Figure 7. Mean ($\pm$ SEM) number of food pellet reinforcements received on a VI schedule by groups of HPM- and sham-irradiated rats. Test conditions on the abscissa describe test treatments on different days of the experiment. OFF DAYS = Combined data from the day preceding the onset of HPM testing and the days between each HPM test. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$.

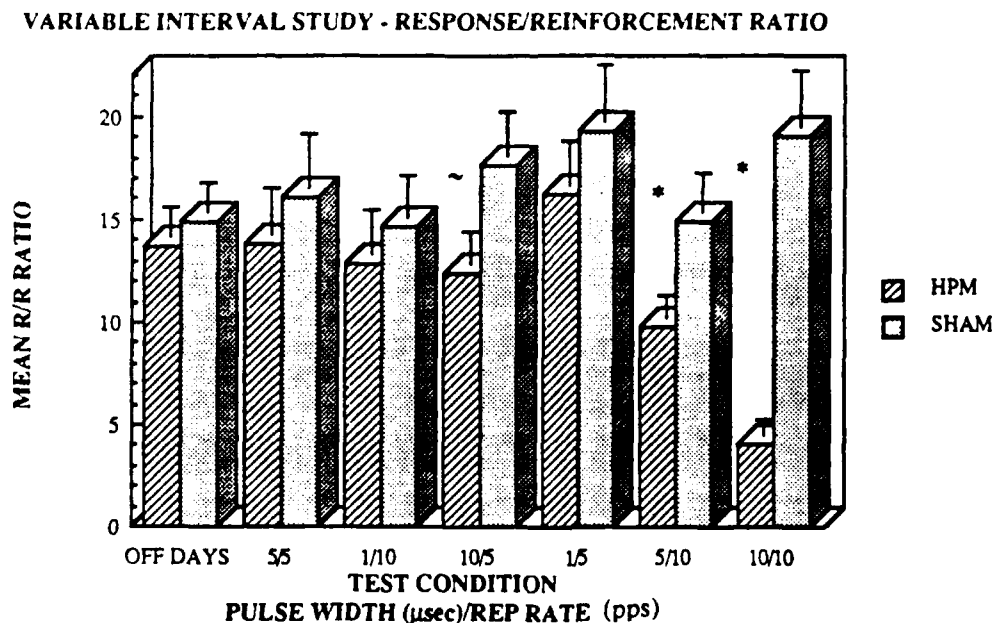


Figure 8. Mean ($\pm$ SEM) response to reinforcement ratio on a VI schedule by groups of HPM- and sham-irradiated rats. Test conditions on the abscissa describe test treatments on different days of the experiment. OFF DAYS = Combined data from the day preceding the onset of HPM testing and the days between each HPM test. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$; ~ = $0.05 < p < 0.06$.

Analysis of response data from the six 5-min test intervals constituting each 30-min VI session indicates no significant differences between experimental and control groups during training. Table 2 (Appendix) depicts the effects of the various HPM-irradiation protocols on VI responding by 5-min intervals during the 30-min session. Irradiation under the 5 μ s/10 pps, 10 μ s/5 pps and 10 μ s/10 pps protocols caused significant decreases in responding during the initial 10-15 min of testing. Only irradiation under the 10 μ s/10 pps protocol caused a decrease in responding over the entire test session. There were no residual effects of HPM irradiation as evidenced by the return of response rate to control values on the Retest day.

There were no significant differences between the HPM- and SH-irradiated groups in body weight or preirradiation colonic temperature on any day of training or HPM testing. The HPM irradiation produced significantly higher increase in colonic temperature (Fig. 9) [$F(1,16) = 30.4$, $p < 0.001$] and irradiation protocols resulting in higher power densities caused greater increases [$F(5,80) = 35.9$, $p < 0.001$]. The HPM-irradiated group had greater increase in colonic temperatures following irradiation under the 10 μ s/5 pps, 5 μ s/10 pps, and 10 μ s/10 pps irradiation protocols ($p < 0.01$).

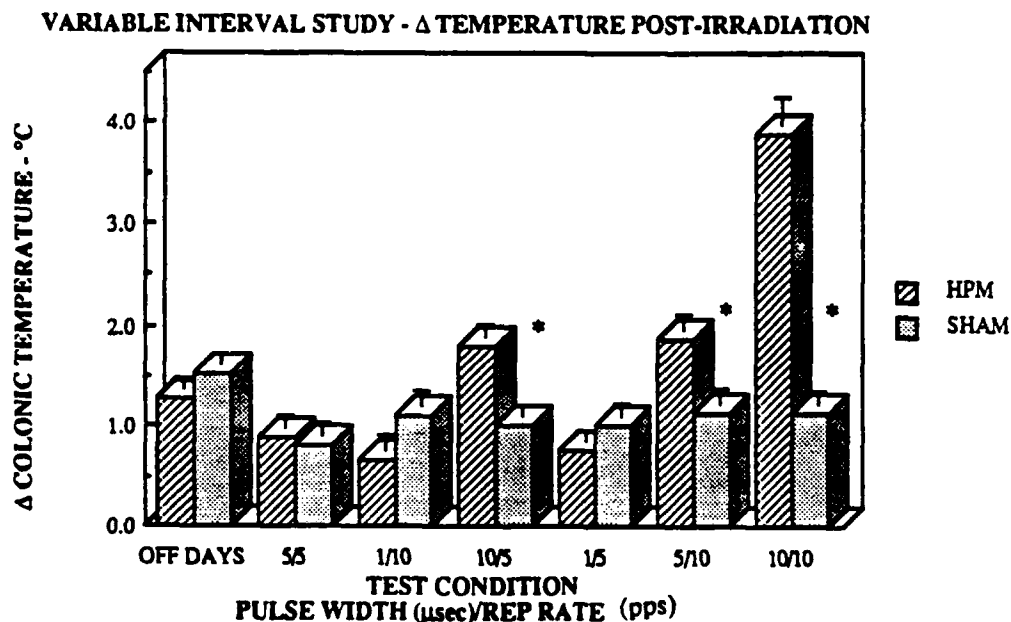


Figure 9. Mean ($\pm$ SEM) postirradiation change colonic temperature ($^{\circ}$ C) of groups of HPM- and sham-irradiated rats used in the VI experiment. Test conditions on the abscissa describe test treatments on different days of the experiment. OFF DAYS = Combined data from the day preceding testing and the days between each HPM test. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$.

Interesting, all HPM and SH groups except the 10 μ s/10 pps HPM group continued to show elevated colonic temperatures following behavioral testing (Fig. 10). The high-power-density 10 μ s/10 pps group failed to show temperature elevation [$F(1,16) = 10.3$, $p < 0.001$]. While the effect of irradiation condition (HPM or SH) alone was not significant ($p = 0.06$), there was a significant irradiation condition (HPM or SH) by irradiation protocol interaction [$F(5,80) = 5.6$, $p < 0.001$] indicating a more rapid decline toward baseline in postbehavioral testing change in colonic temperature in the 10 μ s/10 pps HPM group relative to the SH group.

VARIABLE INTERVAL STUDY - POST-BEHAVIORAL TESTING Δ TEMPERATURE

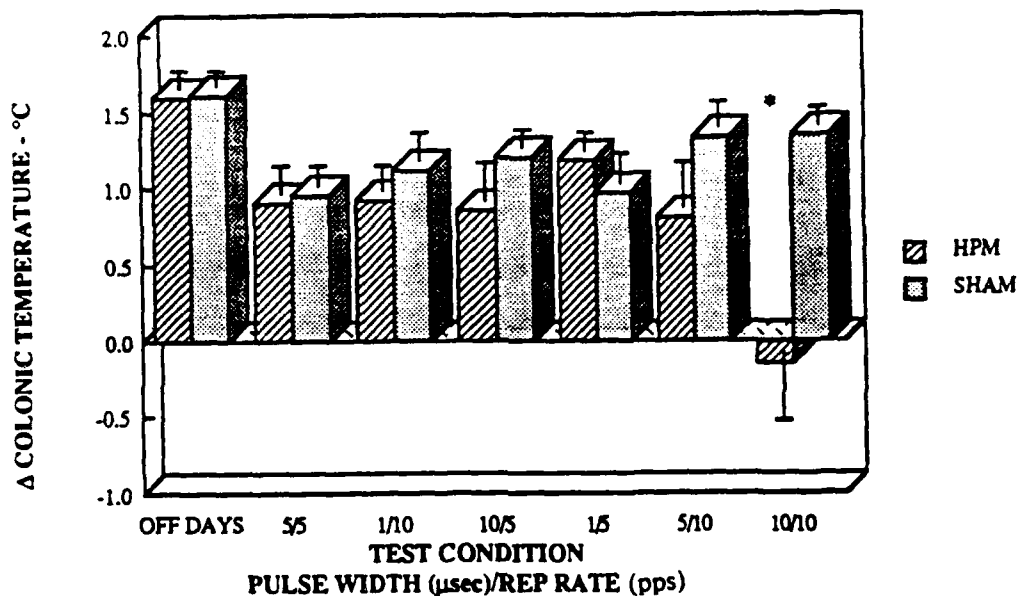


Figure 10. Mean ($\pm$ SEM) Change colonic temperature ($^{\circ}$ C) from preirradiation to postbehavioral testing of HPM- and sham-irradiated groups of rats used in the VI experiment. Test treatment conditions on the abscissa describe test treatment on different days of the experiment. OFF DAYS = Combined data from the day preceding testing and the days between each HPM test. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$.

Discrete-Trial Avoidance Behavior

There were no significant differences between the HPM and SH irradiation in the acquisition of avoidance responding. Despite significant increases in postirradiation colonic temperature among the HPM group under several irradiation protocols (*vide infra*), there were no significant differences between the groups on any test day in the number of avoidance responses, escape responses, number of full shocks received or intertrial interval responses made. There were no differences between the HPM- and SH-irradiated groups on any test day in either avoidance or escape latency. Further, analyses of all key variables by 5-min intervals during the 30-min test period indicate no differences on any variable on any day of testing.

There was no difference in body weight between the HPM experimental and SH control group during either training or HPM testing. Preirradiation colonic temperatures did not differ during training; but the SH-irradiated group had a significantly higher than normal preirradiation colonic temperature before SH irradiation under the 10 μ s/10 pps protocol (SAR = 26.2 W/kg) [$F(1,15) = 9.2$, $p < 0.01$]. Postirradiation change in colonic temperature (Fig. 11) of HPM-irradiated animals was significantly higher following irradiation under the 10 μ s/5 pps, 5 μ s/10 pps and 10 μ s/10 pps protocols ($p < 0.01$), and 5 μ s/5 pps protocol ($p < 0.05$). There were no differences between the groups in postbehavioral testing change in colonic temperature.

DISCRETE TRIAL AVOIDANCE STUDY - Δ TEMPERATURE POST-IRRADIATION

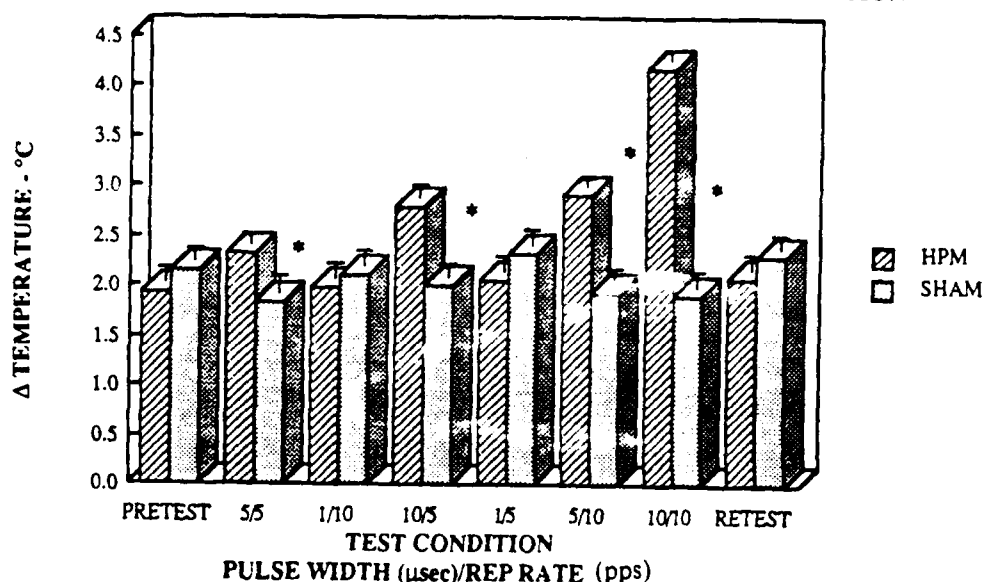


Figure 11. Mean ($\pm$ SEM) postirradiation change colonic temperature ($^{\circ}$ C) of groups of HPM- and sham-irradiated rats used in the discrete-trial avoidance study. Test conditions on the abscissa describe test treatments on different days of the experiment. PRETEST = Baseline test before the start of the experiment; RETEST = Retest day to reestablish baseline performance. Numerical values represent the microwave pulse width (μ s) and pulse per second (pps) protocol used to test for HPM effects. * = $p < 0.05$.

Passive-Avoidance Memory Testing

On Day 1 there was no significant difference between HPM- and SH-irradiated groups in latency to enter the darkened side of the 2-compartment avoidance chamber before shock (Fig. 12). When retested on Day 2, rats that had been irradiated on Day 1 had a significantly [$t(16 \text{ df}) = 3.13$, $p < 0.01$] shorter latency to reenter the darkened (shocked) side of the avoidance chamber than controls (Fig. 12). None of the SH-irradiated rats reentered the darkened side during the 120 s allowed while 5 of the 9 irradiated rats reentered. The proportion of HPM animals reentering the darkened side was significantly greater than that of SH animals ($Z = 2.63$, $p < 0.05$). Rats irradiated for 10 min under the 10 μ s/10 pps protocol had a significantly [$t(16 \text{ df}) = 11.6$, $p < 0.01$] greater increase in postirradiation colonic temperature ($+4.4 \pm 0.2^{\circ}$ C) than SH-irradiated controls ($+1.3 \pm 0.2^{\circ}$ C).

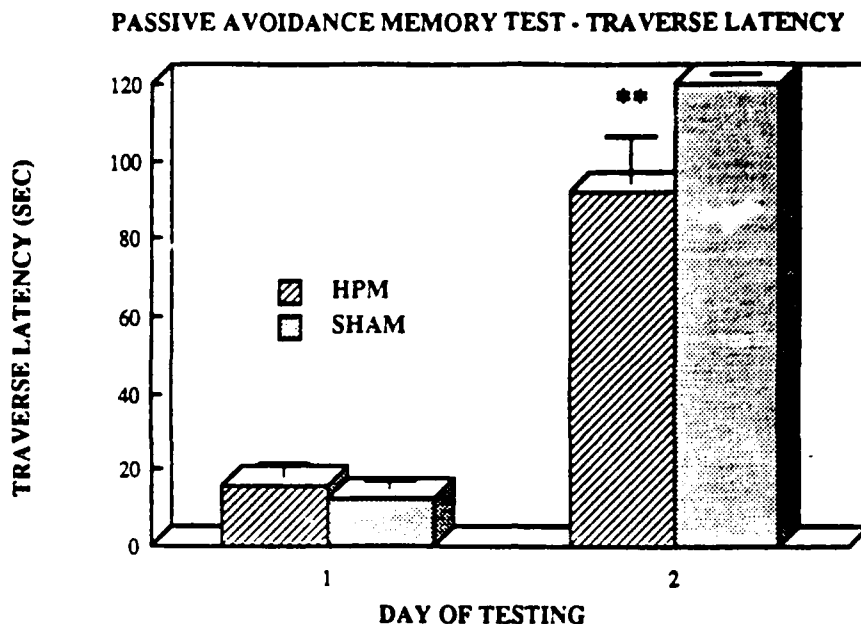


Figure 12. Mean ($\pm$ SEM) traverse latency (sec) of groups of HPM- and sham-irradiated rats in the passive avoidance memory experiment. Following a traverse on Day 1, rats were given a footshock in the dark side of the avoidance chamber. Rats were retested for traverse latency on Day 2. ** = $p < 0.01$.

Passive-Place Avoidance: Experiment 1

There were no significant differences between the test groups in body weight, preirradiation or postbehavioral testing colonic temperature. On Day 1 there was no difference between groups in latency to enter the darkened side of the chamber (Fig. 13). The latency to traverse to the darkened side on the 2d day was significantly higher for the HPM- than the SH-irradiated group (Fig. 13) [$F(1,16) = 10.1$, $p < 0.01$]. The HPM irradiation caused a significantly [$F(1,16) = 81.1$, $p < 0.001$] greater increase in postirradiation colonic temperature ($+2.5 \pm 0.1^\circ\text{C}$) than did SH irradiation ($+0.9 \pm 0.1^\circ\text{C}$).

Passive-Place Avoidance: Experiment 2

There were no significant differences between the SH 1-min and 5 min groups on any variable and their results have been combined for simplicity and to increase statistical power due to the small number of animals tested. On Day 1 there were no differences between SH and HPM groups in body weight or preirradiation colonic temperature. Latency to enter the darkened side on Day 1 was equivalent for all groups (Fig. 14). Postirradiation change in colonic temperature was significantly higher in the HPM 5-min group ($+2.6 \pm 0.1^\circ\text{C}$) than either the SH ($+0.9 \pm 0.1^\circ\text{C}$) or the HPM 1 min ($+0.9 \pm 0.1^\circ\text{C}$) which did not differ from each other [$F(2,15) = 32.6$, $p < 0.01$]. Latency to enter the darkened side on Day 2 was significantly higher in HPM groups than SH-irradiated controls [$F(2,15) = 3.8$, $p < 0.05$]. There were no differences between groups on any temperature measure on Day 2.

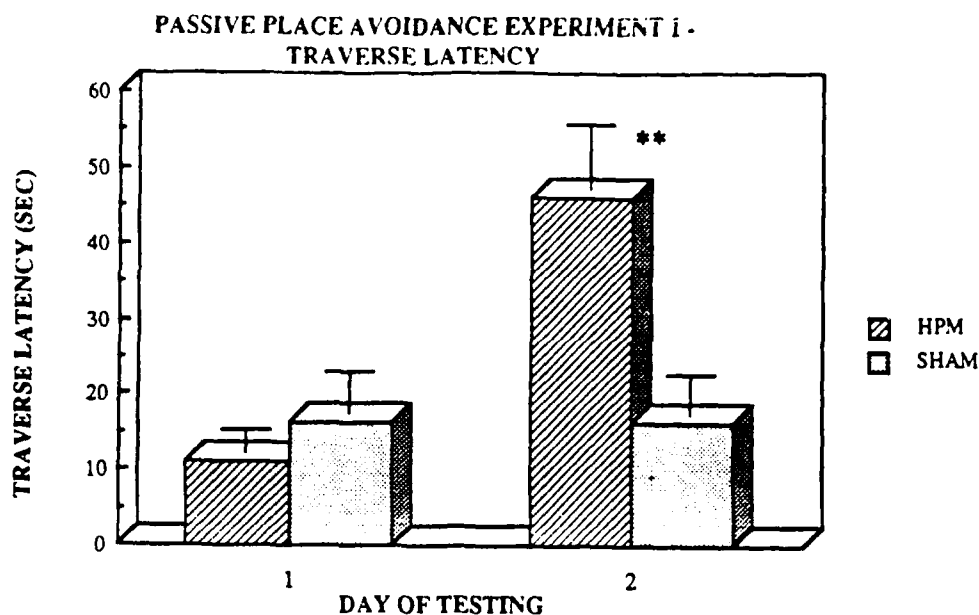


Figure 13. Mean ($\pm$ SEM) traverse latency (sec) of groups of HPM- and sham-irradiated rats in passive avoidance aversion Experiment 1. Following a traverse on Day 1, rats were HPM- or sham-irradiated in the dark side of the avoidance chamber. Rats were retested for traverse latency on Day 2. ** = $p < 0.01$.

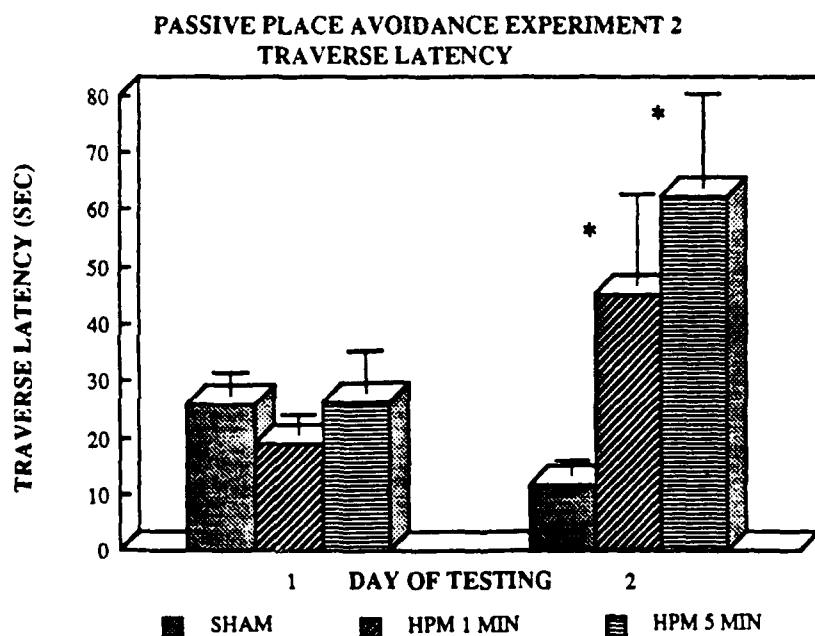


Figure 14. Mean ($\pm$ SEM) traverse latency (sec) of groups of rats that were HPM-irradiated for 1 (HPM 1 MIN) or 5 (HPM 5 MIN) or sham-irradiated in passive avoidance aversion Experiment 2. Following a traverse on Day 1, rats were HPM- or sham-irradiated in the dark side of the avoidance chamber. Rats were retested for traverse latency on Day 2. * = $p < 0.05$.

DISCUSSION

Locomotor Activity

Locomotor activity was significantly reduced following HPM irradiation under a 10 μ s/10 pps protocol (SAR = 26.2 W/kg, average-power density = 180 mW/cm²). The locomotor activity of rats in the HPM group on the Retest day (Fig. 2) indicates that there were no persistent cumulative effects of HPM irradiation on baseline activity rate. The results of this locomotor activity experiment contrast in some ways with the results of our earlier study (7). First, the threshold for suppression of locomotor activity was somewhat higher than that seen in our initial locomotor test (7) where significant suppression was noted under the 10 μ s/5 pps condition (power density = 90 mW/cm², SAR = 13.1 W/kg). The higher threshold in the present experiment was confirmed by the lack of suppression under the 5 μ s/10 pps condition which yielded a power density identical to the 10 μ s/5 pps condition.

One issue raised by our earlier locomotor activity study was the possibility of a testing order effect due to the increasing power levels of the successive HPM-irradiation conditions. When the test order used in the earlier study was reversed in the present experiment, producing decreasing power levels with each test, no effect of HPM irradiation was seen, suggesting that our original results could be attributable to a testing order effect. Second, the preirradiation colonic temperatures of rats in the SH group were consistently higher than that of the HPM-irradiated group and above normal baseline values for Long-Evans rats. In retrospect, there is a possible explanation for this difference. Due to space limitations in the PTF, the rats were housed in pairs with one HPM and 1 SH animal per cage. Just before the 1st HPM-irradiation session, a coin toss determined that the HPM animal in each pair would be the first to be tested. The act of opening the cage and removing the HPM animal was apparently sufficient to excite the SH animal, resulting in an increase in preirradiation colonic temperature when the SH animal was removed 10 min later. Test procedures have been revised to avoid this problem in future experiments.

One other key difference between the 2 experiments was the ambient environmental conditions in the PTF due to the time of year the different studies were conducted. The initial experiment was conducted in late July and early August of 1987; a typically wet but warm period in Los Alamos. Mean relative humidity was $51.1 \pm 0.2\%$ and mean PTF anechoic chamber temperature was $23.7 \pm 0.2^\circ\text{C}$. The present experiment was conducted during February of 1988, a dry period with mean relative humidity of $21.2 \pm 0.1\%$ and mean PTF anechoic chamber temperature of $21.1 \pm 0.1^\circ\text{C}$. Possibly the higher temperature and humidity during the 1st locomotor activity experiment were contributing factors to the apparently lower threshold. Such a difference would support the conclusion that the changes seen in behavior during both experiments are attributable to thermal factors. However, direct comparisons of temperature and humidity factors between the 2 experiments are difficult because of the initial differences in colonic temperature between groups in the present experiment.

While the threshold for suppression of locomotor activity was higher in the present experiment, the conclusion that the behavioral disruption was attributable to the thermal contribution of HPM irradiation remains valid since decreased locomotor activity was accompanied by increased colonic temperature. It is also clear from both experiments that an abrupt threshold for suppression of locomotor activity exists.

Variable-Interval Behavior

Responding on the VI schedule was significantly reduced for 30 min following HPM irradiation under a 10 μ s/10 pps protocol (power density = 180 mW/cm²; SAR = 26.2 W/kg). The HPM irradiation under 10 μ s/5 pps and 5 μ s/10 pps protocols (power density = 90 mW/cm²; SAR = 13.1 W/kg) caused reductions in responding for shorter periods (Table 2, Appendix). In this case, increased disruption by higher SAR irradiation protocols suggests a power-dependent relationship of HPM.

Despite some minor differences, the results of the present VI behavior experiment closely duplicate those of our original experiment (7). In the original experiment, responses and reinforcements significantly decreased in the HPM group following irradiation under the 10 μ s/5 pps protocol (power density = 90 mW/cm²; SAR = 13.1 W/kg), thus providing an apparent threshold for this effect. While suppression of responding for the entire 30-min session following 10 μ s/5 pps and the equivalent 5 μ s/10 pps exposures in the present experiment were statistically marginal (Fig. 6), the 5-min interval data for the first 15 min of VI testing demonstrates clear behavioral suppression (Table 2). The nearly identical effect of 2 different pulse width and PRF protocols with equivalent energy deposition allows a high degree of confidence that a threshold for behavioral disruption exists very near these values. One reason for the slightly higher threshold for behavioral suppression in this experiment compared to our earlier experiment (7) may be a difference in preirradiation training. In our initial test using VI behavior, animals had a total of 30 days of training while in the 2d series rats were trained for 46 days. While the experiments were designed to be replicates, unavoidable delays forced the postponement of testing beyond the originally scheduled date. Furthermore, differences between the 2 experiments in relative humidity and temperature (as discussed for the locomotor activity test results) may also have contributed to the slight variability in results between tests.

The results also indicate that the increased energy deposition of the 10 μ s/10 pps protocol (power density = 180 mW/cm²; SAR = 26.2 W/kg) caused a greater suppression of behavior. This suppression was apparent during all six 5-min intervals of the VI testing. The effect of the various irradiation protocols was also apparent in the response to reinforcement ratio (Fig. 8). This result confirms that rats irradiated under the highest energy deposition protocols were responding less often than SH-irradiated controls, but that reinforcement rate was relatively stable until the highest power protocol was tested.

A postbehavioral testing decline in change of colonic temperature was seen in HPM animals after irradiation under the 10 μ s/10 pps protocol, and there was a significant interaction between irradiation condition (HPM or SH)

and irradiation protocol indicating a power-density-dependent decline in colonic temperature (*vide infra*).

Discrete-Trial Avoidance Behavior

Despite HPM-irradiation-induced increases of colonic temperature, none of the HPM-irradiation protocols tested resulted in disruption of any measure of discrete-trial avoidance behavior. These results closely duplicate those of an earlier discrete-trial avoidance behavior experiment (7). First, significant changes in colonic temperature following irradiation were noted under the irradiation protocols common to both experiments (i.e., 5 μ s/5 pps and 10 μ s/5 pps) and under the 5 μ s/10 pps and 10 μ s/10 pps protocols used only in the 2d experiment. Second, no changes in avoidance performance were noted in either experiment regardless of the irradiation protocol used. No significant difference between HPM- and SH-irradiated groups was noted in the number of full shocks received in the present experiment indicating that differences in number of full shocks received in our earlier experiment (7) were probably not related to HPM irradiation.

The results of this experiment also extend the results of the original experiment. First, the 10 μ s/10 pps protocol resulted in energy deposition twice that of the original experiment without disrupting avoidance behavior. Second, the analysis of 5-min interval data indicates that behavior is not disrupted during the period immediately following irradiation when increases in colonic temperature were presumably greatest. Thus, this aversively motivated behavior appears extremely resistant to perturbation by HPM irradiation. These findings suggest that the ability of HPM irradiation to disrupt behavior depends, in part, on the behavior being examined.

Passive-Avoidance Memory Test

The results indicate that a threshold for disruption of memory processing occurs at HPM power densities somewhere between the 90 mW/cm² (SAR = 13.1 W/kg) produced under the 10 μ s/5 pps/10 min protocol (7) and the 180 mW/cm² (SAR = 26.2 W/kg) produced under the 10 μ s/10 pps/10 min protocol in the present experiments. In our initial experiments using the 10 μ s/5 pps/10 min protocol (7), only 1 animal of the 11 tested returned to the previously shocked side of the avoidance chamber. However, when the shock stimulus was followed by HPM irradiation in the present experiment, 5 of 9 irradiated rats demonstrated avoidance failures upon retesting. In similar experiments, where other environmental insults have followed the shock stimulus, such avoidance failures or reduced return latencies have been interpreted to be indicative of deficits in memory processing (12-16).

Passive-Place-Avoidance Studies

The results of the passive-place-avoidance paradigm indicate a significant increase in avoidance of the side of the 2-compartment avoidance chamber associated with HPM irradiation as evidenced by an increased return latency. In experiment 1, irradiation in the darkened side under the 10 μ s/5 pps/10-min protocol was sufficient to elicit this aversion. Recall that the average power density of 90 mW/cm² (SAR = 13.1 W/kg) yielded by this protocol is at or near the threshold required to disrupt VI responding. In Experiment 2, increased avoidance was apparent following irradiation for 1 min or 5 min

at a high-peak-power density of 9.0 kW/cm^2 . That no increase in colonic temperature was detected following 1-min exposure to 9.0 kW/cm^2 peak-power density, 108 mW average-power density microwave irradiation is not unexpected. Since dosimetry measures showed a 0.5°C colonic temperature rise at 1 min with 180 mW/cm^2 average-power density, a lesser temperature increase would be predicted at 108 mW/cm^2 .

Thus, the aversion noted in this experiment supports the findings of Klauenberg *et al.* (6), who reported behavioral disruption of rotarod task and HPM-induced startle responses in rats during exposure to 0.5 kW/cm^2 , 1.11-1.26 pps, $85 \mu\text{s}$ pulsed HPM. The total energy load produced by ten 0.5 kW/cm^2 pulses, in their experiment was significantly less than that produced by 9.0 kW/cm^2 for 1 min; suggesting that there was no colonic temperature increase in that study either.

GENERAL DISCUSSION

Current occupational exposure standards for microwave irradiation give no guidance on possible effects of HPM irradiation. The present experiments are intended to provide preliminary data for eventual safety standard formulation. Testing was conducted to determine if HPM irradiation alters behavioral measures of performance, memory and passive-place-avoidance formation. The testing was designed to determine power-density and SAR thresholds for possible effects of irradiation in a mammalian model and to study the interaction of pulse width and PRF with possible effects.

The results of the behavioral experiments conducted with a peak-power density of 1.8 kW/cm^2 indicate a threshold for disruption of behavioral performance corresponding to an average-power density of 90 mW/cm^2 , an average colonic SAR of 13.1 W/kg and a peak colonic SAR of 52.0 mW/kg . Under these conditions, there was a significant rise in colonic temperature in all experiments. Behavioral disruption was most apparent immediately following irradiation when colonic temperature was presumably still elevated. No significant differences between HPM- and SH-irradiated groups in VI performance were noted in the absence of a significant increase in colonic temperature.

The results are consistent with the reports of De Lorge (28) indicating deficits in VI performance directly related to increases in SAR and with the report (4) that exposure to high peak-power 5.6 GHz HPM at SARs from 0.2 to 4.41 W/kg did not disrupt the operant performance of rhesus monkeys. Based on this evidence, we suggest that the majority of the behavioral effects observed in these experiments were attributable to the thermal effects of irradiation. There is, however, one exception to this conclusion. Rats in the passive-place-avoidance experiment exposed to a peak-power density of 9.0 kW/cm^2 ($12 \mu\text{s}$ pulse width, 1 pps) showed significantly greater avoidance latencies following 1 min of irradiation. No increase in colonic temperature relative to controls was noted under these conditions. Thus, the possibility arises that HPM irradiation at higher peak powers may be aversive in the absence of thermal interactions. However, it is important to note that under the extreme near-field irradiation conditions used in this experiment, it is possible to cause localized heating in body regions not sampled by our colonic temperature

techniques. Further, this small thermal input would likely be compensated for in the living animal. The extreme near-field conditions used also prevented us from estimating SAR values for this experiment. Further experiments are planned to determine SAR values for the near-field region and to study the issue of localized heating.

Results of the discrete-trial avoidance experiment indicate that the levels of irradiation used were not sufficient to significantly disrupt highly motivated avoidance behavior even during the initial 5 min of the test period when colonic temperature elevations were greatest. While it is possible that the HPM irradiation affected only systems mediating appetitive behavior, the high strength of conditioning achieved in the avoidance paradigm must be considered. That is, the shuttle-avoidance paradigm may result in conditioning too strong to be affected by HPM irradiation and may be too insensitive to behavioral disruption to be used as a screening test. However, the absence of an effect of HPM irradiation on avoidance behavior suggests that motivational factors may serve to ameliorate behavioral disruption.

One interesting outcome of the VI experiment (Fig. 10) was the rapid recovery to preirradiation baseline colonic temperatures among the HPM-irradiated animals in the 10 μ s/10 pps group following behavioral testing. One explanation for the relatively rapid postbehavioral testing decline from the elevated postirradiation colonic temperature in the 10 μ s/10 pps group may be a compensatory response to the thermal consequences of irradiation. Similar differences between HPM- and SH-irradiated groups in postbehavioral testing colonic temperature were noted in the locomotor, as well as earlier experiments (7) suggesting a general HPM effect on thermoregulatory processes. Such an effect is consistent with known effects of microwaves on thermoregulation and metabolism (see reference 29 for review).

We suggest that the most important experimental variable affecting behavioral performance in the present experiments was the power density of the HPM irradiation, since PRF appeared to have little or no effect on experimental outcome. Where pulse width and PRF conditions yielded equivalent power densities (i.e., 5 μ s/10 pps and 10 μ s/5 pps) virtually identical effects on colonic temperature and behavior were observed. However, in most experiments PRF and pulse width were not independently controlled. Thus, additional experiments will be required to determine whether this relationship will continue as higher peak-power and lower pulse-width protocols are tested.

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APPENDIX

TABLES 1 AND 2

TABLE 1

CALCULATED AVERAGE SAR VALUES (All Values in W/kg)						
	<u>1 μsec pulse</u>		<u>5 μsec pulse</u>		<u>10 μsec pulse</u>	
	<u>5 Hz</u>	<u>10 Hz</u>	<u>5 Hz</u>	<u>10 Hz</u>	<u>5 Hz</u>	<u>10 Hz</u>
	1.3	2.6	6.5	13.1	13.1	26.2
	3.1	6.2	15.4	30.9	30.9	61.8
	0.8	1.6	4.4	8.8	8.8	17.6
Colonic SAR						
Brain SAR						
Skin SAR						
CALCULATED PEAK SAR VALUES (All Values in MW/kg)						
	<u>1 μsec pulse</u>		<u>5 μsec pulse</u>		<u>10 μsec pulse</u>	
	<u>5 Hz</u>	<u>10 Hz</u>	<u>5 Hz</u>	<u>10 Hz</u>	<u>5 Hz</u>	<u>10 Hz</u>
	5.2	10.4	26.0	52.0	52.4	104.8
	12.4	24.8	61.8	123.6	123.6	247.2
	3.5	7.0	17.4	34.8	34.8	69.6
Colonic SAR						
Brain SAR						
Skin SAR						

TABLE 2

**EFFECT OF DIFFERENT HPM IRRADIATION PROTOCOLS ON NUMBER OF
RESPONSES MADE DURING 5-MIN INTERVALS OF VARIABLE INTERVAL TESTING**

Interval	IRRADIATION PROTOCOL TESTED						
	<u>1/5</u>	<u>1/10</u>	<u>5/5</u>	<u>5/10</u>	<u>10/5</u>	<u>10/10</u>	<u>Retest</u>
0-5 MIN	Ø	Ø	Ø	▼	▼	▼	Ø
6-10 MIN	Ø	Ø	Ø	▼	▼	▼	Ø
11-15 MIN	Ø	Ø	Ø	Ø	▼	▼	Ø
16-20 MIN	Ø	Ø	Ø	Ø	Ø	▼	Ø
21-25 MIN	Ø	Ø	Ø	Ø	Ø	▼	Ø
26-30 MIN	Ø	Ø	Ø	Ø	Ø	▼	Ø

Ø - No statistically significant difference between HPM and sham-irradiated groups in the number of responses made during test interval.

▼ - Statistically significant decrease in number of responses made by HPM-irradiated group ($p < 0.05$)