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LACK OF BEHAVIORAL EFFECTS OF HIGH-PEAK-POWER MICROWAVE PULSES FROM AN AXIALLY EXTRACTED VIRTUAL CATHODE OSCILLATOR

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NOTICES

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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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A battery of behavioral experiments was conducted to evaluate possible biological effects of high-power microwave (HPM) pulses generated by the Transformer Energized Megavolt Pulsed Output (TEMPO) microwave emitter, an axially extracted Virtual Cathode Oscillator (VIRCATOR). Free space electromagnetic energy in the frequency range 2.01 to 2.57 GHz (mean frequency = 2.11 ± 0.09 SD) was radiated by conical horn in a TM01 mode. The overall pulse width was approximately 85 ns, with maximum power densities ranging as high as 24.11 kW/cm^2 per pulse (mean peak-power density = 10.79 kW/cm^2 ± 0.35 SEM).

Exposure to HPM pulses immediately before a test session as well as at 3 intervals during a 30-min test session had no effects on an appetitively motivated variable interval 10-s (VI-10) operant task. Postirradiation effects on aversively motivated behavior were determined with a discrete-trial avoidance behavior paradigm.

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19. ABSTRACT (Continued)

Postirradiation effects on memory processing were determined using a passive-avoidance task. The aversive properties of TEMPO HPM pulses were determined with a passive-place-avoidance procedure. The results indicate no deleterious effects of TEMPO HPM irradiation after up to 50-HPM pulses presented over a 5-min period. The results suggest that peak power is not as important as average power in causing deleterious biological effects.

TABLE OF CONTENTS

List of Figures

LACK OF BEHAVIORAL EFFECTS OF HIGH-PEAK-POWER MICROWAVE PULSES FROM AN AXIALLY EXTRACTED VIRTUAL CATHODE OSCILLATOR

INTRODUCTION

The development of high-power microwave (HPM) generators capable of producing extremely high-peak-power pulses for defense, commercial, and biomedical applications (1,2) has raised concern for the safety of the personnel operating such equipment. Occupational safety standards for radiofrequency exposure are based on the average-power density of irradiation and may lack applicability in situations where peak-power density is quite high while average-power density is relatively low.

While some biological effects have been attributed to peak-power conditions in earlier reports (3,4), it is only with the development of HPM sources with higher peak powers that attention has focused on this issue. D'Andrea et al. (5) found no deleterious effects of 5.6-GHz HPM pulses [0.8 μs pulses, 400-kW peak power, specific absorption rate (SAR) = 0.20 to 4.41 W/g] on the behavior of rhesus monkeys under 3 components of an operant schedule. In another study, using Long-Evans rats trained to perform a time-related observing task, D'Andrea and Cobb (6) reported exposure in a waveguide (1.3 GHz, 10 pps, 3 μs pulse duration) caused significant reduction in response rates and significant increases in reaction times at peak powers of 496.7 kW and 336.7 kW but not 146.7 kW. Wachtel and colleagues (7,8) reported that irradiation of mice (1.25 GHz, SAR = 20 MW/kg, 10 μs pulses, peak-power density = 20 kW/cm^2) caused behavioral effects ranging from induced reflexive movements to decreased locomotor activity to death depending on the number of pulses delivered. Two series of experiments examining possible behavioral, thermoregulatory and biochemical effects of HPM have been conducted using a 1.30-GHz HPM source at the High-Power Microwave Laboratory of Los Alamos National Laboratory (LANL), Los Alamos, New Mexico (9,10). The experiments were designed to evaluate behavioral and thermoregulatory responses across a range of power density and SAR values, from nonthermal to clearly thermal. Several of the experiments were conducted using repeated testing, after exposure, 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 μs to 5 μs to 10 μs on different test days]. In those experiments significant suppression of operant responding and locomotor activity was noted, when tested after exposure, at an average-power density of 90 mW/cm^2 (peak-power density = 1.8 kW/cm^2, SAR = 13.1 W/kg). The behavioral changes observed were directly correlated with increased colonic temperature and thus directly related to SAR.

Virtually all of the experiments just described were conducted using high-power klystron-type microwave sources. To achieve higher peak powers with shorter pulse durations, virtual cathode oscillator (VIRCATOR) sources have been developed (2). Initial reports (11,12,13) from experiments using the Gypsy and the Transformer Energized Megavolt Pulsed Output (TEMPO) microwave sources at the Air Force Weapons Laboratory (AFWL) Electron Beam Facility at Kirtland Air Force Base, Albuquerque, New Mexico, indicated disruption of several behavioral tasks. Cordts et al. (11) reported exposure to a single 140-ns microwave pulse (1.64 GHz, 1-8 kW/cm^2) reduced drinking in a thirst satiation task, but did not affect performance of a rotarod or single-trial avoidance task. Klauenberg et al. (12) reported disruption of rotarod performance and the induction of a startle response in Fisher 344/N rats by a series of 10 HPM pulses (1.11 to 1.26 GHz, 85-ns pulses, 1 pps, 0.5 to 1.0 kW/cm^2 power density). However, Klauenberg et al. (13) later reported the effects on rotarod and startle observed in earlier tests (12) were absent when rats were housed in a sound-attenuating isolation chamber and exposed to pulsed TEMPO HPM at 2.45 GHz. Similarly, D'Andrea and his colleagues (14), using the same TEMPO system operating at a higher peak-power density (8.5 kW/cm^2) and higher frequency (2.3 GHz, 80-ns pulses), reported no effects on an appetitively motivated auditory frequency discrimination task in rhesus monkeys when tested in a sound-attenuating isolation chamber.

The purpose of the present experiments was to determine if performance of complex behaviors by rats could be disrupted by exposure to high-peak-power pulses produced by the TEMPO system.

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, rats for all experiments except those involving variable-interval (VI) behavior were housed 2 per cage in suspended polycarbonate cages (46 cm x 24 cm x 15 cm) with wood chip bedding, and were provided free access to water and Teklad 4% rat diet. Rats used in experiments involving VI behavior were singly housed and access to food was necessarily restricted. Animals were initially maintained at the Health Research Laboratory of the LANL Life Sciences Division. Before HPM testing, the animals were transported to a Portable Test Facility [PTF (19)] located at the AFWL facility. Ages and body weights of rats as well as environmental conditions for individual experiments are presented at the beginning of each experiment described later.

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 holding cage was modeled after the cage used by Popovic et al. (15) which forced the animals to remain parallel to the long axis of the cage, but provided sufficient space to minimize stress responses associated with restraint (16,17). During the training phase of all experiments requiring repeated testing, rats were acclimated to the holding cages for several days before HPM testing.

Temperature Measurement

A digital telethermometer (Bailey Instruments, Clifton, NJ, Model BAT-8) with a 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.

Computer Control Systems

All behavioral test systems were controlled by a MICRO/PDP 11/73 microcomputer (Digital Equipment Corporation, Westminster, MA) using a State Systems interface [Kalamazoo, MI;(18)]. Schedule contingencies were programmed and responses and reinforcers (for operant conditioning), as well as avoidance and escape responses, were recorded using a State Systems operating system. Specifications of the specific behavioral test apparatus used are provided in the section describing each experiment.

Statistical Methods

Single factor interactions were analyzed with 1-way analyses of variance (ANOVA) (19) with a posteriori contrasts by Duncan's and Scheffe's multiple range tests (20,21) or Student's t-tests. A nonparametric analysis of the number of animals scoring avoidance failures in passive-place-avoidance paradigms was conducted with the test for significant differences between two proportions (22). Multiple factor analysis was conducted using an n-way ANOVA and covariance program (23). Repeated measures were analyzed using the method of Winer (19). For both 1-way and repeated measures ANOVA, missing data were deleted from the analysis. Cases were omitted from the analysis if the values on any dependent variable were missing. The degrees of freedom reported for each statistical test were adjusted to reflect missing data. All values are reported as the mean $\pm$ the standard error of the mean (SEM).

Exposure Chamber and Electronics Housing

Due to noise associated with HPM pulse generation by the TEMPO source, animals were placed in an acoustically isolated exposure chamber (designed and constructed by the Radiation Sciences Division, USAFSAM) for HPM or sham irradiation. The exposure chamber (67.3 cm (h) x 73.4 cm (w) x 50 cm (d)) was constructed of Dow 5-cm Styrofoam (R value @ 24 °C = 10) and lined with 0.32-cm Plexiglas sheet. The interior of the box was illuminated with a 600 W projection lamp (General Electric Quartzline Model DYH) reduced by rheostat to 30% to 50% of possible total output.

The illumination lamp and all other electronic support equipment were contained in a sealed enclosure that was shielded from HPM irradiation and positioned directly above the exposure chamber. The shielded enclosure was constructed of 0.16-cm aluminum sheet with an 0.16-cm copper sheet wrap. The inner aluminum and copper enclosure was covered with 11-cm thick Eccosorb AN-79 anechoic material. The interior dimensions of the shielded enclosure were 61 cm (h) x 61 cm (w) x 38.7 cm (d).

A color charge-coupled device (CCD) television camera (Panasonic Digital Model 5000) was positioned in the shielded enclosure to permit observation of rats in the exposure chamber below. Also contained in the shielded enclosure were a random noise generator (General Radio Co, Model 1382) and a power amplifier (Eico, Model 3070) connected to a speaker (Realistic, 8 Ohm, 3 W, Model 12-1844). Speaker output was directed into the exposure chamber to mask the noise generated by the TEMPO pulser. The sound level output of the speaker was determined with a sound level meter (Thermo Systems Inc., Model 1680) with a range of 36 to 120 dB over a 31.5 to 8,000 Hz frequency range. The sound level inside the sealed lower exposure chamber during a series of TEMPO shots was determined to be 67 dB. The sound level produced by the noise generation system was then set to 73 dB. More tests indicated no increase in sound level due to TEMPO pulses was detectable with the random noise generator on. Also contained in the shielded enclosure was a pellet feeder, (Coulbourn Instruments, Lehigh Valley, PA., Model E14-12), which delivered a food pellet via 2.54 cm OD Tygon tubing to an operant test system in the exposure chamber.

High-Power Microwave Exposure System

The TEMPO pulser used for these experiments was an axially extracted VIRCATOR located at the Electron Beam Facility of the AFWL at Kirtland AFB, New Mexico (see reference 2 for a complete technical description). Free space electromagnetic energy in the frequency range 2.01 to 2.57 GHz (mean frequency = 2.11 $\pm$ 0.09 SD, 166 observations) was radiated by a 1.22 m (d) conical horn in a TM01 mode. The overall pulse length was approximately 85 ns, with peak-power densities ranging from 0 to 24.11 kW/cm^2 per pulse (mean peak-power density = 10.79 $\pm$ 0.35 (SEM) kW/cm^2,

median peak-power density = 10.36 kW/cm^2, 166 observations). Charge voltage was ± 18 kV; diode voltage was 0.6 to 1 MV; diode current was 50 kA. Animals were irradiated at a distance of 2.5 m from the horn at an angle of 10° west of the antenna center line. A diagnostic field probe was located 10° east of the center line at the same distance. The waveform emitted by the TM01 mode horn is toroidal and polarized with the electric field in the radial direction. A description of the waveform, average-power density and system diagnostics has been published elsewhere (2). Sham-irradiation sessions were conducted using procedures identical to those used during HPM testing but with an aluminum foil shield placed over the horn to reflect all radiation. Thus, noise and environmental conditions associated with HPM pulse generation were present during sham-irradiation sessions.

Electromagnetic-Pulse Interference

Initial behavioral tests using the TEMPO pulse were hampered by electromagnetic-pulse (EMP) interference emanating from the pulser. The EMP interference was determined to be the result of a high-power EMP generated by the TEMPO source. To minimize EMP interference, the computer, module rack and 2 operant and 2 discrete-trial avoidance systems were placed in the PTF anechoic chamber which is a doubly electrically shielded room (see reference 9 for details). The power was filtered through the chamber filter, a voltage conditioner (TOPAZ 02406-06Q3) and a strip-type spike suppresser (Tripp-Lite Model Sk6-6) before powering either the computer or the module rack. Cables to and from the AFWL anechoic chamber were disconnected immediately before each 10-pulse burst and then were grounded and reconnected to the computer system after the last pulse. No data collection was possible during this approximately 12-s period. These precautions allowed testing without EMP interference to the test systems.

Behavioral Observation

The behavior of each animal during HPM testing was recorded on videotape (JVC BR 3100U VHS recorder; Sony, VO-5600 recorder; Datavision Video Products, DT-1 Date/Time Generator) and observed (Sony PVM-8020 Video Monitor) in the PTF.

BEHAVIORAL AND PHYSIOLOGICAL EXPERIMENTS

Variable-Interval Behavior

Food-deprived rats were trained for 19 days (M-F) to press an operant lever for a food reinforcement, initially on a Fixed-Ratio schedule and ultimately on a VI schedule. The VI schedule training produced a high rate of operant responding with a very stable rate of reinforcement. Rats in this paradigm were tested under 2 different experimental protocols. In the first protocol, rats were tested immediately following irradiation with a 10-

pulse burst (1 pps) of HPM. In the second protocol, the same rats were irradiated with 10-pulse bursts at 3 different time points during a 30-min operant test session.

Subjects and Environmental Condition

N = 20 (10 HPM/10 Sham)
Age = 78 days on first day of training
Mean HPM group body weight = 201.6 ± 3.2 g
Mean sham group body weight = 203.4 ± 3.7 g
Mean PTF room temperature = 21.3 ± 0.3°C
Mean PTF relative humidity = 31.4 ± 0.9%

Behavioral Apparatus

Operant chambers (Coulbourn Instruments, Lehigh Valley, PA, Model 10-10) were housed 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 (BioServ, Inc., dustless precision pellets for rodents, 45 mg, product #0021; Coulbourn pellet feeder, Model E14-12) to a central delivery magazine.

"Infield" Operant Test System

Two Plexiglas operant chambers for use in electromagnetic fields were designed and constructed using the Coulbourn Model E10-10 operant chamber as a model (9). Each chamber measured 30.5 cm (h) x 30.0 cm (w) x 27.7 cm (d) (exterior). Walls were constructed of 0.64 cm Plexiglas and a grid floor was constructed of sixteen 27.0 x 0.64 cm diameter Lucite rods placed 1.9 cm apart. The front wall of each chamber served as a slide/drop door.

One end of each chamber housed a food cup and 2 operant levers. The food cup (2.7 cm (h) x 3.8 cm (d) Lucite tube) was cut on one side 0.95 cm from its center, a base attached, and was affixed to the end of the chamber midway between the levers and 0.64 cm above the floor. Operant levers, located 2.54 cm from the sides of the chamber and 4.45 cm above the floor, were constructed of 0.64-cm Lucite (3.16 w x 5.08 cm) with a 4.13 cm (h) x 4.00 cm (1) x 0.032 cm thick perpendicular black plastic blade affixed to the exterior portion of the lever. The interior portion of the lever extended 2.22 cm into the chamber and rotated through 30° arc when pressed with a force of 0.02 N, thus passing the opaque blade between fiberoptic cables mounted outside the chamber, interrupting a fiberoptic light beam. This signal was transmitted from the anechoic chamber by fiberoptic cable to a junction box outside the anechoic chamber where it was converted to an electric signal and transmitted to the computer using a Coulbourn photobeam detector Model S23-01. Reinforcement

output from the SKED-11 operating system was sent to a Coulbourn pellet feeder (Model E14-12) and a pellet reinforcement (BioServ, Inc., dustless precision pellets for rodents, 45 mg, product #0021) was delivered through a 1.59-cm diameter Tygon tube to the operant chamber.

Training Schedule

Initial training for this experiment was conducted in the PTF at LANL before animal transport to the test facilities at the AFWL. Animals were placed on restricted diets for 2 weeks before training and handled for 3 min daily for 3 days before the onset of training. Two test groups were assigned on the basis of equivalent body weight and colonic temperature prior to the onset of training. Animals were allowed free access to water, but were placed on a restricted diet (averaging 13 g of Teklad 4% rat diet per day following testing, depending on individual body weights). After training began, the supplement of the Teklad diet was diminished to an average of 5 g/day as reinforcements received during operant training increased. Four of the standard Coulbourn operant chambers and both of the "infield" operant chambers were used during training. The order of animal testing was rotated each day to ensure that all rats had experience in both operant systems. Although response rates were lower in the plastic system than in the standard system, reinforcement rates were within 10%.

Initially, (Day 1), 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 11 only the FR-1 component of this schedule was retained. On Day 12, the variable-interval 10-s (VI-10) schedule was initiated. This schedule resulted in a high response rate and a very stable reinforcement rate. Animals were trained in 3 groups of 6, with testing order rotated for each session. After training on Day 14, the rats were transported in an enclosed, temperature-controlled vehicle to the AFWL where they were again housed in the PTF which was moved on the same day. Beginning on Day 15, animals were placed in holding cages (see description) for 10 min each day, before each training session, to habituate them to eventual exposure conditions. Colonic temperatures were recorded before and after this habituation procedure. Daily operant training continued for 5 additional days before HPM testing began.

High-Power Microwave Test Procedures

The HPM testing using the TEMPO source was conducted on 2 consecutive days (Days 20 and 21). Immediately before HPM or sham irradiation, each animal's body weight and colonic temperature were recorded. Rats were placed in standard holding cages and carried to the exposure chamber within the AFWL

anechoic chamber. The exposure chamber was located approximately 35 m from the PTF test room. A warning was sounded after the anechoic chamber had been cleared of personnel indicating that the TEMPO would be fired in 3 min. Thus, actual irradiation of the animals (10 pulses @ 1 pps) occurred approximately 5 min after the initial colonic temperature measurements. Colonic temperature was recorded approximately 60 s after the cessation of irradiation, and again following the 30-min VI-10 operant session. Mean values for body weight, colonic temperature values, the number of lever press responses made, the number of food pellet reinforcements received, and the ratio between responses made and reinforcements received for the 2 test days were compared to the same measures for the 3 days preceding HPM testing (Days 17 - 19). On the 2 HPM test days, only the standard Coulbourn operant systems were used for testing.

Infield TEMPO testing occurred on Days 22 and 23, with one-half of the animals tested each day, using the infield operant test system (see previous description). Before HPM or sham irradiation, body weights and colonic temperatures were recorded and the animals were placed in standard holding cages for transport to the anechoic chamber. Rats were placed in the infield operant chamber and the door to the exposure chamber was sealed. The 30 min VI-10 session was divided into six 5-min bins and 10-pulse bursts (1 pps) were presented at the beginning of the 2nd, 4th and 6th bins. Due to EMP interference, recording and control cables were disconnected during pulse delivery and reconnected within 2 s of the cessation of irradiation. Final colonic temperatures were recorded after the test session.

Discrete-trial Avoidance Behavior

In this experiment, rats were trained to avoid an electric footshock. In previous experiments, this aversively motivated task has been well learned and has proven difficult to disrupt with HPM irradiation even at power-density values that have resulted in significant heating (9,10,32). The purpose of the present experiment was to determine if high-peak-power pulses of shorter duration than those used in previous studies would be effective in disrupting any facet of avoidance behavior.

Subjects and Environmental Conditions

N = 20 (10 HPM/10 Sham)
Mean age = 78 days on first day of training
Mean HPM group body weight = 324.0 ± 9.5 g
Mean sham group body weight = 313.0 ± 10.1 g
Mean PTF room temperature = 24.1 ± 1.2 °C
Mean PTF relative humidity = 31.4 ± 0.9%

Behavioral Apparatus

Model E-10-16 Coulbourn avoidance chambers were housed in Coulbourn isolation cubicles (40.6 cm (d) x 45.7 cm (h) x 55.9 cm (w) with ventilation fans and baffled air-intake and -exhaust systems. The toggle floor grid of each chamber was connected to a grid-floor shocker (Coulbourn Model E13-08), and a central aluminum divider allowed access between sides through a 6.4 cm 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 TEMPO HPM testing, animals received 19 training sessions (M-F), in the PTF under a 30-trials/session schedule. Animals were tested in 3 groups of 6, with the test order rotated each day. After the Day 14 training session, animals were transported in an enclosed, temperature-controlled vehicle to the AFWL, where they were again housed in the PTF. Beginning with the 15th training session, animals were put in holding cages for 10 min before testing each day (see previous description) to acclimate them to eventual exposure conditions. The acclimation procedure included colonic temperature measurements before and after the 10-min period in the PTF anechoic chamber and following discrete-trial avoidance testing.

The daily training procedure was as follows: after a variable interval from the start of each trial (VI-45 s) a tone was initiated. After 10 s, if the rat had not traversed to the opposite side, a scrambled footshock (0.9 mA, 5-s duration) was administered, while the tone continued. A traverse terminated both tone and shock. A traverse before shock onset was scored as an avoidance response. A traverse (escape) during the 5 s of shock was scored as a full shock. Traverses recorded between tone/shock periods were recorded as intertrial responses. The latencies of avoidance and escape responses were recorded.

High-Power Microwave Test Procedures

During the test period, discrete-trial avoidance testing continued under the same procedures as during training, with HPM or sham irradiation conducted immediately before behavioral testing. Before HPM or sham irradiation, each animal's body weight and colonic temperature were recorded. Rats were placed in standard holding cages and carried to the exposure chamber within the AFWL anechoic chamber. Animals were tested according to the procedures for the VI behavior experiment just described (10 pulses @ 1 pps). Colonic temperature was recorded approximately 60 s after the cessation of irradiation, and again following the 30-min discrete-trial avoidance test session.

Mean values for body weight, colonic temperature, the number of avoidance and escape responses made and their respective latencies, the number of full shocks received and the number of intertrial-interval responses made on the HPM test day were compared to the mean values for the same measures for the 3 days preceding HPM testing.

Passive-Avoidance Memory Testing

Possible HPM effects on memory processing were assessed with a passive-avoidance paradigm. Memory processing is susceptible to disruption for a period of up to 4 h after a learning trial or environmental event (24). A variety of environmental and pharmacological interventions effectively disrupt memory processing when presented following a training trial (retrograde amnesia); the earlier the intervention, the greater the degree of processing disruption (25-31). In a previous HPM experiment (32), a threshold level of irradiation for significant disruption of memory processing was determined. In that experiment a peak-power density of 1.8 kW/cm^2 was presented in 10-μs pulses at 10 pps (average-power density 180 mW/cm^2, SAR = 26.2W/kg) and significant increases in colonic temperature accompanied the disruption of memory processing. The purpose of the present experiments was to determine whether shorter pulses (85 ns) with higher peak-power density (10.79 kW/cm^2) would similarly disrupt memory processing. Rats were placed on the lighted side of a light/dark 2-compartment avoidance chamber and allowed to traverse to the darkened side. Once on the darkened side a footshock was delivered. Following footshock, the animals were immediately HPM or sham irradiated. In one experiment, rats were HPM or sham irradiated with a single burst of 10 pulses. In a second experiment, more rats were irradiated with 5 consecutive 10-pulse bursts for a total of 50 pulses delivered in less than 5 min. On the day following exposure, rats in both experiments were again placed in the lighted side of the 2-compartment avoidance chamber and their latency to return to the previously preferred, but now aversive, darkened side was determined. A short-return latency in this test is indicative of disruption of memory formation.

Subjects and Environmental Conditions

Experiment 1

N = 20 (10 HPM/10 Sham)
Mean age = 82 days on first day of testing
Mean HPM group body weight = 314.2 $\pm$ 35.5 g
Mean sham group body weight = 304.6 $\pm$ 41.9 g
Mean PTF room temperature = 21.7 $\pm$ 0.3°C
Mean PTF relative humidity = 40.4 $\pm$ 0.4%

Experiment 2

N = 10 (5 HPM/5 Sham)
Mean age = 105 days on the first day of testing
Mean HPM group body weight = 416.4 ± 10.5 g
Mean sham group body weight = 419.6 ± 22.9 g
Mean PTF room temperature = 19.6 °C
Mean PTF relative humidity = 37.6 ± 0.9%

Behavioral Apparatus

One Model E10-16 Coulbourn avoidance chamber, housed in a Coulbourn isolation cubicle (40.6 cm (d) x 45.7 cm (h) x 55.9 cm (w)) with ventilation fan and a baffled air-intake and -exhaust system, was used for both experiments. A central aluminum divider with a centered door opening (6.4 cm x 7.6 cm) was modified to accommodate a remotely operated aluminum guillotine door. A Coulbourn Instruments photodetector and photocell assembly (Models S23-01 and T22-01) was arranged such that the photobeam was interrupted when the door was fully raised. Shocks were administered by a Coulbourn grid-floor shocker (Model E13-08). 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).

High-Power Microwave Test Procedures

One day before testing, animals were divided into equivalent groups on the basis of body weights and colonic temperatures. On Day 1, alternately selecting rats from the sham and HPM groups, each animal was weighed, its colonic temperature recorded, and it was immediately placed in the lighted side of the 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 placed in a standard holding cage and carried to the exposure chamber within the AFWL anechoic chamber. Animals were individually HPM or sham irradiated, according to the procedures just described. The 2 experiments were conducted on different days. For Experiment 1 the rats were HPM or sham irradiated with a burst of 10 pulses (1 pps). For Experiment 2, five 10-pulse bursts were presented as rapidly as possible. The TEMPO capacitor banks were recharged between bursts with a mean interburst interval of 44.7 ± 3.3 s for the sham-irradiated group. Following HPM or sham irradiation a final colonic temperature was taken and the animal was returned to his home cage.

After 24 h, in the same order as they were tested on Day 1, animals in both experiments again were placed in the lighted side of the avoidance chamber. After 30 s the guillotine door was

opened, allowing access to the darkened side. Latency to re-enter the darkened side was recorded (maximum 120 s), but no shock was administered after entry.

Passive-Place-Avoidance Testing

The aversive qualities of high-peak-power microwaves were assessed with a passive-place-avoidance test. The theoretical foundation of this experiment is the formation of place aversions by rats (see reference (33) for review). Accordingly, rats will avoid any physical locale that has previously been paired with negative consequences, such as poisoning or footshock. Rats demonstrated a significant aversion to the side of the chamber associated with irradiation in a previous HPM experiment, where HPM irradiation was tested as an aversive stimulus [(32) 10 min of 1.3 GHz irradiation at a peak-power density of 1.8 kW/cm^2 under a 10 μs pulse width/5 pps protocol (average-power density = 90.0 mW/cm^2, SAR = 13.1 W/kg)].

The purpose of this experiment was to determine whether HPM pulses of higher peak-power density but shorter duration were aversive. In this experiment, rats were placed on the lighted side of a 2-compartment light/dark avoidance chamber in the exposure chamber within the AFWL anechoic chamber and allowed to traverse to the darkened side. The HPM or sham irradiation was presented immediately upon entry into the darkened side. The following day, rats were again placed in the lighted side of the avoidance chamber and their latency to reenter the darkened compartment was determined. In this task, a long return latency is interpreted as an aversion to HPM irradiation. Since rats form lasting aversions to the dark side, single trials were used rather than repeated testing.

Subjects and Environmental Conditions

N = 20 (10 HPM/10 Sham)
Mean age = 57 days on first day of testing
Mean HPM group body weight = 254.0 $\pm$ 1.6 g
Mean sham group body weight = 254.0 $\pm$ 2.7 g
Mean PTF room temperature = 22.3 $\pm$ 0.1 °C
Mean PTF relative humidity = 40.7 $\pm$ 0.3%

Behavioral Apparatus

A dimensionally correct replica of the Coulbourn avoidance chamber (Model E10-16) was constructed of 0.95 cm Plexiglas. This cage has been described in detail in an earlier publication (32). A center dividing wall with a 6.4 cm x 7.6 cm door opening was equipped with a pivoting drop door which was operated at a distance from the anechoic chamber and TEMPO building. One side of the avoidance chamber was darkened by black fabric, while the other (the starting side) remained illuminated. Entry into the darkened side of the avoidance chamber was visually confirmed by

the video camera mounted in the sealed enclosure located above the exposure chamber and entry latency was timed by 2 observers.

High-Power Microwave Test Procedures

One day before testing, animals were divided into equivalent groups on the basis of body weights and colonic temperatures. On Day 1 each rat was weighed, its colonic temperature was recorded, and it was placed in the starting side of the avoidance chamber. They remained on the starting side of the avoidance chamber for 3 min with the lights off to reduce heating in the exposure chamber, while the TEMPO recharged. Lights were turned on for 60 s and then the guillotine door was opened, allowing access to the darkened side of the avoidance chamber. Upon entry into the darkened side, the guillotine door was closed and HPM or sham irradiation was administered. Rats were individually irradiated with a burst of 10-HPM pulses presented at 1 pps. The animal was then removed from the chamber and its colonic temperature was recorded.

On the following day, in the same order as they were tested on Day 1, animals were placed in the starting side of the avoidance chamber, inside the microwave exposure chamber. After 2 min of darkness, lights were turned on for 60 s and then the guillotine door opened, allowing access to the darkened side. Latency to enter the darkened side was recorded, but no irradiation was administered following reentry.

RESULTS

Variable-Interval Behavior After High-Power Microwave Irradiation

There were no differences between the HPM- and sham-irradiated groups in body weight, preirradiation, postirradiation or postbehavioral testing colonic temperatures during the 3 days preceding HPM testing or on either of the HPM test days. The HPM irradiation caused no increase in colonic temperature relative to the sham-irradiated controls. There was a nonsignificant tendency toward an increased number of responses made by both groups on the HPM Test Days relative to the Pretest Days (Fig. 1)

A burst of 10-HPM pulses resulted in no significant differences between the HPM- and sham-irradiated groups in the number of responses made. There were no significant differences between the groups before testing and both groups showed an equivalent increase in responding on the HPM test days. Similar results were noted in the number of reinforcements received and the response to reinforcement ratios. There was a nonsignificant tendency toward increased reinforcements and response to reinforcement ratio in both groups on the HPM test days. There

were no statistically significant differences between the groups either before or following HPM testing. It is noteworthy that there was no increase in the variability of any measure during HPM testing compared to baseline conditions. Thus, no effects of HPM irradiation were noted on any of the thermoregulatory or operant-behavioral measures studied following a burst of 10-HPM pulses.

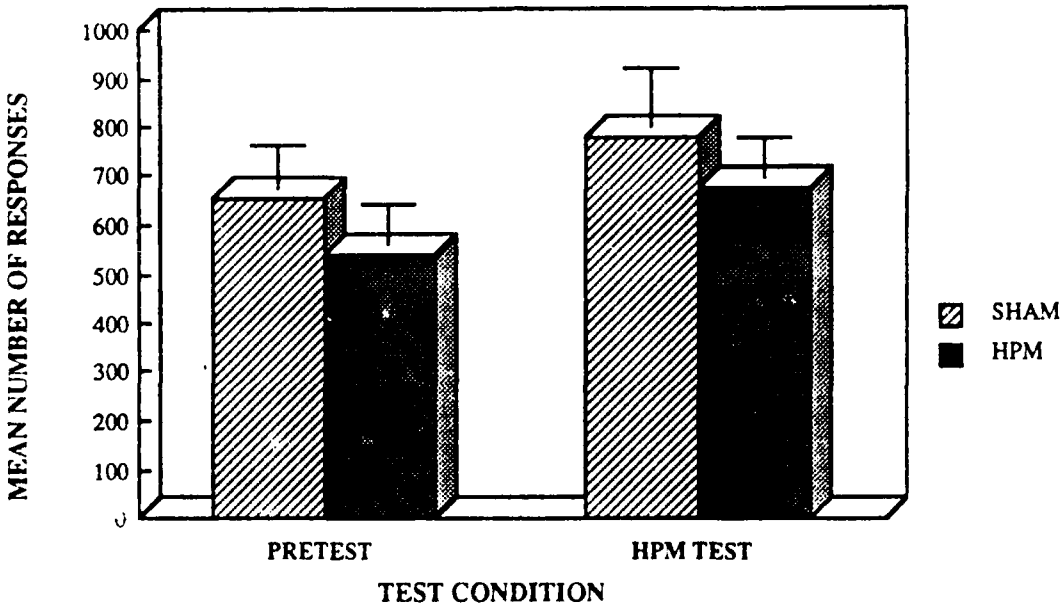

Figure 1. Mean (± SEM) number of lever press responses of HPM- and sham-irradiated groups of rats in post HPM irradiation VI operant test. Test conditions on the abscissa describe test treatments under PRETEST and HPM TEST conditions. PRETEST values represent combined data from the 3 days preceding HPM testing.

High-Power Microwave Irradiation During Variable-Interval Behavior

There were no differences between the HPM- and sham-irradiated groups in body weight or pre- or postirradiation colonic temperatures. There were no statistically significant differences between HPM- and sham-irradiated groups in the number of lever press responses made (Sham = 312.7 ± 30.8, HPM = 307.1 ± 34.8), the number of food pellet reinforcements received (Sham = 113.2 ± 7.9, HPM = 112.9 ± 6.4) or in the response to reinforcement ratio (Sham = 2.7 ± 0.1, HPM = 2.7 ± 0.2). There were no differences between the groups in the number of responses made during any of the six 5-min interval bins (Fig. 2) that comprised the 30-min test session. Thus, no effects of HPM irradiation were noted on any of the thermoregulatory or operant-behavioral measures studied.

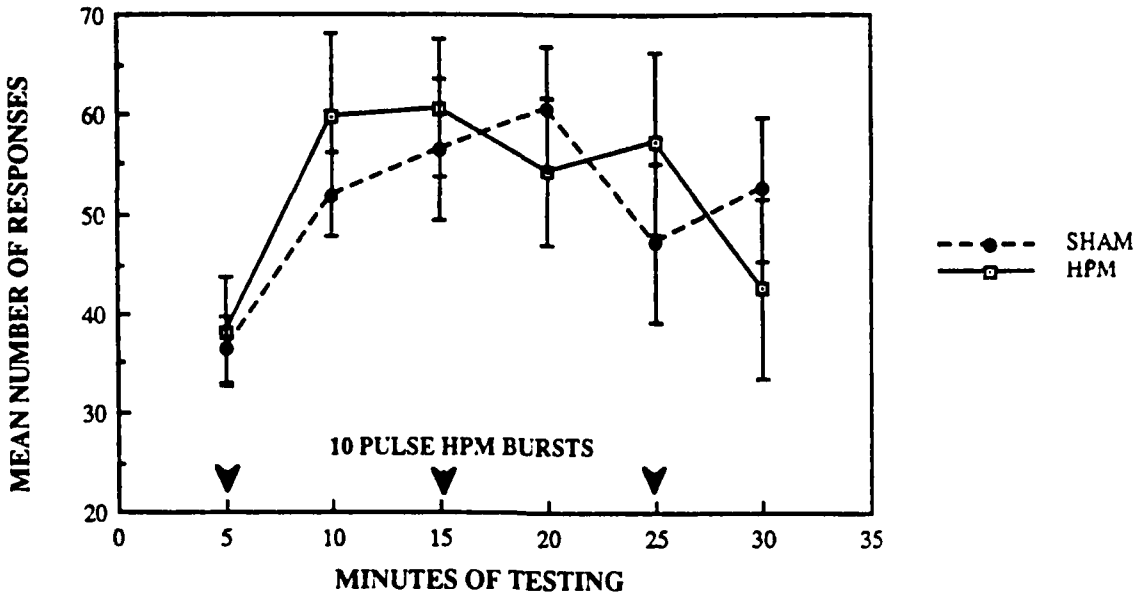

Figure 2. Mean (± SEM) number of lever press responses made during each 5-min interval of operant testing of HPM- and sham-irradiated groups of rats in VI operant test conducted during irradiation. Rats were irradiated or sham irradiated with burst of 10 pulses at 5, 15, and 25 mins during the 30-min session (arrows).

Discrete-Trial Avoidance Behavior

There were no differences between the HPM- and sham-irradiated groups on any thermoregulatory or behavioral measure on any of the 3 days preceding HPM testing. The HPM irradiation did not increase colonic temperature relative to the controls. There were no significant differences between HPM- and sham-irradiated groups in the number of avoidance (Fig. 3) or escape responses or the latency to each response. Similarly, there were no differences between HPM- and sham-irradiated groups in the number of full shocks received or the number of intertrial responses made.

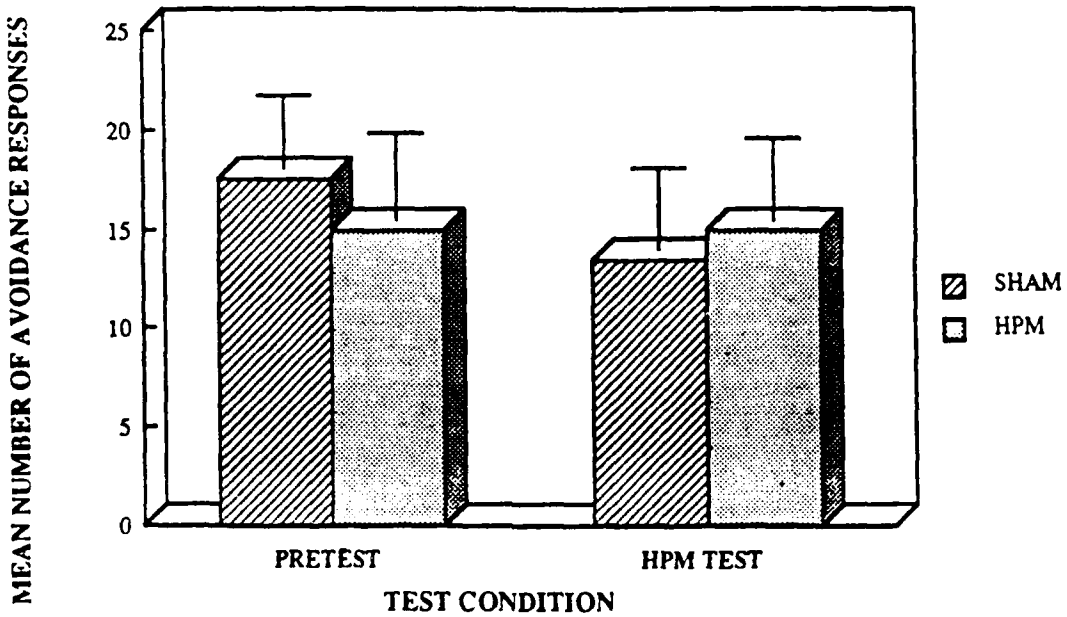

Figure 3. Mean (± SEM) number of avoidance responses of HPM- and sham-irradiated groups of rats in discrete-trial avoidance test. Test conditions on the abscissa describe test treatments under PRETEST and HPM TEST conditions. PRETEST values represent combined data from the 3 days preceding HPM testing.

Passive-Avoidance Memory Testing

Experiment 1

There were no differences between HPM- and sham-irradiated groups in body weight or preirradiation colonic temperature. Sham-irradiated rats had a greater increase in preirradiation to postirradiation colonic temperature (+1.06 ± 0.1 °C) than HPM-irradiated rats (0.61 ± 0.2 °C) ($t = 2.12$, $df = 18$, $p<0.05$). On the initial test day, HPM-irradiated rats had a significantly shorter latency to enter the darkened side of the avoidance chamber (Fig. 4) than did sham-irradiated controls ($t = 2.16$, $df = 18$, $p<0.05$). Note, however, that this measurement preceded irradiation treatment. When retested the following day, one animal from the HPM-irradiated group demonstrated an avoidance failure by entering the side of the avoidance chamber where it was previously shocked (latency = 90.5 s). Not one of the animals in the sham-irradiated group demonstrated an avoidance failure. A test for the significance of difference between 2 proportions indicates no statistically significant difference in the number of animals with avoidance failures. Mean latency to reenter the shocked side of the chamber was not altered by HPM irradiation (Fig. 4).

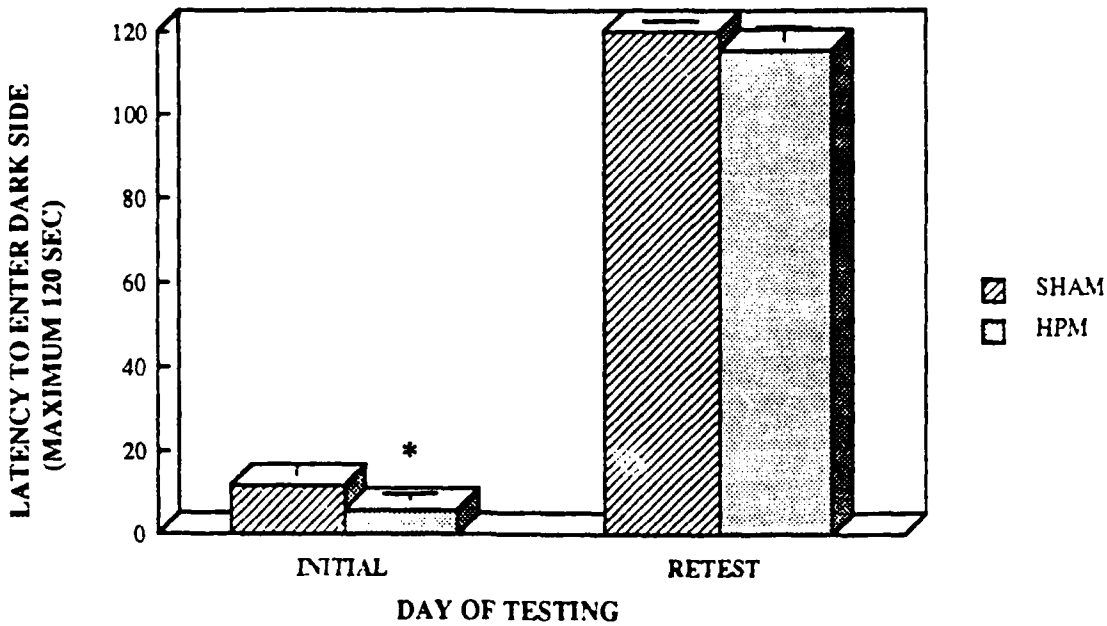

Figure 4. Mean (± SEM) traverse latency (sec) of HPM- and sham-irradiated groups of rats in passive-avoidance memory Experiment 1. Following a traverse on the INITIAL test day, rats were given a footshock in the darkened side of an avoidance chamber. Rats were returned for a RETEST on Day 2. * = $p<0.05$

Experiment 2

Irradiation with 50-HPM pulses over a 5-min period did not result in a significant increase in colonic temperature relative to sham-irradiated controls. There were no significant differences between HPM- and sham-irradiated groups in latency to enter the shocked side of the avoidance chamber before either shock or irradiation (Fig. 5).

One animal in the HPM-irradiated group demonstrated an avoidance failure when retested on the following day (latency = 30.1 s). None of the animals in the sham-irradiated group demonstrated an avoidance failure when retested. A test for the significance of difference between 2 proportions indicates that there was no statistically significant difference in the number of animals with avoidance failures. Mean latency to reenter the shocked side of the chamber was not altered by HPM irradiation (Fig. 5).

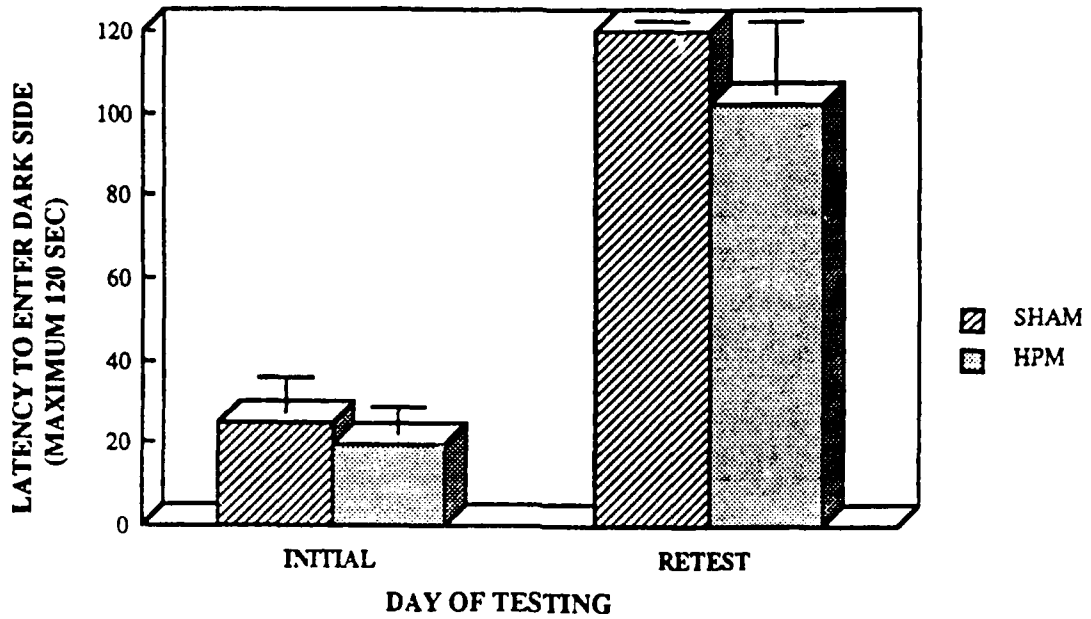

Figure 5. Mean (± SEM) traverse latency (sec) of HPM- and sham-irradiated groups of rats in passive-avoidance memory Experiment 2. Following a traverse on the INITIAL test day, rats were given a footshock in the darkened side of an avoidance chamber. Rats were returned for a RETEST on Day 2.

Passive-Place-Avoidance Testing

There were no statistically significant differences between HPM- and sham-irradiated groups in body weight, preirradiation or postirradiation colonic temperature. There were no significant differences between the groups in latency to enter the darkened side of the avoidance chamber for the first time (Fig. 6). When retested the following day there were again no differences between HPM- and sham-irradiated groups in preirradiation or postirradiation colonic temperature. Latency to reenter the darkened side was shorter on Day 2 than on Day 1 for both groups, but there were no significant differences in latency between groups (Fig.6).

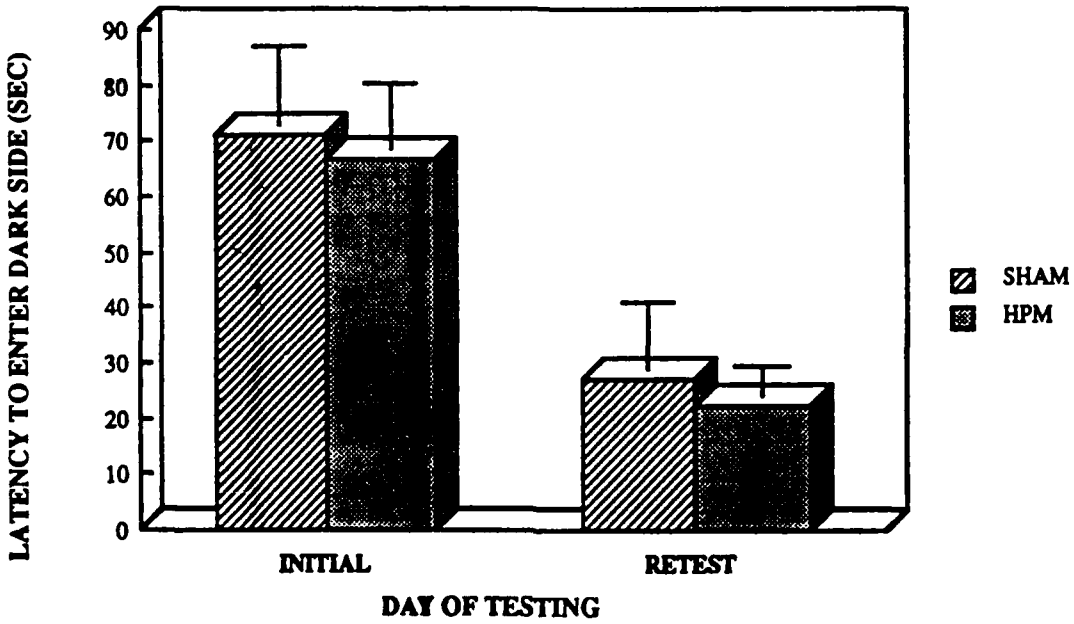

Figure 6. Mean (± SEM) traverse latency (sec) of HPM- and sham-irradiated groups of rats in the passive place-avoidance aversion experiment. Following a traverse on the INITIAL day of testing, rats were HPM or sham irradiated in the darkened side of an avoidance chamber. Rats were RETESTed for traverse latency on Day 2.

DISCUSSION

The results of the behavioral experiments reveal no consistent effect of TEMPO HPM pulses at a carrier frequency averaging 2.11 GHz with pulses of 85 ns in duration and a mean peak-power density of 10.79 kW/cm^2. The number of responses and reinforcements on the VI operant test following HPM irradiation increased slightly in both HPM- and sham-irradiated groups relative to pretest baseline conditions. This difference may be attributable to a later starting time (60 min) on the HPM Test days and a consequent increase in the time since the last feeding. While there were inherent differences between the groups, these differences remained proportional following HPM irradiation. Similarly, increasing the number of pulses presented and presenting them during a 30-min VI behavior session did not affect performance under the VI schedule. Although the rats had lower response rates in the "infield" chamber compared to standard operant chambers, there was no indication of an effect of HPM irradiation on performance. Video observation of

the animals during testing indicated no response of any kind during irradiation. This behavior is highly relevant to the results of the VI experiment conducted during irradiation. During pulse delivery, animal behavior was observed by 3 investigators. In no instance was any response to any pulse train elicited from any animal. So, while recording cables were disconnected during the actual period of pulse delivery, it is unlikely that any relevant information was missed.

There was no effect of HPM irradiation on any measure of aversively motivated discrete-trial avoidance behavior.

The passive-avoidance memory experiments were the most suggestive indicators of possible HPM-pulse effects on behavior. In Experiment 1, one of the HPM-irradiated animals demonstrated an avoidance failure by returning to the previously shocked side of the chamber. While this behavior does not represent a significant proportion of the tested animals, it is an interesting result given the strength of the aversive conditioning in this paradigm. In all of our previous experiments using this paradigm (9,10,32), not a single sham-irradiated animal has demonstrated an avoidance failure. Similarly, in Experiment 2 where 50 pulses were delivered over a 5-min period, 1 of the 5 HPM-irradiated animals demonstrated an avoidance failure. Again, although the proportion of animals demonstrating avoidance failures was not significant, the results indicate that a variation of this paradigm may be sensitive to possible HPM-pulse effects and that additional experiments are warranted.

The passive-place-avoidance experiment indicates that exposure to a burst of HPM TEMPO pulses was not an aversive stimulus. Previous experiments (32) have suggested that HPM irradiation at levels that do not result in significant heating may be aversive. Apparently, the HPM-irradiation parameters used in the present experiment are subthreshold for such an effect.

The results of the present experiments are consistent with the reports of D'Andrea and his colleagues (5) who reported no disruption of the performance of rhesus monkeys on an appetitively motivated task either with 5.6-GHz high-peak-power microwave pulses (0.8 μs pulses, 400 kW peak-power) or when using the TEMPO pulser [2.3-GHz irradiation at a peak-power density of 8.5 kW/cm^2, 80-ns pulses (14)]. The present data are also supported by the Klauenberg et al. (13) report that either sound isolation or increasing carrier frequency to 2.45 GHz blocked startle responses and disruption of rotarod performance observed at 1.11 - 1.64 GHz in the presence of TEMPO-induced noise.

The findings do not support the earlier observations of Cordts et al. (11) or Klauenberg et al. (12) of significant behavioral disruption due to irradiation with HPM. However, there were several areas of difference between the experiments of Cordts et al. (11) and Klauenberg et al. (12) and the present

experiments. The Klauenberg et al. (12) experiments reported behavioral disruption that was concurrent with and limited to the occurrence of the TEMPO pulse. Due to EMP interference, concurrent data collection was not possible in the present experiments (vida supra). Discharge of the TEMPO results in a noise level of approximately 110 dB per pulse, as measured at the location of the animal exposure in the absence of an acoustically shielded isolation chamber. Recent bioeffects experiments using the TEMPO have employed an acoustically shielded isolation chamber (13). With the acoustically shielded exposure chamber sealed (as in the present experiments), the measured noise level at the location of animal exposure was determined to be 67 dB. With the random noise system generating an output of approximately 73 dB, no noise associated with the TEMPO firing was measured inside the exposure chamber. Thus, when sound level factors were excluded, no consistent effects of TEMPO HPM pulses were observed. Review of the videotape records of animal behavior during each HPM pulse burst revealed no instance where the pulse was associated with any change in the behavior of the animal. That is, no startle response was observed during any of the HPM tests. However, this response does not rule out the possibility of behavioral effects as the paradigms and rat strain used by Cordts et al. (11) and Klauenberg et al. (12,13) were not the same as those used in the present experiments and their experiments were conducted with carrier frequencies of 1.62 and 1.11 - 1.26 GHz, respectively.

The present results also differ from our own earlier experiments using klystron-based HPM systems (9,10,32). In our earlier experiments clear thresholds for behavioral disruption were noted using the same paradigms that were used in the present studies; however, discrete-trial avoidance behavior was unaffected by HPM irradiation in both studies. The critical difference between our past and present experiments is the average and peak powers delivered. In our previous experiments, the maximum peak-power density used was 1.8 kW/cm^2. However, power was delivered at pulse repetition rates of up to 10 pps with pulse durations of up to 10 μs. This combination of irradiation protocols was sufficient to cause significant increases in colonic temperature. In general, the behavioral disruption observed following HPM irradiation in our previous experiments could be attributable to the increase in colonic temperature. In the present experiments, where the mean peak-power density was 10.79 kW/cm^2, the average power was considerably lower due to the 85-ns pulse width and the lower number of pulses delivered. In no instance during the present experiment did HPM irradiation result in an increase in colonic temperature relative to controls. In general, the results of these experiments indicate that HPM TEMPO pulses do not represent a significant risk to biological systems under the conditions tested. The only apparent exception to this conclusion is the possibility of HPM effects on memory processing. Additional work with larger numbers of animals and refinements of the test paradigm will further illuminate this issue.

The results of present and previous experiments (9,10,32) suggest that the peak power of HPM irradiation may not be as important a factor as average power in producing bioeffects. When average power has remained low, no significant behavioral or thermoregulatory effects of HPM irradiation have been noted, regardless of the peak-power density tested.

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