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BEHAVIORAL EFFECTS OF MICROWAVE IRRADIATION ON SQUIRREL MONKEY (<u>SAIMIRI SCIUREUS</u>) PERFORMANCE OF A REPEATED ACQUISITION TASK

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SUMMARY PAGE*

THE PROBLEM

The use of microwave radiation in naval communications and weapons systems requires specification of power densities and parameters of microwaves to which fleet personnel may be exposed. Current scientific reports indicate that microwave radiation can produce behavioral changes and that behavioral measures are a sensitive index of microwave effects. In this study the effects of three different incident power levels of microwaves on learning were investigated in the squirrel monkey, and the minimum power density necessary to significantly affect response acquisition behavior was determined.

FINDINGS

Response acquisition was impaired following 30 minutes of exposure to an incident power density of 53 mW/cm² but not to 11 or 43 mW/cm². A mean 1.9° C increase in rectal temperature above control levels accompanied the behavioral effects and no such effects were observed without concomittant hyperthermia. These effects were transitory, and no evidence of irreversible impairment of learning ability was observed. The threshold power density necessary to significantly disrupt behavior under the conditions of this experiment was estimated to be between 45 and 50 mW/cm².

ACKNOWLEDGMENTS

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*The animals used in this study were handled in accordance with the Principles of Laboratory Animal Care established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science-National Research Council.

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INTRODUCTION

The safety standard for microwave irradiation in the U.S. is currently set at 10 mW/cm² for periods greater than 0.1 hour. In contrast, the standard in the Soviet Union and Eastern Europe is 10 μ W/cm², or one thousand times less. This difference seems to be due to the conclusions drawn by Soviet and Eastern European researchers about the effects of low level microwaves on the central nervous system and on behavior (5). Soviet and Czechoslovakian studies of humans occupationally exposed to low level microwaves list symptoms such as headache, fatigue, dizziness, moodiness, insomnia, and decreased memory (6, 7). Such a syndrome is difficult to quantify and simulate in behavioral experiments with animals.

Microwave irradiation has been shown by American investigators to disrupt various behaviors in animals (1-4). The use of different experimental designs, microwave parameters, and species of subjects precludes a concise systematic summary of microwave effects on behavior at present. Behaviors investigated in animals exposed to microwaves have varied from untrained behaviors, such as spontaneous activity level or food intake, to highly trained behaviors such as operant performance on complex schedules of reinforcement or discrimination tasks.

In the present experiment the effect of microwaves on the acquisition of new behavior was investigated. Overlearned or well practiced behavior patterns and skills are sometimes unaffected by physiological trauma. The acquisition of new skills and behavior, however, may be impaired by the same physiological event that has little effect on previously learned behaviors.

To study the effect of microwaves on the acquisition of behavior, a repeated acquisition task was chosen. This task, which has been used to study the effects of various drugs on behavior (9), requires the subject to learn different but similar behaviors each session. At the start of each session the subject characteristically makes a large number of incorrect responses, which gradually declines over the course of the session as the task is learned. Each session, therefore, provides an individual learning curve for each subject. The effect of microwaves on response acquisition was determined by comparing several indices of performance following microwave irradiation to performance following sham irradiation.

Galloway (3) has reported on the effects of microwaves on a repeated acquisition task with the rhesus monkey as the subject. However, Galloway used 2450 MHz microwaves produced by a diathermy applicator applied directly to the monkey's head for 2 minutes or until convulsions began. Because Galloway's irradiation method produced severe skin burns, only two out of the original four monkeys were able to receive the entire irradiation series. Galloway suggested that his limited data on repeated acquisition indicated that this complex behavior might be more sensitive to radiation effects than a simple, schedule-controlled behavior under similar radiation conditions. The purpose of the present study was to investigate the effect of low level microwaves on the acquisition of behavior under more standardized and precisely quantified exposure conditions and with a larger number of subjects. A primary objective was to determine the minimum power level (inreshold) necessary to significantly produce an effect on behavior.

PROCEDURE

SUBJECTS

Six experimentally naive male squirrel monkeys, <u>Saimiri sciureus</u>, were maintained at approximately 90 percent of their free-feeding body weight. The monkeys were housed in individual but adjacent home cages, with water continuously available in the cages. Home-cage temperature was maintained at approximately 24°C, and the 24-hour light-dark cycle was 15 hours light and 9 hours darkness.

APPARATUS

The exposure chamber's exterior measured 2.5 m in length, 1.96 m in width, and 1.83 m in height; it was shielded with copper and then completely lined with 8-inch pyramidal absorber (AAP-8) obtained from Advanced Absorber Products, Amesbury, MA. The exposure chamber was equipped with a closed-circuit TV camera used to monitor the animals during exposures. A radar set, AN/SPS-4 (Raytheon Manufacturing Co., Waltham, MA), was the source of 5.62 GHz microwaves pulsed at a repetition rate of 600/sec. Pulse widths were 0.5 μ sec at an average incident power density of 11 mW/cm², and were 2 μ sec at 43 and 53 mW/cm². A wave guide connected to a standard gain horn, Narda model 643 (Narda Microwave Corp., Plainview, NY), was used to irradiate the monkey's ventral surface while it was restrained in an upright seated position in a Styrofoam restraint chair.

Field measurements were made with a Narda microïine isotropic probe, model 8323, in the three dimensions at 5-cr. intervals in the region to be occupied by the monkey. Incident power densities were measured at the approximate location of the monkey's head.

The monkeys were restrained in a Styrofoam chair and irradiated in the far field except for the 53 mW/cm² level which was at 90 percent of the minimum far field distance in a large anechoic chamber. The formula 2 D^2/λ , where D = diagonal of the horn, was used to define the far field. Figure 1 shows the confinement chair in the exposure chamber.

Air in the anechoic exposure chamber was circulated via ventilation fans at a rate exceeding 28 m^3 per minute. Temperature of the air in the chamber fluctuated with building temperature; typical variation was between 23° C and 26.5° C and usually increased during the day. Humidity of the air drawn into the exposure chamber varied somewhat with meteorological conditions but averaged about 54 percent. White noise to mask extraneous sounds was present in the exposure chamber.



Figure 1. A Styrofoam squirrel monkey couch inside the irradiation chamber. A radar set (AN/SPS-4) provided pulsed microwaves via a standard gain horn to the restrained monkey's ventral surface. Animal rectal temperature was measured with a Yellow Springs Instruments tele-thermometer model 43TA (Yellow Springs Instrument Co., Yellow Springs, OH), with the rectal probe inserted approximately 4 cm into the animal's rectum.

The operant chamber where the behavioral tests were conducted was in a separate room adjoining the exposure facility. A Plexiglass chamber measuring 30 cm wide by 42 cm high by 30.5 cm deep in the monkey's compartment was enclosed within a larger sound- and vibration-attenuated outer chamber. Three circular 2.56-cm diameter Plexiglass response keys extending 2 mm out from the front intelligence panel were mounted horizontally 5.72 cm apart, center to center, and 23 cm above the chamber floor. A minimum force of approximately 0.35 N was required to close the microswitch behind each response key. General illumination was provided by a white $7-\frac{1}{2}$ Watt house light. A ventilation fan and white noise measured at 80 dB were present. Reinforcement was the delivery of a 190-mg Noyes Precision food pellet (The P.J. Noyes Co., Lancaster, N.H.).

In an adjoining room solid state switching circuitry recorded responses, delivered reinforcers, and scheduled stimuli and response contingencies.

METHOD

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Each monkey received 66 preliminary training sessions on a repeated acquisition task prior to exposure to any microwave irradiation. During these preliminary sessions the monkeys were adapted to 30 minutes of restraint in the Styrofoam restraint chair, placed inside the exposure chamber prior to behavioral testing, and were trained unrestrained to press the response keys. The large number of preliminary sessions was required because, on a repeated acquisition task, the number of errors not only declines within a single session but also gradually declines across sessions until a relatively stable baseline level of errors is reached.

Experimental sessions began after preliminary training and were conducted five days a week. Each monkey was placed in the restraint chair, the temperature probe was inserted, and the animal was placed in the irradiation chamber for 30 minutes. During this 30-minute period the animals were irradiated at a preselected power density of 11, 43, or 53 mW/cm^2 or were sham irradiated at 0 mW/cm². The subject was then removed from the irradiation chamber and restraint chair and transported to the next room for testing on the repeated acquisition task. The time between removal from the exposure chamber and the start of the behavioral testing session was approximately 120 seconds.

The repeated acquisition task required the monkeys to press the three response keys in a particular sequence to obtain food. The required sequence was different during each session; therefore, each session provided a measure of acquisition behavior as the novel response sequence was learned. Each response in the sequence was cued by one of four distinct visual stimuli projected behind the three transparent response keys. The four stimuli were red light, green light, three white horizontal lines on a dark background, and three white vertical lines on a dark background.

If the three response keys are designated by position as left, center, and right, a sample sequence will be left, center, left, right. The monkeys were required to press a particular response key when a particular stimulus was present. For example, red color, press left key; green color, press center key; vertical lines, press left key; horizontal lines, press right key and obtain a food pellet. No particular stimulus was consistently correlated with any particular response or position from reinforcement within the sequence as the order of the stimuli was randomly selected at the start of a session and then fixed for the remainder of the session. No response key was correct twice in succession, and responses on all three keys were required each sequence. Responses and positions in the sequence were counterbalanced so that each response was required equally often in each position in the sequence. Each correct response in the sequence changed the stimulus, and the fourth correct response produced a food pellet, and the stimulus condition was resumed for the first response in the sequence. Responses not in the required sequence (errors) were followed by a 5-second period when all lights in the chamber were extinguished and the response keys were dark and ineffective in producing food.

Each behavioral session lasted until 60 food pellets were collected or 50 minutes had elapsed, whichever occurred first. Infrequent instances of equipment failure, power failure, fire alarm drill, and air conditioning failure necessitated the discarding of some data.

Table 1 shows the number of experimental exposure sessions and sham exposure sessions at each power density. Each monkey received a minimum of seven and maximum of eleven exposures at each power level.

Table 1

Power Density (mW/cm ²)	Experimental Exposure Sessions	Sham Exposure Sessions
11	7-10	7-9
43	9-11	3-5
53	7-11	5-7

Frequency of Exposure Sessions Per Monkey

RESULTS AND DISCUSSION

In a recent study (2) of the effects of microwaves on operant behavior in the squirrel monkey it was concluded that behavioral effects were directly related to hyperthermia and that consistent behavioral changes occurred only when rectal temperatures increased greater than 1°C. Figure 2 shows



Figure 2. The mean change in rectal temperature as a function of power density when exposure duration was 30 minutes. The solid line connects group means and 0 on the abscissa represents the sham exposure condition.

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the mean increase in rectal temperature as a function of power density when exposure duration was 30 minutes. The data in Figure 2 are the temperature changes between the start and the end of the 30-minute period of restraint in the microwave exposure chamber. The group mean temperature increases after 30 minutes at 0, 11, 43, and 53 mW/cm² were 0.79, 0.86, 2.37, and 2.66°C, respectively. Thirty minutes of exposure to 11 mW/cm² produced little increase in rectal temperature above the control (sham) level while 30 minutes of exposure at 43 or 53 mW/cm² increased rectal temperature substantially above control levels. Observation of the monkeys immediately following exposures at 43 mW/cm² and 53 mW/cm² revealed reddening of, and moisture on, the skin areas of the monkeys' hands and feet and occasionally profuse saliva around an animal's mouth.

A major dependent variable in the repeated acquisition task was the number of errors a subject made under experimental (microwave irradiation) and control (sham irradiation) conditions. Within each session the number of errors systematically decreased from the start to the end of a session, indicating that the monkeys learned each sequence. Figure 3 shows the mean number of errors per block of ten reinforcements for a representative monkey, M42, with microwave power density as a parameter. The mean number of errors was greatest during the initial portion of a session and decreased as the session progressed; i.e., as the number of cumulative reinforcements increased during a session. In short, the monkeys learned the required sequences. The difference in number of errors after microwave exposure compared to sham exposure shown for M42 in Figure 3 is typical of results with the other five monkeys. No reliable difference between experimental and control performance was found after 11 or 43 mW/cm², but at 53 mW/cm² a greater number of errors was generally made after microwave exposures than after sham exposures.

A comparison between number of errors after microwave exposures and number of errors after sham exposures for all six monkeys is shown in Figure 4. Because there was considerable variability among the six monkeys in the absolute value of the mean number of errors made during control sessions, Figure 4 shows mean errors as a percentage of errors on comparable control sessions. This method of data presentation takes into account, for example, the fact that an increase in 5 errors is relatively greater when increasing from 5 to 10 than from 30 to 35 although the absolute increase is the same. In Figure 4, values above or below the 100 percent no-difference line indicate, respectively, more or fewer errors after microwave exposures. Inspection of Figure 4 shows that no consistent deviation from the 100 percent line occurred at power densities of 11 or 43 mW/cm². At a power density of 53 mW/cm² subjects typically, although not invariably, made more errors after microwave irradiation than sham irradiation. At the 53 mW/cm² level the increase in percentage of errors ranged from a group mean of 31 percent during the 1-10 reinforcement block to a mean of 122 percent during the 31-40 reinforcement block. In Figure 4 it can also be seen that the increases in errors following microwave exposure were not restricted to the initial portion of a session but persisted throughout the entire session. It should be noted, however, that the absolute number of errors decreased over reinforcement blocks so



Figure 3. Mean errors per session over blocks of ten reinforcements for monkey 42 with power density as a parameter. The solid lines represent performance following microwave exposures and the dashed lines represent performance following sham exposure.

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that percentages of control values did not indicate larger absolute differences in the number of errors near the end of a session.

To clarify trends in the number of errors after microwave and after sham exposure the mean errors per reinforcement as a percentage of comparable control data for each subject are shown in Figure 5. The statistic "number of errors per reinforcement" was used as a measure of the ratio of errors to correct responses (1 reinforcement = 4 correct responses) rather than the total number of errors per session because occasionally a monkey failed to collect the 60 reinforcers before the end of 50-minute session time. On such sessions total errors would not distinguish between a low number of total errors resulting from a high response rate but a low ratio of incorrect to correct responses. The data shown in Figure 5 agree with those shown in Figures 3 and 4 and indicate that 30 minutes of exposure at 11 or 43 mW/cm² did not increase the number of errors compared to control performance, but 30 minutes of exposure to 53 mW/cm² typically increased the number of errors relative to control performance.

Several studies (2, 8) have shown that response rate was affected by microwave irradiation. On the repeated acquisition task the number of errors is analogous to accuracy, while reponses per minute is analogous to speed of performance. Figure 6 shows the correct responses per minute and errors per minute as a percentage of comparable responses per minute on control sessions. Values below the 100 percent no-difference line indicate a decrease in responses per minute decreased monotonically with inereased power density. Although no trends in mean errors per minute were apparent as a function of power density, there was a large increment at the highest power density. Figure 7 shows overall response rate, the sum of errors and correct responses divided by total session time, excluding the time out periods, as a percentage of comparable data on control days. It can be seen that the effect of microwaves on response rate was a large increment in variability.

A recent finding on the effects of microwaves on response rate was reported by de Lorge (2). In de Lorge's study, squirrel monkeys responded on levers for food prior to irradiation, while they were irradiated, and following irradiation. This procedure contrasts with the more common one of exposing the animal to microwaves first and then testing for a behavioral effect after removal from the field. de Lorge (2) found that rectal temperature typically decreased about twice as rapidly at the offset of irradiation than it increased at the onset of irradiation. After a pause of about 10 minutes the monkeys began responding on the levers again. In the present study no behavioral measurements were obtained until after the monkey was removed from the microwave field so that it was not possible to determine if the "off effect" suppression of response rate occurred. However, such an effect implies that response rate would be lowest at the start of a session and increase as the session progressed. Figure 8 shows the mean time to the first key press in a session as a percentage of the mean time to the first key press after sham exposures for power densities of 43 and 63 mW/cm². As the group means show, the monkeys paused



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Figure 7. Total correct responses summed with total error responses divided by total session time (excluding time out periods) yield overall responses per minute. Overall responses per minute following irradiation are shown as a percentage of corresponding data following sham irradiation with incident power density as a parameter.

over twice as long before they began responding after irradiation than after sham irradiation. This result is compatible with an "off effect" of response suppression and suggests that it would be informative if future research studies on the effects of microwaves on behavior more closely examined the time course of microwave effects; i.e. prior to exposure, during exposure, and post exposure.

Overall examination of the results shown in Figures 1-8 supports the conclusion that behavioral changes following microwave irradiation in the squirrel monkey are directly related to hyperthermia. Exposure to a power density of 11 mW/cm^2 , which did not significantly increase rectal temperature above control levels, did not produce a behavioral effect. Exposure at 43 and 53 mW/cm², which produced a greater increase in rectal temperature, correspondingly produced a greater change in behavior. Furthermore, with only the highest power density employed in this study, 53 mW/cm^2 , there was often a correlation between how much the monkey's temperature increased and the effect of the microwaves on the monkey's acquisition behavior. For example, Figure 2 shows that M40 and M35 showed the largest increases in rectal temperature and M37 and M47 the smallest increases at 53 mW/cm². Figure 5, an index of overall errors per session, shows that at 53 mW/cm², the two monkeys showing the least deviation from control performance were M37 and M47, the two monkeys showing the least temperature increase. The two animals showing the greatest increase in percentage of errors per reinforcement were M3 and M42. Monkeys M36 and M42 exhibited the second and third largest increases in rectal temperature, respectively. Similarly, the two monkeys whose temperatures increased the most at 53 mW/cm^2 (M36 and M40, Figure 6) exhibited the greatest reduction in correct responses per minute relative to control performance at 53 mW/cm². The two monkeys whose temperatures increased least, M37 and M47, showed little deviation from control values in correct responses per minute.

The present data also relate to a conclusion expressed by Hunt et al (4) that the effect of microwaves on the behavior of animals trained on some task was on the performance of the task and not interference with the trained skills since the behavior returned to preexposure proficiency without retraining. The monkeys in the present experiment were exposed to 30 minutes of microwaves two or three times a week for a period of approximately 15 weeks. Figure 9 shows the mean $(\pm 1 \text{ standard deviation})$ errors per reinforcement on control sessions over the time course of the experiment. The mean errors per reinforcement after sham exposures actually decreased during the first month when the sham exposures were interspersed among exposures at 11 mW/cm^2 . This decrease is best attributed to the increased number of training sessions on the repeated acquisition task. The mean errors per reinforcement on control sessions showed little change during the following three months when the exposures at 43 and 53 mW/cm^2 were interspersed among the control sessions. These data indicate that the effects of microwaves on learning were transitory and did not produce permanent impairment of the learning process.

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CONCLUSIONS

Conclusions drawn from the present data should be restricted to the microwave and exposure parameters used in this experiment. Extrapolation to other species and parameters is tenuous at present.

The main objective of this study was to determine if microwaves disrupt an organism's ability to acquire new behaviors. To the degree that the repeated acquisitions task serves as an accurate model of new learning, the data show that microwaves at 53 mW/cm² temporarily disrupted learning performance but did not interfere permanently with the monkey's capacity to learn new response sequences. There was no evidence of permanent impairment in learning ability. The highest power level used, 53 mW/cm², consistently disrupted the monkeys' behavior and generally increased the number of errors, decreased the correct responses per minute, and increased the time to the first response. Both the speed and accuracy of performance decreased. However, the behavior returned to baseline values under sham exposures conducted after exposures at the highest power level. Comparison of repeated acquisition performance prior to any microwave exposure with performance after 16 weeks of intermittent exposure did not indicate any decrease in ability to acquire new response sequences.

The data are compatible with the hypothesis that behavioral changes were directly related to hyperthermia in the monkey. Power densities that did not increase rectal temperatures above control levels did not consistently disrupt behavior. In general, the power density that produced the greatest increase in temperature also produced the greatest perturbation of behavior.

The threshold for behavioral effects in the squirrel monkey after 30 minutes of irradiation with pulsed 5.62 GHz microwaves was estimated to be between 45 and 50 mW/cm². This threshold value compares to an estimated threshold for behavioral effects in the squirrel monkey of between 40 and 50 mW/cm² for 60 minutes of exposure to constant wave 2450 MHz microwaves (2).

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