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The present study was one of several designed to measure specific operant behaviors and discover effects of exposure of animals to extremely low frequency magnetic and electric fields of low intensity in support of the U.S. Navy's attempt to scientifically explore the biological effects of these fields. Measurements of immediate memory, operant responding, and reaction time were obtained on two rhesus monkeys. No significant changes could be related to the exposure of these animals to a magnetic field of 10 gauss at 75 Hz or to the magnetic field combined with an electric field of 4 v/m at 75 Hz. The results provide supportive evidence that these specific electromagnetic fields have no general behavioral influence on non-human primates.

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OPERANT BEHAVIOR OF RHESUS MONKEYS IN THE PRESENCE

OF EXTREMELY LOW FREQUENCY-LOW INTENSITY

MAGNETIC AND ELECTRIC FIELDS: EXPERIMENT 1

John de Lorge

Bureau of Medicine and Surgery MF51.524.015-0014BX7X



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SUMMARY PAGE

THE PROBLEM

Recent Navy research has been concerned with the biological effects of electromagnetic radiation in the extremely low frequency (ELF) region. The present study is one of several designed to measure specific psychological behavior and discover effects of exposure of animals to ELF magnetic and electric fields of low intensity.

FINDINGS

Measurements of immediate memory, operant responding, and simple reaction time were obtained on two rhesus monkeys during 50 daily sessions. No significant changes could be related to the exposure of the animals to a magnetic field of 10 gauss at 75 Hz or to the magnetic field combined with an electric field of 4 v/m at 75 Hz. These results provide supportive evidence that these specific electromagnetic fields have no general behavioral influence on non-human primates.

ACKNOWLEDGMENTS

The author is appreciative of the advice provided by Doctors D. E. Beischer and J. D. Grissett. Special appreciation goes to E. A. Molina and the Electronics Services Division and to B. Boss and R. J. Franklin who assisted in the experiment.

Experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences--National Research Council.

INTRODUCTION

A number of recent studies have shown that animal behavior might be affected by electric and magnetic fields of low intensity alternating at extremely low frequencies (ELF). Current interest in the area has arisen partly because of the effects of meteorological and geomagnetic conditions on various animals, including man (19), and partly because of the increasing use of power generating and transmission systems. Although most previous research was primarily concerned with the ability of animals to orient to static magnetic fields (1,2), contemporary investigations have explored more specific behavioral effects of static and alternating fields (15).

Much of the work in the magnetic area has emphasized the intensity of the fields as a parameter and used animals as subjects. For example, classical conditioning studies have shown that carp can be conditioned to a 100-gauss signal (9) and pigeons can be conditioned to a 0.8-gauss signal (16). Also, operant conditioning techniques have been used to explore avoidance responding in rats prenatally exposed to a 0.5-30 gauss field (14). One study demonstrated that humans increased their reaction time in a 5-11 gauss field alternating at 0.2 Hz but not in a static field or a field alternating at 0.1 Hz (4). Yet, studies with squirrel monkeys have failed to discover any reaction time effects of magnetic fields at various intensities and frequencies (6,7). Although the area is cluttered with conflicting results, at least one investigator has concluded that a primary effect of ELF magnetic fields is to produce heightened reactivity to novel stimuli (14), which might explain the reported aversiveness of pulsed magnetic fields (17).

Research on the behavioral effects of electric fields in the ELF range has frequently utilized man as the subject and has been concerned with the frequency parameter. Perhaps it is no coincidence since the 2-10 Hz frequencies generally dealt with are in the range of human brain waves. One investigator has shown that human reaction time can be either increased at 3-6 Hz (11) or decreased at 9 Hz (10), while another investigator failed to discover uny significant changes at all (8). It has also been determined that the circadian rhythm in man is influenced by an electric field (20). Findings have been just as tentative when other animals were used as subjects in similar research. The nocturnal activity of mice has increased at 60 Hz (13), the operant response rate of rhesus monkeys has increased in a 7-Hz field, but not in a 10-Hz field (5), and the operant response rate of rats has been depressed by a moderately intense 50-Hz field (18).

The purpose of the present experiment was to develop and verify a technique for synthesizing previous research on the behavioral effects of low intensity ELF fields. This synthesis involved an exploration of operant response rates, reaction time performance, a measure of immediate memory, and general motor activity. Since the present study was exploratory and since a large number of hours of behavior was recorded providing both experimental and control data, only two subjects were used.

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METHOD

SUBJECTS

Two male rhesus monkeys (Macaca mulatta), approximately 6 years old, were the subjects. When the animals, AP6 and AR4, were infants, they were subjected to 14 days of ionizing radiation. AP6 was given 10 rads per day for a total of 140 rads, and AR4 was given 5 rads per day for a total of 70 rads. Prior to the present experiment both animals were subjects in an experiment on operant schedules of reinforcement. For the present experiment they were trained at approximately 90 per cent of their ad libitum body weight. During the experiment their body weights differed; AR4 remained at approximately 90 per cent while AP6 gained to 110 per cent.

APPARATUS

The animal chamber was made of wood and fiberboard with plastic and formica internal walls and mounted on rubber vibration isolators. Internal dimensions were 39 inches high by 27-1/2 inches wide by 29-1/4 inches deep. The floor was 7/8-inch phenolic rods spaced 7/8 inch apart and arranged perpendicularly to the sides of the chamber. When the middle rod was stepped on, a microswitch was actuated to measure general motor activity. Air entered the chamber through vents at the top and exited at the bottom of the rear wall. The temperature was controlled by air conditioners in the room. While an animal was in the chamber, the temperature typically increased approximately 2° F. The front of the chamber was a large hinged door. Inset 7 inches into the chamber was a secondary wall of black synthane which contained a small guillotine door on the right and a work panel on the left comprised of stimuli projectors, signal lights, manipulanda, and food and water receptacles. When the animal was in the chamber in a work position, he faced the front wall with the work area to his right and the guillotine door to his left. Figure 1 illustrates the animal's view of the work panel. The match-to-sample discs (B) were about eye level when the subjects were sitting. A switch button was on the right of the food receptacle (fd), and a similar button was on the left of the water receptacle (H2O). A light was directly above the water receptacle and the food receptacle contained a similar light. When these lights were on, a force of approximately 700 grams could actuate the food or water buttons. The fixed interval and reaction time response lever (C) centered between the food and water receptacles was an aluminum rod 1-3/16 inches long and 1/2 inch in diameter. The lever was 8 inches above the floor and could be actuated by an upward force of 200 grams.

The stimulus lights (D) were in a vertical line above the response lever. The lower red light was the reaction time discriminative stimulus; the green light above this was the fixed interval discriminative stimulus. The top match-to-sample disc (B) was approximately 15 inches above the floor. The other two match-to-sample discs were 3 inches below and 2 inches on either side of the top one. Each disc was 1-1/4 inches in diameter. Forces of approximately 300 grams operated the discs. An Industrial Electronics Equipment inline digital display projector with Grason-Stadler stimuli, model A509-2A,



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Line drawing of the front panel of the monkey chamber. A denotes speaker inputs; B denotes the three match-to-sample discs; C indicates the FI and reaction time lever; D indicates the FI (upper) and reaction time (lower) stimulus lights. The circles to the left of the water aperture and to the right of the food aperture denote the respective manipulanda. The circle above the water aperture represents the water available stimulus. was mounted behind each of the three match-to-sample discs. A small grouping of holes (A) in the wall 8 inches directly above the top disc was for the reaction-time tone. A second grouping of holes directly above the first grouping and 7-1/2 inches from the top of the work panel was for a white masking noise. The speakers for these two tones were located on the hinged front door of the chamber and the magnetic field from the speaker magnets was not detectable within the animal chamber. All manipulanda and stimuli utilized nonferrous metal or plastic wherever possible. Feeding and watering devices were located out of the coil's magnetic field above the chamber. A 15-watt fluorescent houselight provided illumination through a window in the ceiling of the chamber, but the magnetic field from the houselight starter could not be detected within the chamber. The work panel was cleaned at the end of each session and the waste tray was cleaned every second day.

The magnetic field was produced by two vertical Helmholtz coils wound on parallel wood frames and aligned in a north-south direction. Three thousand feet of No. 10 copper wire was wound on each frame. The average magnetic field produced was 10 gauss and varied ± 0.5 gauss within the animal chamber placed in the center of the coils. The front of the animal chamber faced east when in the coils. A Bell 620 gaussmeter was used to measure the magnetic field. Figure 2 contains a line drawing of the coils and animal chamber.

The electric field was developed across two sheets of copper screen 30x52 inches mounted on each side of the animal chamber as shown in Figure 2. The calculated intensity of the electric field was 4 volts per meter (peak-to-peak voltage). Both fields were oscillated in phase at 75 Hz. The strength of the fields was periodically checked and the amplifier and oscillator output was continuously monitored with an oscilloscope. Although the experimental control and recording equipment were located in a distant room, the oscillator and power amplifier along with capacitors for tuning the coils were located in the same room as the coils. Adjustment of the air conditioners in the room compensated for the heat generated by the system. The average animal chamber temperature before the fields were activated was 73° F and, in the presence of the fields, it was 72° F during the experiment.

PROCEDURE

The animals were trained on three tasks with food or water as reinforcement. During the experiment the animals obtained all their daily food intake (0.86 gm Purina Monkey Chow Tablets) while performing the tasks except for a small portion of fruit after the experimental session and except for their weekend food. The animals were first trained to press a button next to the food receptacle when the receptacle was illuminated. A button press produced a food pellet in the receptacle. Then, they were trained to press the button next to the water receptacle when a nearby light was on. Immediate conditioned reinforcement for appropriate performance on the tasks was the illumination of the food or water lights indicating reinforcement was available. The three tasks were Fixed Interval (FI), Reaction Time, and Match-to-Sample.



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A schematic side view of the animal chamber inserted in the two coils (A). The front view (B) illustrates the manner in which the electric field screens were mounted on the chamber sides. The dotted circle indicates the minimum perimeter of the coils around the chamber.

F1. Each animal was initially trained to raise the response lever following a standard shaping procedure (3). When the animals were consistently working this task in the presence of a green light on the work panel, the time between reinforcements following a lever lift was increased to two minutes. This schedule remained in effect until the experiment began, when an F11-minute was instituted for the first 13 sessions. Thereafter, the schedule was an F120-second. These schedule changes were imposed in an attempt to maintain an equivalent reinforcement density in each of the three different tasks.

Reaction Time. The animals were next trained to lift the lever in the presence of a red light on the work panel. The lever lift produced a tone. When the lever was released, the tone and red light went off and reinforcement became available. Gradually, the time between red light presentations, the intertrial interval (ITI), was lengthened and responses during this interval reset the interval. After the animals learned to discriminate the red light, the time between lever lift and tone onset (foreperiod) was lengthered. Lever releases during the foreperiod extinguished the red light and reset the ITI. Finally, a limited hold procedure was introduced so that if the tone came on and the lever was not released within 3.0 seconds, the tone and red light went off and the ITI was reset. Initially, the ITI was variable between 1 and 30 seconds, but during the experiment it was fixed at 10 seconds.

<u>Match-to-Sample</u>. The animals were initially trained to press the top disc when it was illuminated to obtain reinforcement. Next, each disc press resulted in the illumination of the left or right lower disc with the matching stimulus. When the illuminated lower disc was pressed, reinforcement became available. Each time a disc was pressed the stimulus was removed. Finally, both lower discs were illuminated, one with the matching stimulus, one with a non-matching stimulus. A correct lower disc press resulted in reinforcement. An incorrect disc press turned all stimuli off for 10 seconds, followed by the reappearance of the same stimulus on the top disc. Each correct disc press was also followed by a different stimulus on the top disc, although the top disc remained off until reinforcement was obtained. During training the stimuli shown on the discs were in sequence and the correct and incorrect discs alternated, but during the experiment the sequence of stimuli and the correct-incorrect disc changes occurred randomly. The time from reinforcement and the next illumination of the disc (ITI) during the experiment was 10 seconds.

Each task was available for three 15-minute components during an experimental session. Each component was followed by a 5-minute extinction period (ext) in which no tasks were available. The sequence was as follows: F120-second, ext, Reaction Time, ext, Match-to-Sample, ext. The sequence was repeated three times each experimental session.

The animals were given 26 experimental sessions after being trained for 39 sessions (AP6) and 44 sessions (AR4). Prior to session 27 the magnetic field was activated. The field remained continuously on and prior to session 39 the electric field was also turned on. Prior to session 45 both fields were turned off and six additional sessions in the absence of the field occurred before the experiment was terminated.

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RESULTS AND DISCUSSION

Although behavioral changes occurred, such changes were not related to the electromagnetic fields. Instead, most changes in performance were a consequence of continuous experimental sessions and elapsed time, or of equipment problems. Figure 3 illustrates the development of FI behavior during the course of the experiment for both subjects. Reinforcement time was the time elapsed between the reinforcement available stimulus and a button press on one of the reinforcement buttons. Reinforcement time was not reliably obtained during the first 26 sessions and neither was post reinforcement pause time or running rate during the first 4 sessions. Data for sessions 34, 35, and 36 were omitted because of a problem with the food delivery mechanism.

Response rate in Figure 3 was calculated by including only the time from the initial response following the start of an FI interval to the last response in that interval (running response rate). In the lower portion of Figure 3 it is seen that the response rates were increasing to session 14 when the FI 1-minute was changed to FI 20-second. This change had very little effect on the response rate but AR4 tended to stabilize at this rate until session 45. AP6 continued to gradually increase his response rate until the experiment ended. At session 40 an equipment problem in the match-to-sample component gradually led to extinction on that task for AR4 and, hence, a compensatory increase in his FI response rate occurred by session 45. Although this difference in response rates for AR4 was significant (t = -4.14, p < .01), AP6 failed to be substantially affected by the match-to-sample malfunction, did not entinguish on that task, and therefore did not show a significant FI response rate increase. Even though the fields were turned off at session 45, it is doubtful that this manipulation was responsible for AR4's rate increase.

As seen in the top portion of Figure 3, both subjects also showed significant decreases in time to obtain reinforcement (t = 3.14 for AP6 and t = 3.00 for AR4; p < .02). These significant decreases occurred between No Field (2) sessions and the sessions in the presence of the combined fields. Such decreases in reinforcement time normally occur with increased sessions and therefore were probably not caused by the absence of the fields.

Post reinforcement pause time was the time elapsed between a reinforcement and the next lever response and is shown in the middle portion of Figure 3. During the Fl 1minute schedule pause time was quite high, but when the Fl was changed to 20 seconds at session 14, large differences in post reinforcement pause time were not seen between either subjects or sessions. Although AP6 was highly variable on this measure, such variability was often due to his not responding at all for some of the Fl components.

Figure 4 contains distribution curves of the mean rates of responding in successive 2.0-second segments of the F120-second schedule. The data were obtained from the last six sessions prior to the introduction of the magnetic field (No Field 1), nine sessions with the magnetic field (B Field), six sessions with the magnetic and electric fields (B & E Fields), and the final six sessions in the absence of the fields (No Field 2). Figure 4 illustrates that responding within the F1 components differed only slightly as the conditions

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Fixed Interval behavior of AP6 (triangles) and AR4 (circles). A, B, and C on the abscissa indicate changes in the ELF fields.

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The mean response rate per 2.0-second segment as a function of the ELF fields. The responses were sorted according to the successive 2.0-second segment following the start of an FI 20-second component wherein it occurred.

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changed. This difference was most certainly due to the time course of events. Comparisons of No Field (1) and the B Field or No Field (2) and the B & E Fields show no obvious differences.

In general, when the response rates during the initial No Field sessions were low, the rates in the presence of the fields were lower. However, this decrement normally occurs with increased sessions on FI schedules. At the highest rates, during the last 2.0-second segment of the FI, there were essentially no differences associated with the presence or absence of the fields.

The reaction time task produced some of the most stable behavior. The lower portion of Figure 5 demonstrates the median reaction time for both animals. No significant differences in reaction time were related to the presence or absence of the fields. Also, no relationships between the fields and either intertrial interval responses or anticipatory responses were observed.

Figure 6 contains histograms of the distribution of reaction times as a function of the field conditions and confirms the lack of a reliable reaction time relationship to the presence or absence of the fields. The shape of the distributions remained essentially the same under all four conditions, although AP6 had a relatively greater number of 0.4-second reaction times during the No Field (1) sessions.

Performance on the match-to-sample task was also not related to the presence of the fields. An illustration of a classic learning curve was produced when both animals continued to decrease their error rate until the equipment problem developed as seen in the top portion of Figure 7. Although less consistently, their latency to respond to the matching stimulus also gradually decreased and became less variable. AR4 was significantly faster during the presence of the magnetic field (t = 2.52, p < .05). This difference was more than likely due to continued experience and seems to be the anticipated outcome of a developing decrease in the variance of this data. Even though much of the match-to-sample data was lost, there was at least one session's data in all except the No Field (2) condition. No obvious dependence on the fields was observed.

Finally, the lower portion of Figure 7 illustrates the general motor activity of the animals. Here too, we see no relationship between the presence and absence of the fields. However, there was a five-day activity cycle for both animals. Generally, they were more active on Monday than they were on Friday, if the data of the first week are ignored. The cyclic activity was probably related to the policy of running the experiment only on week days. In anticipation of this phenomenon, experimental manipulations were made only in the middle of the week.

Statistical tests on the means of the data from the five sessions prior to a field change and five sessions following a change were made for each animal on a total of 42 comparisons with Student's "t" Tests (12). This approach produced comparisons of the initial No Field sessions with the magnetic field sessions, the magnetic sessions with the magnetic and electric field sessions, and the magnetic and electric field sessions with the final No

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Figure 5

Reaction Time measures for AP6 and AR4. A, B, and C on the abscissa indicate changes in the ELF fields. Intertrial interval responses were those lever lifts occurring before the reaction time light appeared, and anticipatory responses were those occurring after the light appeared but before the tone was presented.



Figure 6

Histograms representing average frequencies of reaction times that occurred during the first second following the reaction time tone. Reaction times greater than 1.0 second and less than 3.0 seconds appear in the 1.0+ column.

60 MATCH TO SAMPLE 50 AP6 PERCENT ERRORS AR4 ~ 40 30 20 10 0 MATCH TO SAMPLE 2.0 MEDIAN LATENCY (SEC) 16 1.2 0.8 04 0 B &E FIELD NO FIELD (1) B FIELD NO FIELD (2) ACTIVITY 200 160 FREQUENCY 120 80 40 0 ò ю 15 гò 25 45 50 5 30 35 40 CONSECUTIVE SESSIONS B C

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Field sessions. The analysis partially accounted for the gradual changes that occurred on the various tasks with continuous sessions. Only the four previously mentioned tests produced significant "t's".

CONCLUSIONS

The lack of observable ELF effects in the present study agrees with previous findings in our laboratory (6,7). The behavior investigated is either not susceptible to the ELF fields or the specific fields used in the study have no general behavioral effects on this particular organism. Neither of these assumptions is entirely supported or denied by the literature. Gavalas, et al (5) demonstrated that rhesus monkeys increased their response rate in a 7-Hz electric field although the field parameters and behavioral task differed from those in the present study. When the same investigators (5) explored the effects of a 10-Hz electric field, they failed to observe a behavioral effect. Spittka, et al (18) with a more intense field observed that the response rate of rats was depressed by a 50-Hz electric field of 0.5 Kv/m. Perhaps the specific frequency of the field is a more important variable then the specific behavior.

On the other hand, some investigators (4,10,11) found reaction time in man to be affected by magnetic and electric fields. Yet, these same investigators concurrently reported that differences in the field frequency either failed to influence reaction time (4) or influenced it in the opposite direction (10,11). Another study on reaction time in man (8) was inconclusive as to whether ELF fields increased or decreased a human's reaction time. Human reaction time obviously employs different skills than the reaction time task as presently defined for the rhesus. Regardless of such differences, reaction time has not been reliably shown to be influenced by ELF fields in either primate species.

In most cases, only historical precedent provided a rationale for expecting a specified behavior to be influenced by the ELF field. However, in the present experiment it was thought that the match-to-sample performance could be changed due to the task's dependence upon immediate memory and attentiveness. Relationships between immediate memory, attention, and the hippocampus have been demonstrated and, since the hippocampal slow wave is around 5 Hz, there is a possibility that ELF waves at the same frequency or one of its harmonics (75 Hz) might disrupt memory or orientation and thereby be observable in match-to-sample performance. However, no match-to-sample effects of the ELF fields were observed.

In conclusion, the present study discovered no behavioral effects of a low intensity 75-Hz electric or magnetic field. Much of the contemporary research indicates that general effects do exist and that ELF fields act as stressors on animals (14, 17). The failure to confirm previous findings in the present investigation is probably because of the specific frequency employed. A replication of the study with other frequencies might discover behavioral effects of a general nature.

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