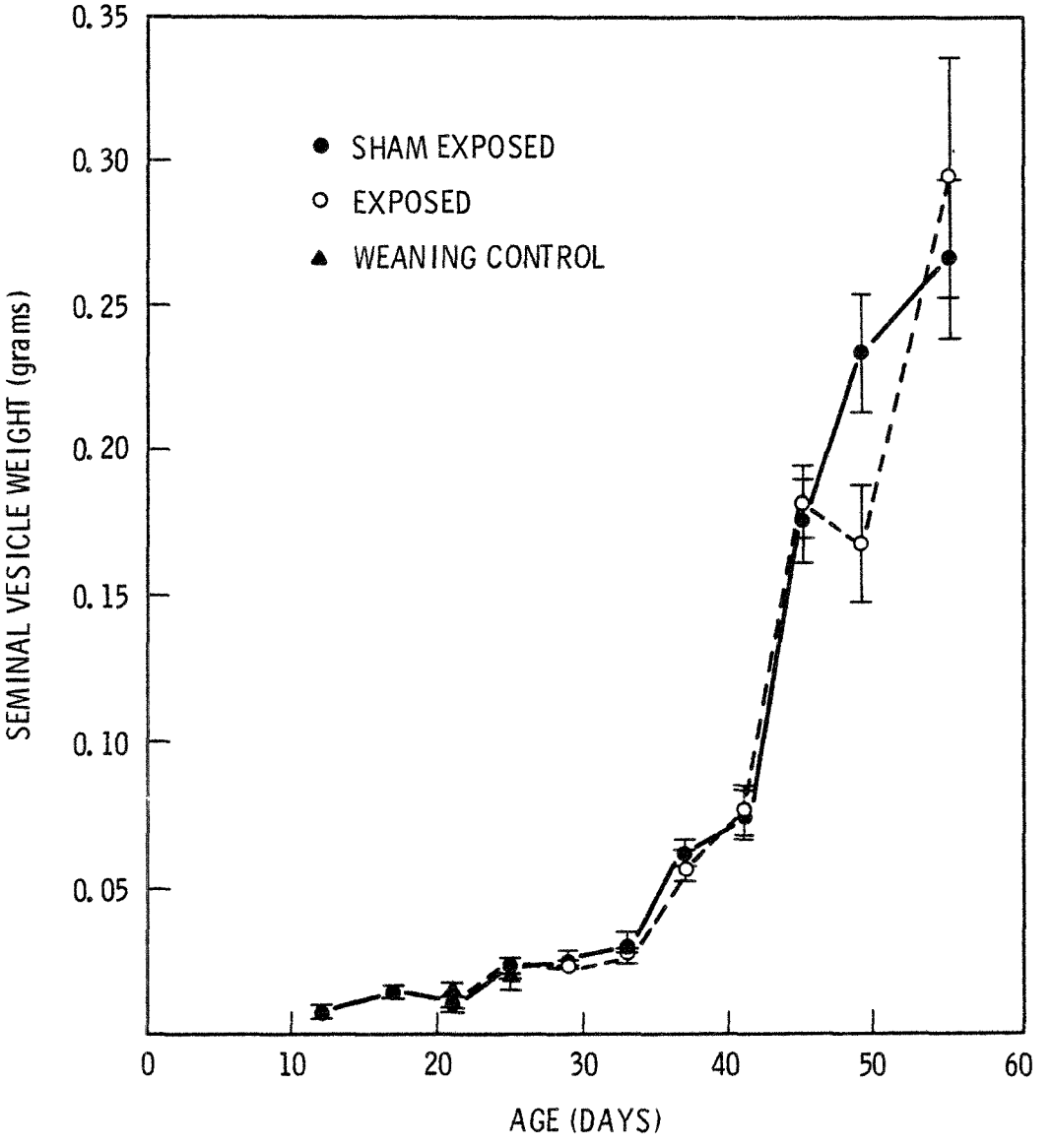



FIGURE IX-7. Mean (± S.E.) Seminal Vesicle Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

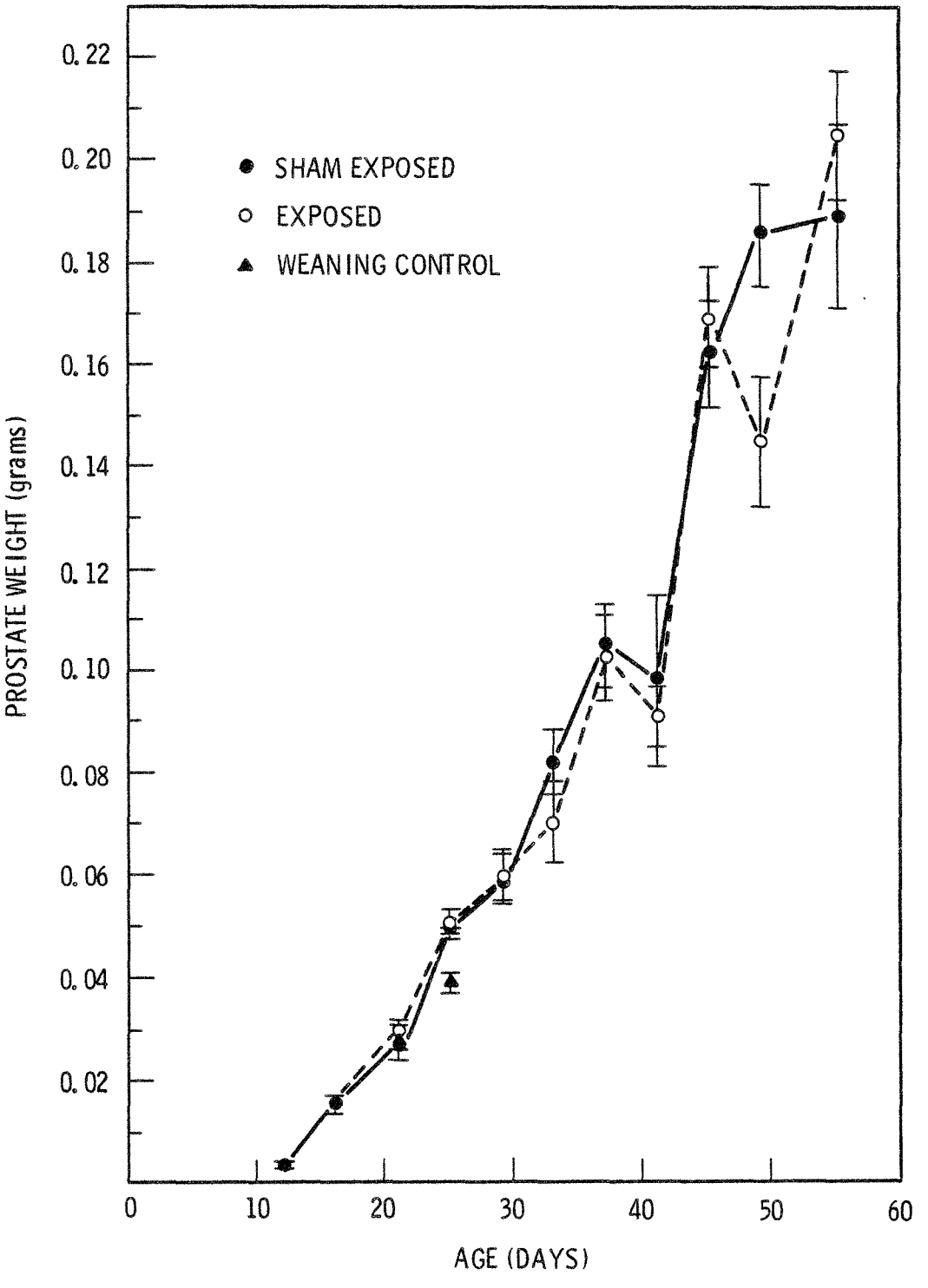



FIGURE IX-8. Mean (± S.E.) Prostate Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

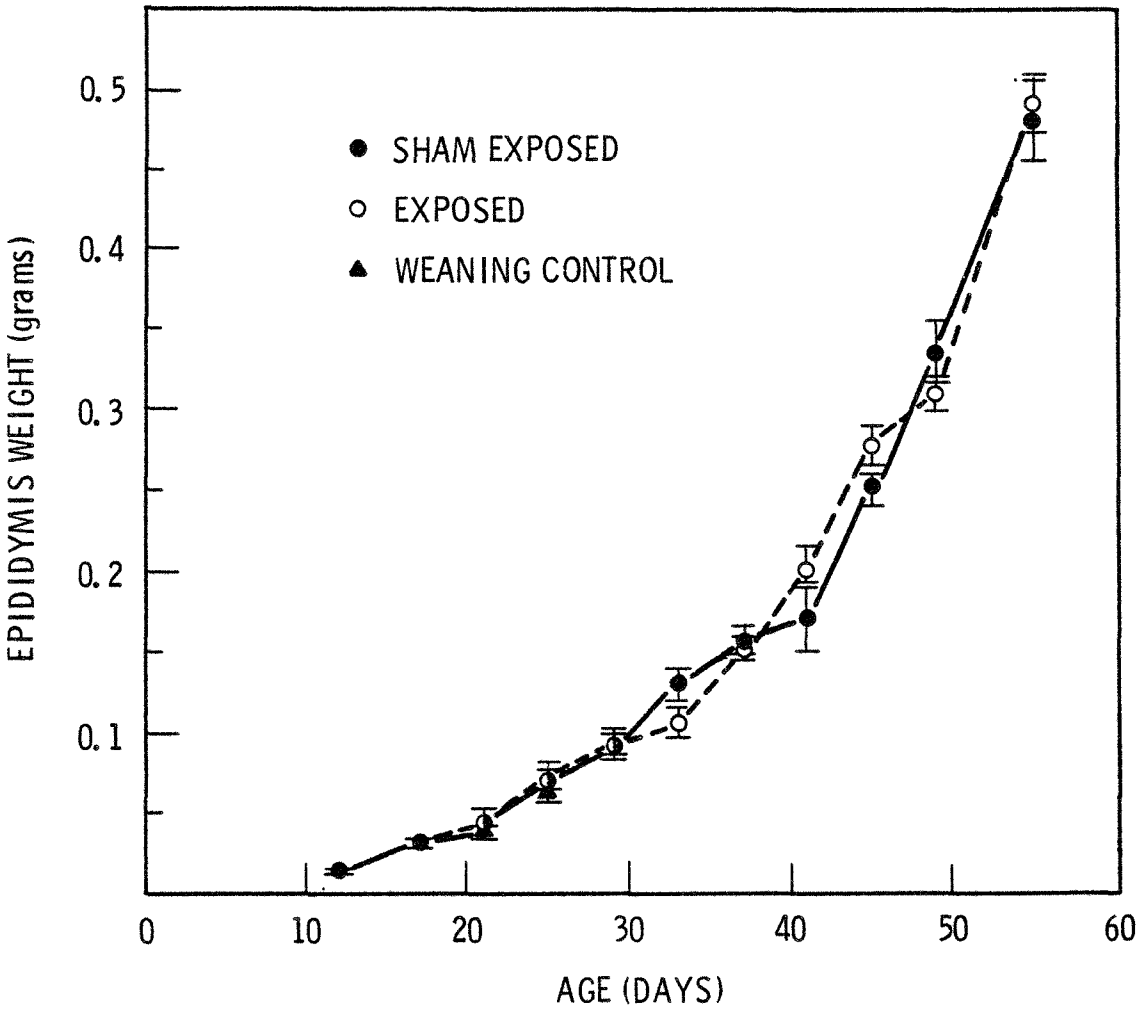



FIGURE IX-9. Mean (± S.E.) Epididymis Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

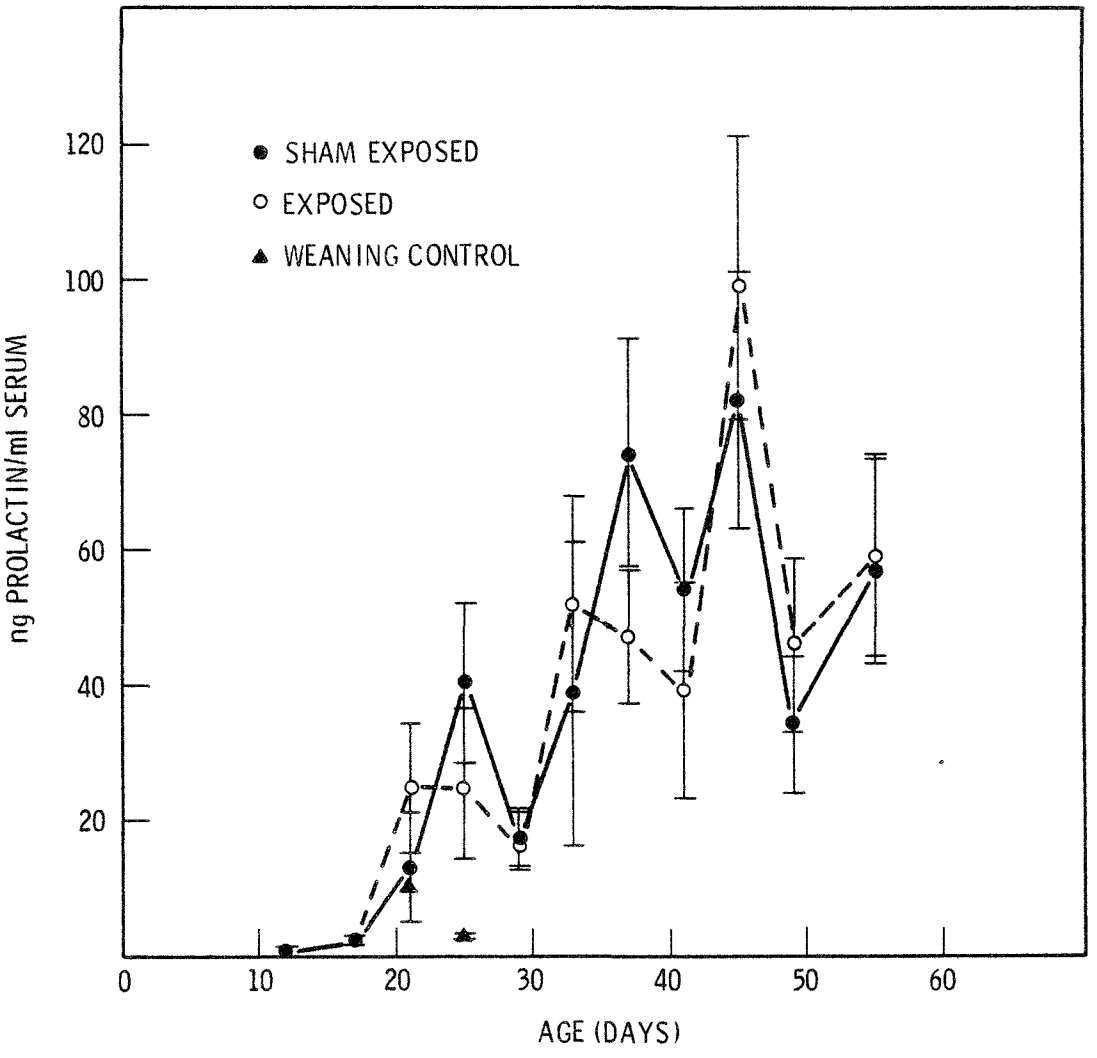



FIGURE IX-10. Mean (± S.E.) Serum Prolactin Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

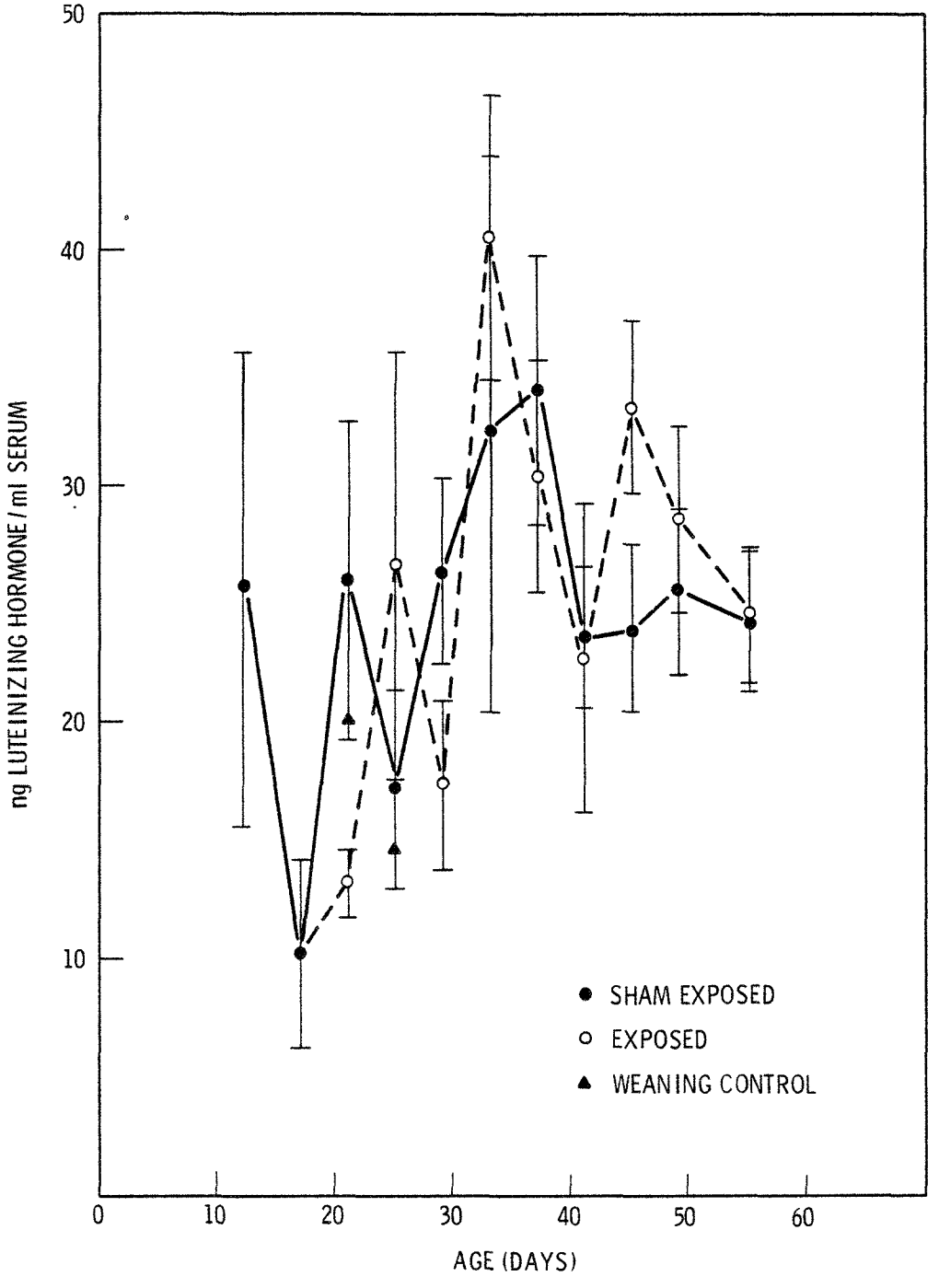



FIGURE IX-11. Mean (± S.E.) Luteinizing Hormone Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

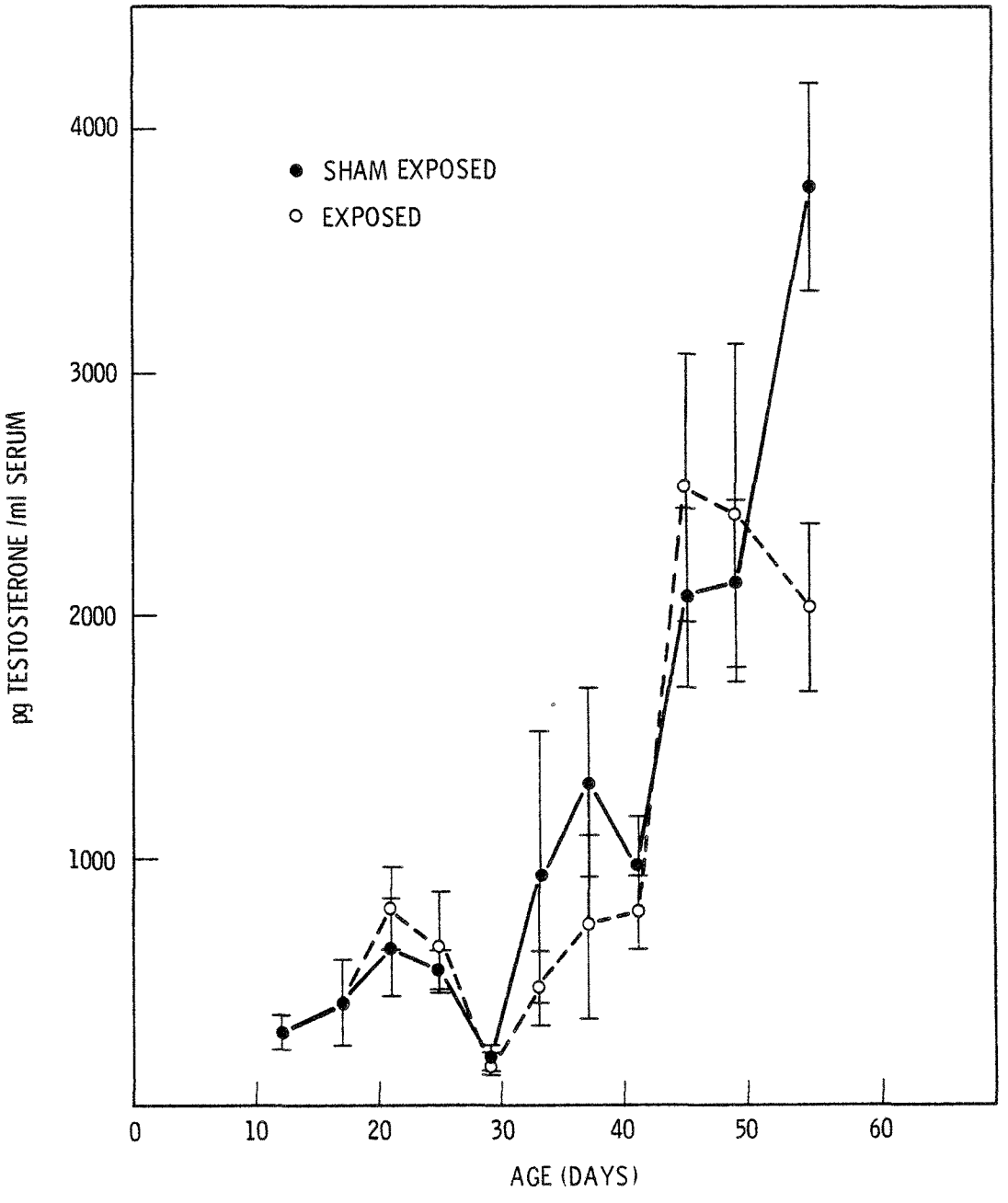



FIGURE IX-12. Mean (± S.E.) Testosterone Concentration of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

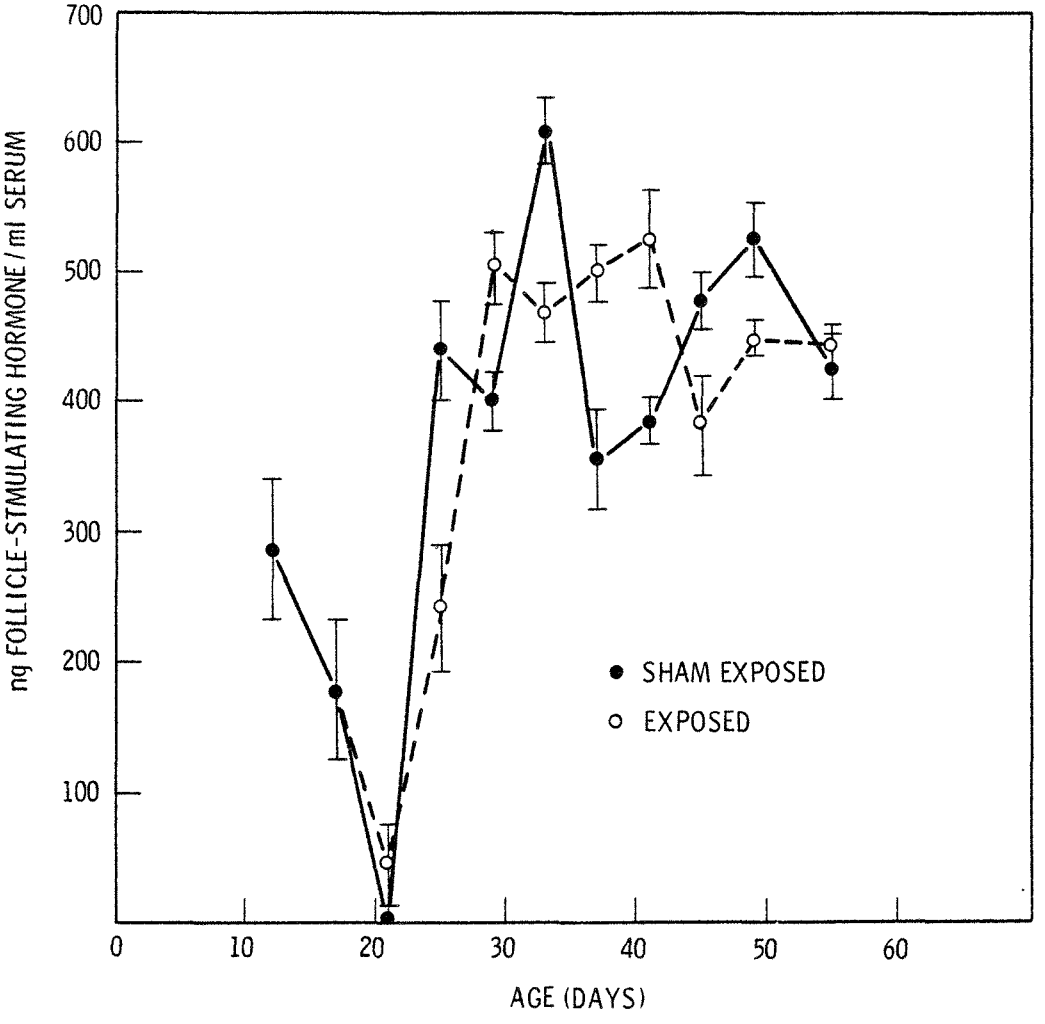



FIGURE IX-13. Mean (± S.E.) Follicle Stimulating Hormone Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

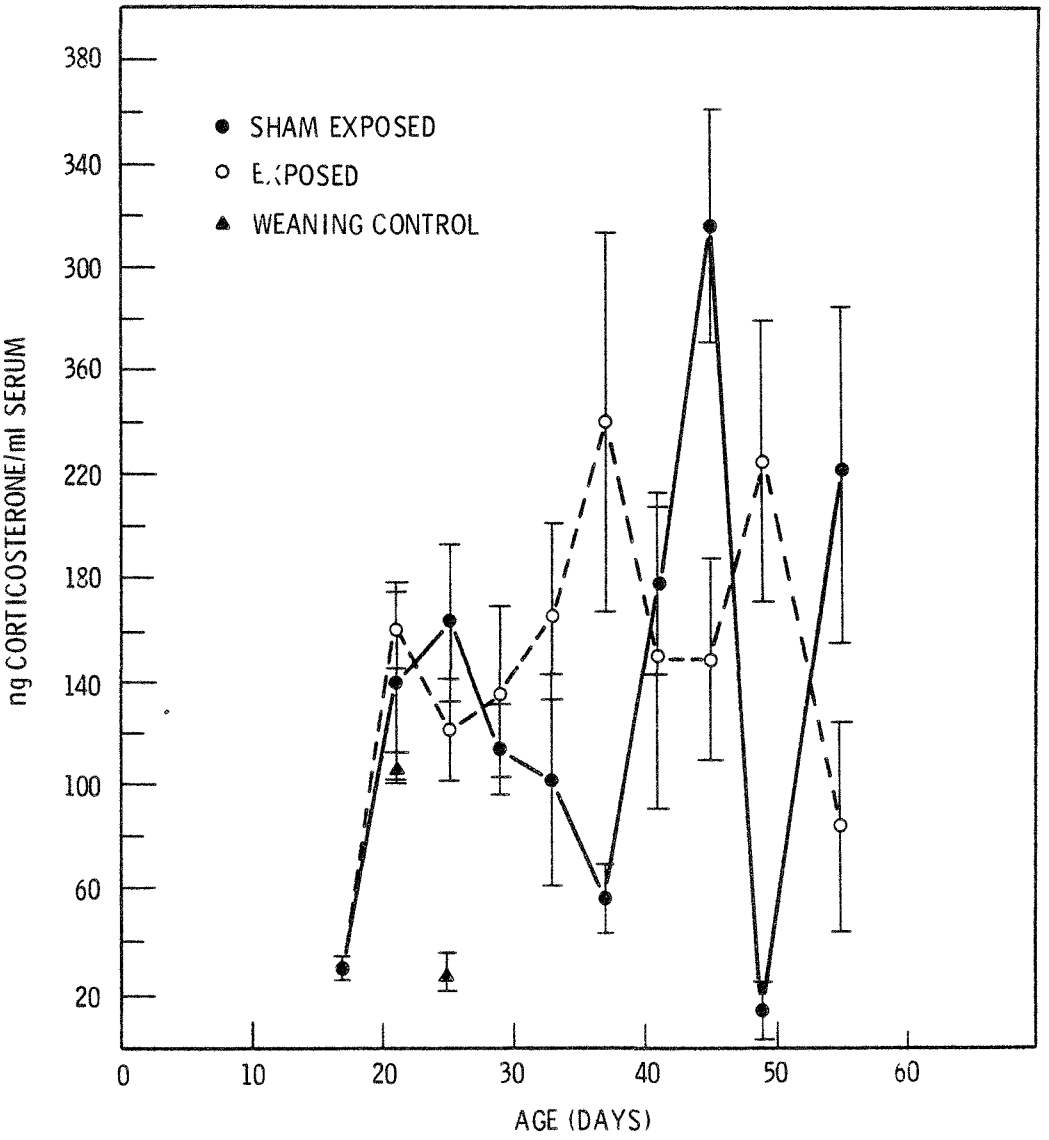



FIGURE IX-14. Mean (± S.E.) Corticosterone Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

## D. DISCUSSION

Exposure of rats to 60-Hz electric fields at 100 kV/m for 30 or 120 days appears to reduce the mean level and the variability of circulating adrenocorticoids in adult rats. Similar effects may occur when rats are exposed during early development, i.e., 20-56 days of age. Currently, we have no evidence that the rats are stressed, and based on the results of other measures (see Section VII and X), it seems unlikely that the apparent lower cortical steroid levels are representative of the exhaustion stage of adaptation to extreme stress. No other endocrine parameters have shown consistently significant differences or notable trends as a result of electric-field exposure.

## E. FUTURE PLANS

A replicate of the experiment in which rats are exposed for 120 days is in progress. In addition, the experiment to examine different sampling procedures on serum hormone levels will be replicated. Future studies will concentrate on neurotransmitters and brain biochemistry.

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# X. CARDIOVASCULAR FUNCTION

Principal Investigators: D. I. Hilton and R. D. Phillips

## ABSTRACT

Electrocardiogram (ECG), heart rate, blood pressure and vascular reactivity measurements were made on rats exposed to 60-Hz electric fields. Heart-rate measurements were made on animals after exposure to 100 kV/m for 1 or 4 mo. Blood pressure and vascular reactivity measurements were made on animals after exposure to 100 kV/m for 35 days or 4 mo. In addition, to test for physiological reserve capabilities, rats were exposed to 100 kV/m for 1 mo and then subjected to cold stress. Heart rate, deep colonic temperature and skin temperature were recorded before, during and after cold exposure. Plasma corticosterone and thyrotropin levels were also measured, both in rats exposed to electric fields alone and in those exposed to electric fields and then subjected to cold stress. Blood pressure and heart-rate measurements were also made on rats during exposure to electric fields. No significant differences in heart rates, ECG patterns, blood pressure or vascular reactivity were found between exposed and sham-exposed animals during exposure or after 1 or 4 mo of exposures. We also found that the physiological and endocrine response to cold stress did not differ between electric-field-exposed animals and sham-exposed controls.

## A. INTRODUCTION

A number of investigators have reported effects of high-strength electric fields on the cardiovascular system. Clinical investigations in the USSR (Asanova, 1966) revealed that substation workers who are occupationally exposed to electric fields exhibit signs of unstable pulse and arterial blood pressure. Cerretelli and Malaguti (1976) reported that arterial blood pressure in rabbits exposed to 50-Hz fields at 80 kV/m for 500 hr tended to be higher than in controls. Coate and Teeters (1970) measured blood pressure in dogs exposed to 45-Hz or 75-Hz electric

fields at 10 kV/m, a magnetic field of 1 gauss and a 0.5-mA ground current. He found that the animals had significantly elevated arterial pressure after 3 wk of exposure. The response of the cardiovascular system to controlled hemorrhage was measured in dogs exposed to 15-kV/m fields for 5 hr (Gann, 1976, personal communication). Electric-field-exposed animals, when allowed to hemorrhage, showed lower mean arterial and pulse pressures than controls. In addition, heart-rate values of exposed animals were considerably higher than those of controls during bleeding.

Studies have also been made to assess whether electric-field exposure could produce stress-related physiological responses. Marino et al. (1977) reported lower serum corticosterone levels and enlarged adrenals and pituitaries in rats exposed to 15-kV/m fields for 1 mo. Investigations by Poznaniak et al. (1977) showed increased levels of serum corticosterone in mice in response to the electric field being turned on and off at 25 or 50 kV/m.

Many of the reported effects of electric-field exposure on cardiovascular function might be responses to secondary factors associated with the electric-field exposure systems. These factors include: 1) corona discharges resulting in audible noise and ozone production; 2) discharge shocks to the animals because of differences in electrical potential between the animal and the caging, feeding or watering systems; 3) audible noise from the transformer; 4) electric-field-induced vibration of the electrodes and/or caging system. Since reported effects may be responses to secondary field factors, we are presently investigating the possibility

of cardiovascular and endocrine changes in rats exposed to 60-Hz electric fields, using an exposure facility that is free of secondary factors such as corona, ozone, shocks and significant cage vibration.

## B. METHODS

Techniques for obtaining electrocardiograms and heart-rate measurements from rats were described in the last Annual Report (Phillips et al., 1978). Two additional studies involving heart-rate measurements were conducted during this past year.

Heart rates were measured on 20 female Sprague-Dawley rats after exposure to a 100-kV/m electric field for 1 mo. The main purpose of this experiment was to obtain cardiovascular data on female animals comparable to those described in males in the last report, since the longer-duration studies (i.e., 120 days) required the use of females.* These females, like the males in the previous experiments (Phillips et al., 1978), were acclimated to the restraint holders used during physiological measurements in order to minimize the possibility of stress-related effects of confinement. Heart rates were measured within 1 hr after the end of the electric-field exposure. Heart-rate measurements were also taken on female rats after exposure to a 100-kV/m field for 4 mo.

Blood pressure and vascular reactivity were measured on rats that were exposed to 100-kV/m fields for 35 or 120 days. Methods for these procedures were modified from those reported by Phillips and Kimeldorf

*Adult males (older than ∿ 100 days of age) are too large to fit in the holders used to contain the animal during measurements.

(1963). In both experiments, all the animals underwent the same acclimation in the restraint holder as was used for ECG and heart-rate measurements. After the last exposure session, each animal was anesthetized with ethrane (Enflurane, Ohio Medical Products) and the left femoral artery and vein were cannulated with polyethelene tubing (I.D., 0.58 mm; O.D., 0.965 mm). The arterial cannula was connected to a pressure transducer (Micron, MP-15) and pressures were recorded on a polygraph (Grass Model 7). After surgical procedures, the incision was infused with a 2% solution of lidocaine HCl. Each animal was then placed in a restraint holder and allowed to recover for 1 hr. Systolic, diastolic, pulse and mean systemic pressures were monitored continuously for 1 hr. Heart rates were determined by counting pressure pulses per min.

After the recovery period, when the animals were fully conscious, a solution of 5% dextrose in sterile water was given as a control injection. After baseline measurements were obtained, a single injection of phenylephrine (Neo-Synephrine HCl, Winthrop), 1 μg in 1 ml of 5% dextrose in sterile water, was given and the change in mean systemic pressure was recorded during the injection. Vascular reactivity was scored as the increase in the mean systemic arterial blood pressure that was caused by the phenylephrine injection. Diastolic, systolic and pulse pressures, and heart-rate values were recorded $\sim$ 10 sec after injection. Fifteen minutes after the first injection a second identical dose of phenylephrine was given, and measurements of the same parameters were made.

Two experiments were conducted to assess for physiological reserve capabilities of rats subjected to cold stress. In both experiments, rats were exposed or sham exposed to a 100-kV/m electric field for 1 mo.

In the first experiment, animals were acclimated to restraint holders during the last 7 days of exposure ($\sim$ 2 hr/day). These acclimations were carried out during the 4 hr/day when the exposure system was de-energized for animal care. After the last day of exposure, each animal was placed in a restraint holder, and heart rate, deep colonic temperature and skin temperature were monitored. Deep colonic temperature was recorded using a thermistor probe inserted, via the rectum, 4 cm into the descending colon. Skin temperature was recorded with a surface thermistor probe attached to the dorsal surface at the base of the tail. Baseline values were taken for 30 min (at 5-min intervals). Each animal was then placed in a cold box (-13 $\pm$ 1°C) for a period of 1 hr and recordings of all parameters were continued. Measurements were also continued for 1 hr after cold exposure.

In the second experiment, 20 male rats were exposed and 20 were sham exposed to a 100-kV/m field for 1 mo. Basal plasma corticosterone and thyrotropin levels were measured in 10 exposed and 10 sham-exposed animals within 1 hr after exposure. The remaining animals in the exposed and sham-exposed groups (N = 10/group) were placed in a cold box (-13 $\pm$ 1°C) for 1 hr prior to blood sampling.

The same experimental procedures were used for obtaining blood from animals maintained at room temperature as for those exposed to cold stress for 1 hr. Exposed and sham-exposed animals were taken alternately from the electric-field exposure room to another room, where they were anesthetized with a fluothane (Halothane, Ayerst)/$NO_2$/$O_2$ mixture. Blood was drawn via intracardiac puncture, and immediately

centrifuged. The plasma portion was frozen and stored for later analysis. A 3-min time limit was imposed for the procedure, from the time of first handling the animal to the time it took to obtain the blood sample. All sampling (basal and cold stress) was made between 8:00 a.m. and 11:30 a.m. The plasma from each rat was divided into two samples; one sample was analyzed fluorometrically for corticosterone by Battelle's analytical chemistry laboratory, using a modified procedure of Steenburg and Thomasson (1964). The other sample was analyzed at the University of Rochester, using a radioimmune assay technique (Murphy, 1967). Concentrations of thyrotropin were measured at the University of Rochester, by Nancy Lebda, using reagents supplied by Dr. A. F. Parlow of the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Hormone Distribution Program.

Blood pressure and heart-rate measurements were also made on rats during exposure to a 100-kV/m field. The procedure for obtaining blood pressure has already been described. To avoid 60-Hz interference with the electrical signals generated from the blood-pressure transducer, the entire length of the transducer cable was wrapped in grounded, conductive wire braid. After the animals were allowed to recover from surgery, 300 units of heparin sodium (Panheprin, Abbott) were introduced into the femoral artery to help prevent clotting in the arterial cannulae during the exposure and recording period. A Faraday shield was placed over the sham-exposed animal, and measurements of heart rate, pulse and mean blood pressures were recorded simultaneously on exposed and sham-exposed animals. After 0.5 hr of baseline measurements, the field was energized to 100 kV/m for 1 hr while measurements

were continued. Cardiovascular parameters were also recorded for 0.5 hr after terminating exposure.

## C. RESULTS

Results for ECG and heart-rate measurements on rats exposed to electric fields at 80 kV/m for 8 hr, 40 hr or 1 mo were presented in the last Annual Report. No significant differences were found between exposed and sham-exposed groups.

Figures X-1 and X-2 show the heart-rate measurements taken on female rats exposed to 100-kV/m electric fields for 1 and 4 mo, respectively. Statistical analyses of the data (Student's $t$ test) showed no significant differences between exposed and sham-exposed groups in either experiment.

Tables X-1 and X-2 show the blood pressure and vascular reactivity measurements made on rats exposed or sham-exposed to 100-kV/m electric fields for 35 days or 4 mo, respectively. Statistical analysis of the data from the 35-day exposure showed no significant differences in any of the data between exposed or sham-exposed controls (Student's $t$ test). The only significant difference between exposed and sham-exposed groups after 120 days of exposure was in the baseline pulse pressure prior to the second phenylephrine injection [not shown in Table X-1; mean pulse pressure of the exposed group (56 $\pm$ 1 mm Hg) was significantly ($p < 0.01$) higher than that of the sham-exposed group (51 $\pm$ 1 mm Hg)]. However, the increases in mean pressure in response to the first and second injections of phenylephrine were not significantly different between exposed

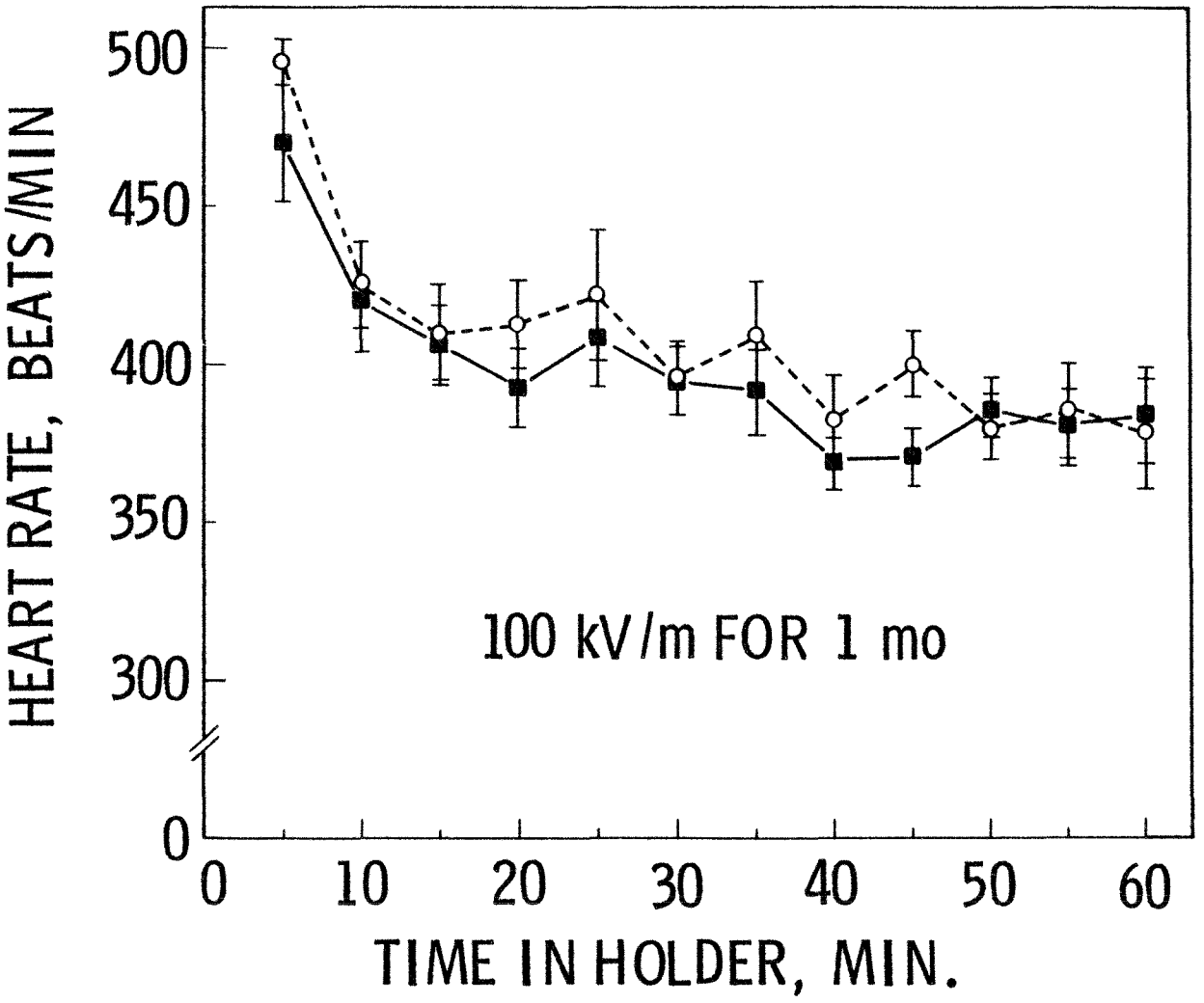



FIGURE X-1. Mean Heart Rates (± S.E.) of Rats After 1 Mo Exposure (■) or Sham Exposure (o) to 60-Hz Electric Fields at 100 kV/m. N = 20/group.

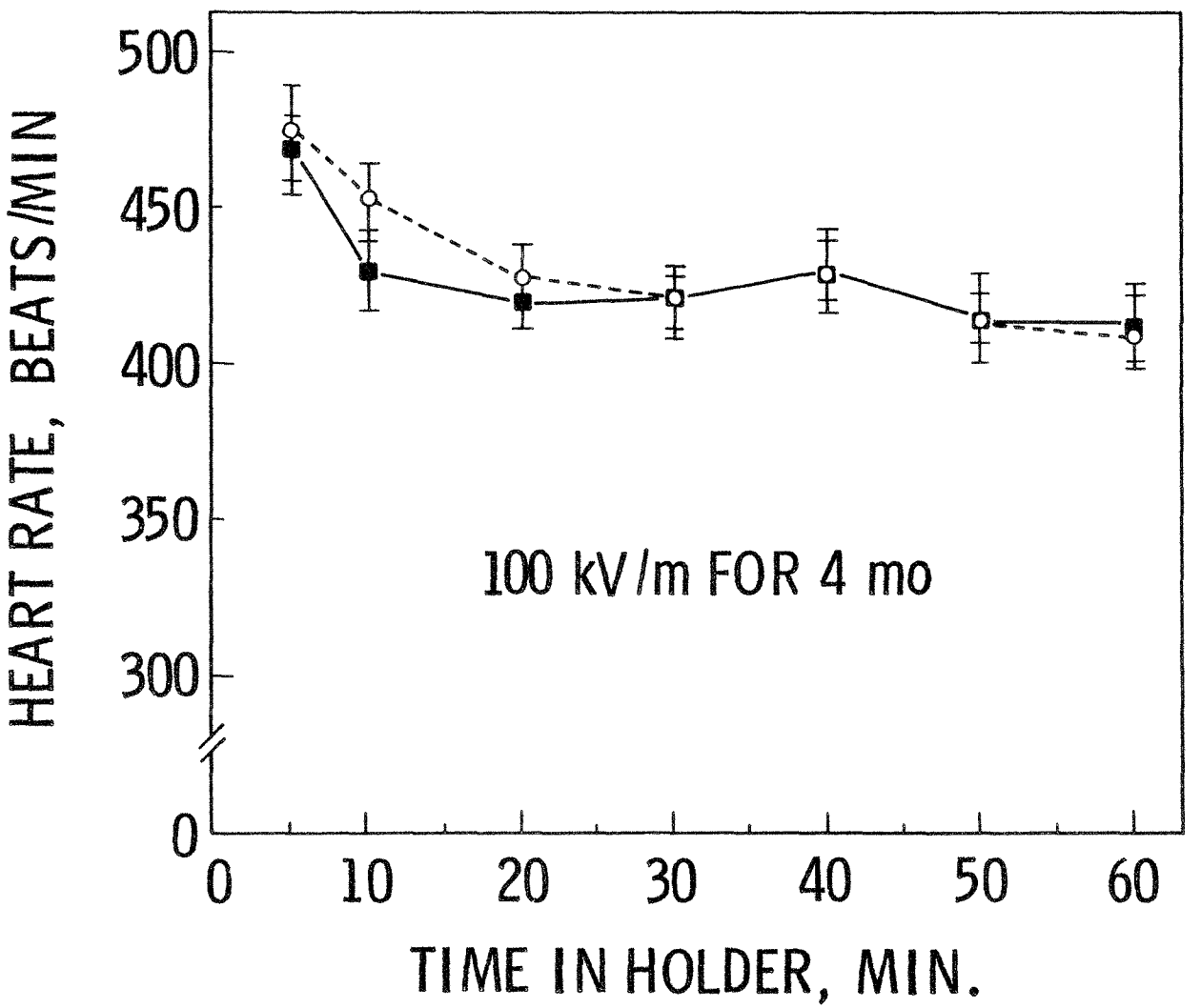



FIGURE X-2. Mean Heart Rates (± S.E.) of Rats After 4 Mo Exposure (■) or Sham Exposure (o) to 60-Hz Electric Fields at 100 kV/m. N = 20/group.

TABLE X-1. Cardiovascular Measurements* of Rats Exposed (N = 12) or Sham-Exposed (N = 13) to a 60-Hz, 100-kV/m, Electric Field for ∿ 20 hr/day for 35 Days*

| | Mean ± S.E. | |
|---|---|---|
| | Exposed | Sham-Exposed |
| Heart Rate (beats/min) | 343 ± 9 | 362 ± 12 |
| Diastolic Pressure (mm Hg) | 95 ± 2 | 97 ± 2 |
| Systolic Pressure (mm Hg) | 155 ± 3 | 160 ± 3 |
| Pulse Pressure (mm Hg) | 60 ± 2 | 63 ± 3 |
| Mean Pressure (mm Hg) | 115 ± 2 | 117 ± 3 |
| Increase in Mean Pressure (mm Hg) - 1st phenylephrine | 34 ± 2 | 34 ± 2 |
| Increase in Mean Pressure (mm Hg) - 2nd phenylephrine | 37 ± 2 | 35 ± 2 |

*Baseline values are shown for each parameter, with additional values showing the change (increase) in mean blood pressure in response to the first and second doses of phenylephrine.

TABLE X-2. Cardiovascular Measurements* of Rats Exposed (N = 12) or Sham-Exposed (N = 13) to a 60-Hz, 100-kV/m Electric Field for ∿ 20 hr/day for 120 Days*

| | Mean ± S.E. | |
|---|---|---|
| | Exposed | Sham-Exposed |
| Heart Rate (beats/min) | 386 ± 5 | 401 ± 13 |
| Systolic Pressure (mm Hg) | 153 ± 3 | 154 ± 2 |
| Diastolic Pressure (mm Hg) | 99 ± 2 | 102 ± 2 |
| Pulse Pressure (mm Hg) | 54 ± 2 | 52 ± 2 |
| Mean Pressure (mm Hg) | 118 ± 2 | 119 ± 3 |
| Increase in Mean Pressure (mm Hg) - 1st phenylephrine | 43 ± 2 | 44 ± 2 |
| Increase in Mean Pressure (mm Hg) - 2nd phenylephrine | 42 ± 1 | 45 ± 1 |

*Baseline values are shown for each parameter, with additional values showing the change (increase) in mean blood pressure in response to the first and second doses of phenylephrine.

and sham-exposed groups. Mean systolic/diastolic pressures in exposed and sham-exposed groups were, respectively, 157/102 and 159/105 after the first injection, and 159/105 and 157/104 after the second injection.

Figures X-3 through X-5 show heart rate, skin temperature and colonic temperature, respectively, of animals exposed to acute cold after exposure or sham exposure to a 100-kV/m electric field. The responses of exposed and sham-exposed animals to cold stress were identical. Recovery after cold exposure was also the same in both groups of rats.

Table X-3 shows the plasma corticosterone and thyrotropin levels in rats exposed to 100-kV/m fields for 1 mo and in rats subjected to cold stress after electric-field exposure for 1 mo.

Mean plasma corticosterone and thyrotropin levels measured in rats exposed or sham-exposed to electric fields were not significantly different (unequal variance $t$ test, $p < 0.05$). Corticosterone and thyrotropin levels in rats subjected to cold stress after electric-field exposure were not significantly different from controls.

The apparent large difference in mean concentration of corticosterone (by radioimmunological assay) between exposed and sham-exposed animals was not statistically significant because of the very high variance within the sham-exposed group. However, variances were statistically different (F-test, $p < 0.05$) between exposed and sham-exposed groups not exposed to cold stress. Accordingly, this part of the experiment (without cold stress) was repeated. The results are shown in Table X-4. We failed to replicate the previous findings. Both mean values and variances were the same in exposed and sham-exposed groups.

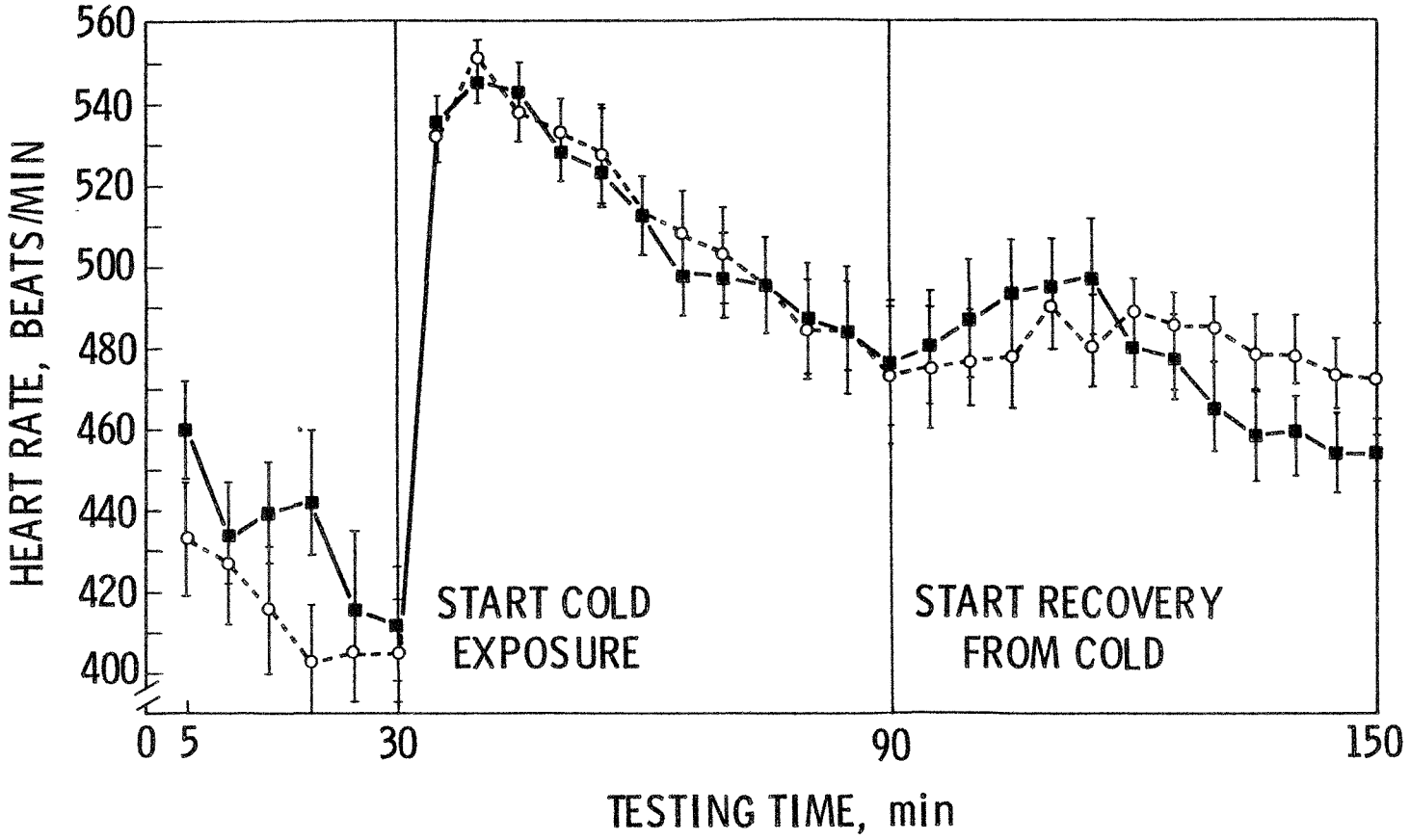



FIGURE X-3. Mean (± S.E.) Heart Rates of Rats Before, During and After Being Subjected to Low Temperature (-13°C). Animals were exposed (■) or sham exposed (o) to 60-Hz electric fields at 100 kV/m for 30 days prior to the cold stress test. N = 10/group.

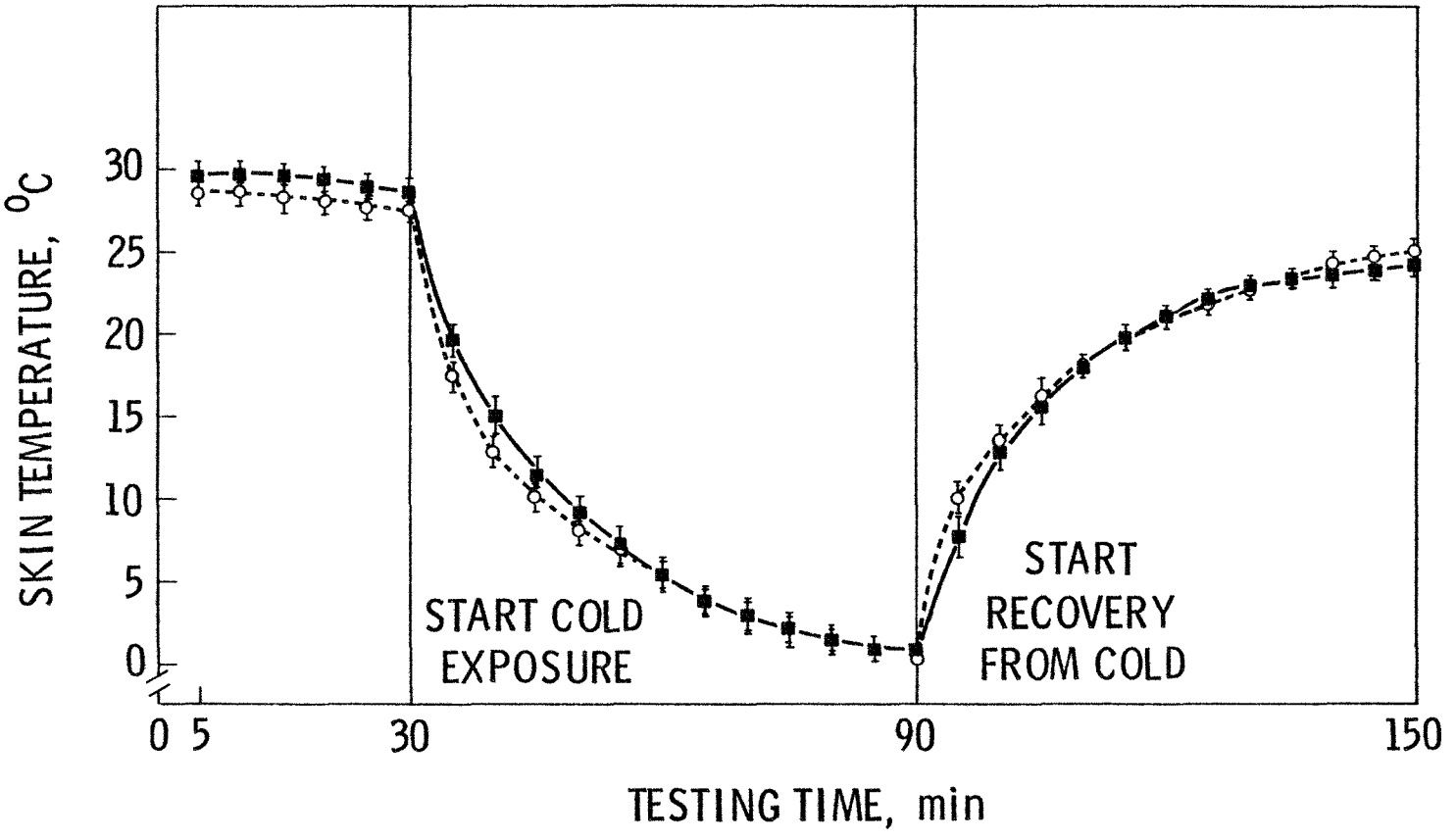



FIGURE X-4. Mean (± S.E.) Skin Temperature of Rats Before, During and After Being Subjected to Low Temperature (-13°C). Animals were exposed (■) or sham exposed (o) to 60-Hz electric fields at 100 kV/m for 30 days prior to cold stress test. N = 10/group.

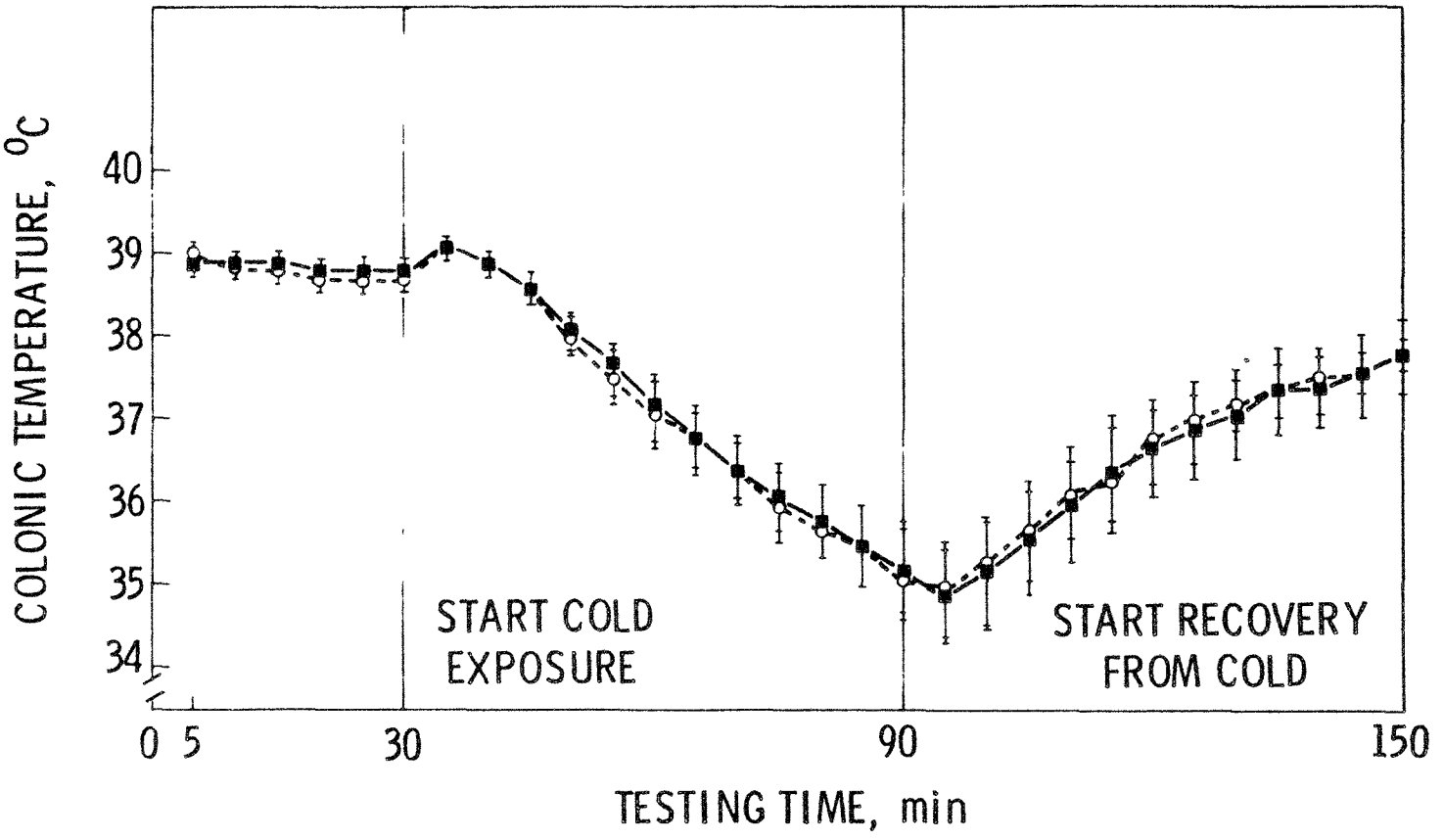



FIGURE X-5. Mean (± S.E.) Deep Colonic (Core) Temperature of Rats Before, During and After Being Subjected to Low Temperature (-13°C). Animals were exposed (■) or sham exposed (o) to 60-Hz electric fields at 100 kV/m for 30 days prior to the cold stress test. N = 10/group.

TABLE X-3. Mean (± S.E.) Plasma Corticosterone and Thyrotropin Levels in Rats Exposed or Sham-Exposed to a 100-kV/m Electric Field for 1 Mo. Blood sampling was done within 1 hr postexposure. In addition to electric-field exposure, half the animals (10 exposed, 10 sham-exposed) were also exposed to cold stress (-13 ± 1°C) for 1 hr prior to blood sampling.

| | Exposed | Sham-Exposed | Exposed + Cold Stress | Sham-Exposed + Cold Stress |
|---|---|---|---|---|
| N | 10 | 10 | 10 | 10 |
| Body Weight (g) | 446 ± 14 | 428 ± 15 | 411 ± 11 | 426 ± 7 |
| Plasma Corticosterone, Fluorometric Technique (ng/ml) | 123 ± 17 | 175 ± 45 | 570 ± 32 | 534 ± 36 |
| Plasma Corticosterone, Radioassay Technique (ng/ml) | 35 ± 8 | 287 ± 138 | 620 ± 28 | 619 ± 40 |
| Plasma Thyrotropin (μ units/ml) | 920 ± 93 | 1122 ± 145 | 546 ± 46 | 577 ± 32 |

TABLE X-4. Mean (± S.E.) Plasma Corticosterone Concentrations (Radioimmune Assay) in Rats Exposed or Sham-Exposed to a 100-kV/m Electric Field for 1 Mo.

| | Corticosterone (ng/ml) | | |
|---|---|---|---|
| | N | Mean | S.E. |
| Exposed | 20 | 42.1 | 11.6 |
| Sham-Exposed | 20 | 35.6 | 9.5 |

Heart rate values and blood pressures of rats during exposure or sham exposure to a 60-Hz electric field at 100 kV/m for 1 hr are shown in Table X-5. Values for both exposed and sham-exposed groups were comparable during the 0.5 hr preceding exposure. Electric-field exposure had no effect on heart rate or blood pressure during the 1-hr exposure period, nor during the 0.5-hr period after exposure.

## D. DISCUSSION AND CONCLUSIONS

Electric-field exposure for periods up to and including 1 mo of duration at 80 or 100 kV/m did not affect the ECG wave pattern or heart rate of rats. Exposures for 1 hr, 35 days or 4 mo at 100 kV/m did not affect heart rate, blood pressure or vascular reactivity. Exposure to a 100-kV/m field for 1 mo did not affect the levels of plasma corticosterone or thyrotropin, and had no effect on the corticosterone, thyrotropin, skin temperature, colonic temperature or heart-rate response to cold stress.

Many of these results cannot be directly compared to other investigators' findings. Cerretelli and Malaguti (1976), Gann (personal communication, 1976), Coate and Teeters (1970) and Poznaniak et al. (1977) each used different species of animals in their studies. Also, in one of those studies, 45-, 50- and 75-Hz frequencies were used, whereas our study used a 60-Hz frequency exclusively. In the experiment by Coate and Teeters (1970), the animals were exposed not only to electric fields, but also to a magnetic field and a ground current. The observed effects might have been due to any one or a combination of these three components.

TABLE X-5. Heart Rate, Mean Blood Pressure, Systolic Pressure, Diastolic Pressure and Pulse Pressure of Rats Before, During and After Exposure or Sham Exposure (N = 12/group) to a 100-kV/m Electric Field for 1 hr*

| | Pre-exposure Baseline | | During Exposure | | Postexposure | |
|---|---|---|---|---|---|---|
| | Exposed | Sham-Exposed | Exposed | Sham-Exposed | Exposed | Sham-Exposed |
| Heart Rate (beats/min) | 419 ± 8 | 417 ± 12 | 406 ± 8 | 417 ± 10 | 395 ± 8 | 408 ± 10 |
| Mean Arterial Blood Pressure (mm Hg) | 121 ± 3 | 122 ± 2 | 120 ± 3 | 118 ± 2 | 119 ± 3 | 115 ± 2 |
| Systolic Blood Pressure (mm Hg) | 155 ± 3 | 158 ± 3 | 151 ± 3 | 152 ± 2 | 151 ± 3 | 148 ± 2 |
| Diastolic Blood Pressure (mm Hg) | 104 ± 2 | 103 ± 2 | 102 ± 2 | 102 ± 1 | 102 ± 3 | 100 ± 1 |
| Pulse Pressure | 52 ± 2 | 54 ± 2 | 49 ± 3 | 50 ± 2 | 49 ± 3 | 48 ± 2 |

*Values before, during and after exposure are the respective means of measurements made over 0.5-, 1- and 0.5-hr intervals.

Marino et al. (1977) reported that exposure of rats to a 60-Hz, 15-kV/m electric field for 30 days resulted in physiological stress. In one of two experiments that we conducted, we found that the exposed group exhibited lower (but not significantly different) corticosterone levels in the plasma. Also, in one of our two experiments, variance among sham-exposed rats was significantly higher than among exposed rats.

## E. FUTURE PLANS

In view of our failure to detect any effects of 60-Hz electric field exposure on cardiovascular function, no further research in this area is planned.

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# XI. NEUROPHYSIOLOGY

Principal Investigator: R. A. Jaffe

## ABSTRACT

Following 30 days of electric-field exposure, superior cervical sympathetic ganglia, vagus and sciatic nerves were removed from rats anesthetized with urethan, placed in a temperature-controlled chamber (37.5 ± 4°C) and superfused with a modified mammalian Ringer's solution equilibrated with 95% $O_2$ and 5% $CO_2$. Several parameters and tests were used to characterize synaptic transmission and peripheral nerve function. These included amplitude, area, and configuration of the postsynaptic or whole-nerve compound action potential; conduction velocity; accommodation; refractory period; strength-duration curves; conditioning-test response; frequency response; post-tetanic response; and high-frequency-induced fatigue. The results of the neurophysiologic tests and measurements so far completed indicate that synaptic transmission is significantly and consistently affected by chronic (30-day) exposure to a 60-Hz, high-voltage electric field. In addition, there are trends in the data that suggest a generalized increase in neuronal excitability in exposed animals.

## A. INTRODUCTION

Several reports in the literature on biological effects of electric fields have suggested that mammalian and avian nervous systems are affected by exposure to extremely low-frequency (16-60 Hz) electric fields (Adey and Bawin, 1977; Carter and Graves, 1976; Dumansky et al., 1976). The purpose of this study is to examine specific components of the mammalian (rat) nervous system in three broad areas (synaptic transmission, peripheral nerve function, neuromuscular function), in order to detect any possible effects of chronic (30-day) exposure to a 100-kV/m, 60-Hz electric field. The experiments are designed to detect potential

effects prior to the development of obvious clinical signs, thereby providing realistic estimates of threshold exposure levels.

In the last Annual Report (Phillips et al., 1978) data were presented which suggested that synaptic transmission in superior cervical sympathetic ganglia of rats was affected by exposure of the whole animal for 30 days to a 100-kV/m, 60-Hz electric field. Specifically, we reported that animals exposed to an electric field showed a slight but detectable shift in the conditioning-test (C-T) ratio ($p < 0.05$ @ 300 msec; $p < 0.08$ overall), which suggested increased excitability. There was also an increase in time-to-half-amplitude during fatigue testing ($p < 0.05$), which suggested a decrease in susceptibility to fatigue by high-frequency stimulation. These findings prompted us to repeat the experiments in order to verify the results. In this report, results from the replicate experiments on synaptic transmission are presented.

Results from experiments on peripheral nerve function also are presented in this report. Data from these experiments strongly suggested that the conduction velocity of the action potential in unmyelinated nerve fibers was increased (+10%; $p < 0.01$) following 30 days of exposure to a 100-kV/m, 60-Hz electric field. This increase in conduction velocity was accompanied by a shift in the strength-duration curve consistent with increased excitability of the nerve membrane. However, the results of two replicate experiments on unmyelinated (vagal) nerve fibers failed to confirm the increased conduction velocity observed in the first experiment. Similar experiments performed on myelinated

nerve fibers failed to reveal any difference between exposed and sham-exposed animals. Details of these experiments are discussed below.

## B. SYNAPTIC TRANSMISSION REPLICATE STUDY

1. Methods. A total of 46 male Sprague-Dawley (Hilltop) rats were examined. One group of 24 rats was exposed to a 100-kV/m, 60-Hz electric field for 30 days. The other group (22 rats) was maintained in identical housing without the electric field (sham-exposed). The exposure system has been described in detail in previous reports (Phillips and Kaune, 1977; Phillips et al., 1978).

Superior cervical sympathetic ganglia, including segments of the cervical sympathetic trunk (CST) and internal carotid nerve (ICN), were removed from 24 electric-field-exposed and 22 sham-exposed rats. Rats were anesthetized with urethan (1.25 g/kg) and the ganglia were removed within 2 hr after completion of the 30-day exposure period for C-T ratio measurements, and between 4 and 6 hr postexposure for the second ganglion fatigue study. Ganglia were maintained in vitro in a specially constructed, temperature-controlled lucite chamber, where they were continuously superfused with a modified mammalian Ringer's solution equilibrated with 95% $O_2$ and 5% $CO_2$.

Suction electrodes were used for both stimulation of CST and recording of ICN activity. Presynaptic nerve fibers were stimulated supramaximally by square-wave pulses of 0.3-msec duration. A signal-averaging computer was used to obtain detailed time and amplitude

information during the course of individual experiments. In addition, postsynaptic compound action potentials were recorded photographically for subsequent analyses.

Several parameters were used to compare the properties of synaptic transmission in the two groups:

(1) Characteristics of the postsynaptic compound action potential [including amplitude, area, configuration, maximum rate of rise (dV/dt), maximum rate of fall (-dV/dt) and time relationships]

(2) Conduction velocity of the compound action potential--measured between the stimulating and recording electrodes, therefore including synaptic delay

(3) Conditioning-test response--an adaptation of the method used by Whitcomb and Santolucito (1976), which measured the excitability and the refractory period of the ganglionic synapses. This is done by applying two stimuli to the preganglionic nerve and measuring the postganglionic action potential heights as a function of the time interval between the stimuli

(4) High-stimulus, frequency-induced fatigue--preganglionic nerve fibers were stimulated at 20 Hz for 60 min while the amplitude of the postganglionic action potential was monitored. Recovery at a stimulus frequency of 0.5 Hz was measured during the subsequent 10 min. In order to duplicate the original experiment on second ganglia, the ganglia used for this fatigue test were left in the urethan-anesthetized animal 4-6 hr before removal and subsequent fatigue testing.

Differences between means of exposed and sham-exposed animals were compared by the Student's $t$ test. A p value less than 0.05 was considered to be significant.

2. Results. No significant differences between electric-field-exposed and sham-exposed animals were found among the parameters used to characterize the postsynaptic compound action potentials except for the rate of rise, dV/dt (Table XI-1). Measurements of the conduction velocity (reduced by inclusion of synaptic delay) are shown in Table XI-2. No significant differences were found between exposed and sham-exposed animals.

The C-T response ratio was different in this replicate study, as in the first study, for electric-field-exposed animals compared to sham-exposed animals (Figure XI-1). Mean values for the postsynaptic compound action potential height ratio at specific conditioning-test intervals are shown in Tables XI-3a and 3b. The tables differ in the method used to acquire the data: In Table XI-3a, C-T ratios were determined by direct measurement of traces on a storage oscilloscope; while in Table XI-3b, C-T ratios were determined by a computer average at four separate trials at each time interval. Although the data presented in both tables are qualitatively identical, slight differences in statistical significance are apparent. In the original experiment (see Phillips, et al., 1978), the C-T ratio at only one of the 11 C-T intervals was significantly ($p < 0.05$) increased for electric-field-exposed animals compared to the sham-exposed controls; whereas in this replicate experiment, seven of the 11 C-T ratios were significantly ($p < 0.05$) increased. A statistical

TABLE XI-1. Characteristics of the Postsynaptic Compound Action Potential Recorded from Rat Superior Cervical Sympathetic Ganglia Following 30 days Exposure to a 60-Hz, 100-kV/m Electric Field.

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p* |
| Amplitude (mV) | 40 | 8.65 ± 0.29 | 30 | 8.22 ± 0.30 | 0.311 |
| Area (mV•msec) | 40 | 46.6 ± 1.7 | 30 | 44.3 ± 1.8 | 0.268 |
| Rate of Rise (+dV/dt) (V/sec) | 40 | 5.00 ± 0.18 | 30 | 4.37 ± 0.21 | 0.024 |
| Time before Peak to Maximum +dV/dt (msec) | 40 | 2.2 ± 0.1 | 30 | 2.1 ± 0.1 | 0.309 |
| Rate of Fall (-dV/dt)(V/sec) | 40 | -4.08 ± 0.30 | 30 | -4.10 ± 0.19 | 0.948 |
| Time after Peak to Maximum -dV/dt (msec) | 40 | 2.4 ± 0.1 | 30 | 2.4 ± 0.1 | 0.789 |

* p values determined using Student's *t* test

TABLE XI-2. Conduction Velocity (Including Synaptic Delay) of the Postsynaptic Compound Action Potential Recorded from the Internal Carotid Nerve of the Rat Superior Cervical Sympathetic Ganglion Following 30 Days Exposure to a 60-Hz, 100-kV/m Electric Field.

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p* |
| Peak Conduction Velocity (m/sec) | 40 | 0.40 ± 0.01 | 29 | 0.38 ± 0.01 | 0.233 |
| Chamber Temperature (°C) | 40 | 32.1 ± 0.1 | 30 | 32.1 ± 0.1 | 0.540 |
| Inflection Conduction Velocity (m/sec) | 40 | 0.69 ± 0.01 | 29 | 0.69 ± 0.02 | 0.868 |

*p values determined using Student's $t$ test

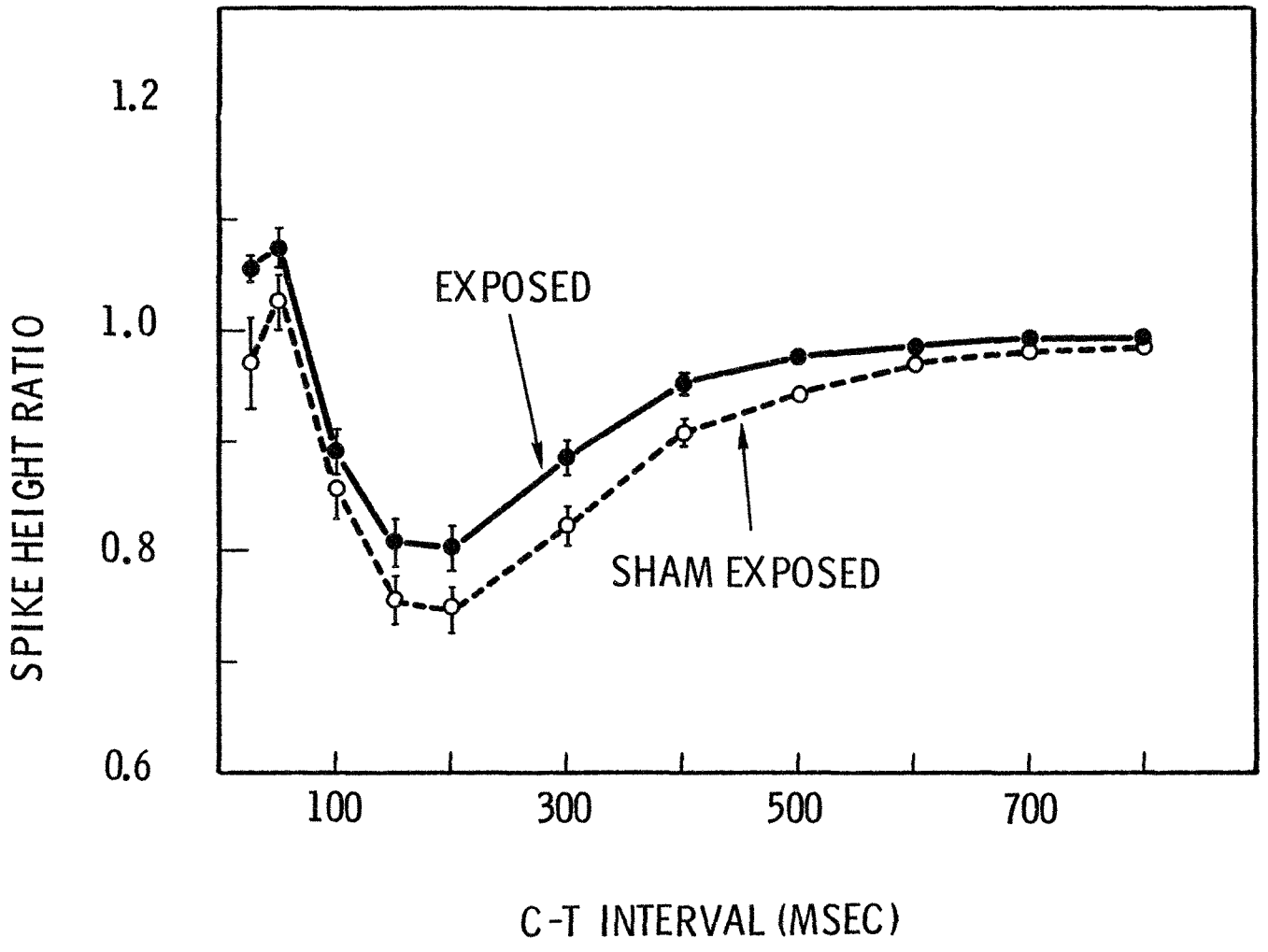



FIGURE XI-1. Comparison of Conditioning-Test Response Ratios of Rats Exposed and Sham Exposed to 60-Hz Electric Fields at 100 kV/m

TABLE XI-3a. Mean Values (± S.E.) for the Postsynaptic Compound Action Potential (Spike) Height Ratio at Specific Conditioning-Test Intervals. Conditioning stimulus rate = 0.5 Hz. Ratios determined by direct measurement from CRT.

| Conditioning-Test Interval (msec) | | C-T Ratio, Exposed | C-T Ratio, Sham-Exposed |
|---|---|---|---|
| 25 | Mean | 1.06* | 1.00 |
| | ± S.E. | 0.01 | 0.03 |
| | N | 17 | 10 |
| 50 | Mean | 1.08 | 1.03 |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 11 |
| 100 | Mean | 0.89 | 0.86 |
| | ± S.E. | 0.02 | 0.03 |
| | N | 19 | 11 |
| 150 | Mean | 0.81 | 0.76 |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 11 |
| 200 | Mean | 0.80 | 0.75 |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 11 |
| 300 | Mean | 0.88* | 0.82* |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 11 |
| 400 | Mean | 0.95* | 0.91* |
| | ± S.E. | 0.01 | 0.01 |
| | N | 19 | 11 |
| 500 | Mean | 0.98* | 0.94* |
| | ± S.E. | 0.005 | 0.007 |
| | N | 19 | 11 |
| 600 | Mean | 0.99* | 0.97* |
| | ± S.E. | 0.004 | 0.005 |
| | N | 19 | 11 |
| 700 | Mean | 1.00* | 0.98* |
| | ± S.E. | 0.003 | 0.004 |
| | N | 19 | 11 |
| 800 | Mean | 1.00* | 0.99* |
| | ± S.E. | 0.002 | 0.003 |
| | N | 19 | 11 |

*Significantly different (Student's $t$ test, $p < 0.05$)

TABLE XI-3b. Mean Values (± S.E.) for the Postsynaptic Compound Action Potential (Spike) Height Ratio at Specific Conditioning-Test Intervals. Conditioning stimulus rate = 0.5 Hz. Ratios determined by computer average of four separate trials at each interval.

| Conditioning-Test Interval (msec) | | C-T Ratio, Exposed | C-T Ratio, Sham-Exposed |
|---|---|---|---|
| 25 | Mean | 1.06 | 1.03 |
| | ± S.E. | 0.01 | 0.03 |
| | N | 17 | 9 |
| 50 | Mean | 1.08 | 1.03 |
| | ± S.E. | 0.02 | 0.03 |
| | N | 19 | 10 |
| 100 | Mean | 0.90 | 0.84 |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 10 |
| 150 | Mean | 0.81 | 0.75 |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 10 |
| 200 | Mean | 0.80* | 0.74* |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 10 |
| 300 | Mean | 0.88* | 0.82* |
| | ± S.E. | 0.02 | 0.02 |
| | N | 19 | 10 |
| 400 | Mean | 0.95* | 0.91* |
| | ± S.E. | 0.01 | 0.01 |
| | N | 19 | 10 |
| 500 | Mean | 0.98* | 0.94* |
| | ± S.E. | 0.005 | 0.008 |
| | N | 19 | 10 |
| 600 | Mean | 0.99* | 0.97* |
| | ± S.E. | 0.005 | 0.005 |
| | N | 19 | 10 |
| 700 | Mean | 0.99* | 0.98* |
| | ± S.E. | 0.003 | 0.006 |
| | N | 19 | 10 |
| 800 | Mean | 1.00* | 0.99* |
| | ± S.E. | 0.002 | 0.002 |
| | N | 19 | 8 |

*Significantly different (Student's $t$ test, $p < 0.05$)

analysis of the original C-T ratio curves yielded a 92% chance that the curves were different ($p = 0.08$, not statistically significant). An analysis of the curves in the present experiment, however, indicates that they are significantly different ($p < 0.05$; multivariate test of the coefficients, obtained from fitting orthogonal polynomials to the curves).

Synaptic fatigue was tested by stimulating the presynaptic nerve trunk at 20 Hz for 60 min. The amplitude of the postsynaptic compound action potential was measured at intervals throughout the period of high-frequency stimulation (20 Hz) and during a 10-min recovery period at the control stimulus frequency of 0.5 Hz. These data are presented in Table XI-4, reported as the ratio of the spike amplitude during fatigue and recovery to the spike amplitude measured prior to the start of the test. There were no significant differences between electric-field-exposed and sham-exposed animals. In addition, the calculated values for times-to-half-amplitude (exposed = 6.7 $\pm$ 1.3 min; sham-exposed = 10.6 $\pm$ 3.1 min) were not significantly different for either group. The variability of the times-to-half-amplitude for the exposed group, however, was significantly less (F-test; $p < 0.001$) than that for the sham-exposed group.

3. Discussion and Conclusions. The difference in the C-T response ratio between exposed and sham-exposed animals suggested in the original experiment is substantiated in the present experiment. The shape of the

TABLE XI-4. Fatigue Response to High-Frequency Stimulation. Superior cervical sympathetic ganglia were stimulated presynaptically at 20 Hz for 60 Min. The amplitude of the postsynaptic compound action potential was measured (computer averaged) at intervals throughout the period of stimulation. Data are expressed as the ratio of the 20-Hz spike amplitude to the amplitude of the spike during 0.5-Hz control stimulation. Stimulus frequency during recovery = 0.5 Hz.

| | FATIGUE | | | | | | | | | | | RECOVERY | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | 1 | 2 | 3 | 4 | 5 | 10 | 15 | 20 | 30 | 45 | 60 | 1 | 2 | 5 | 10 |
| Exposed | | | | | | | | | | | | | | | |
| Mean | 0.79 | 0.70 | 0.63 | 0.56 | 0.54 | 0.44 | 0.40 | 0.42 | 0.41 | 0.41 | 0.39 | 0.68 | 0.75 | 0.82 | 0.84 |
| S.E. | 0.03 | 0.03 | 0.04 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.03 | 0.03 | 0.03 | 0.02 | 0.04 |
| N | 18 | 19 | 18 | 17 | 18 | 17 | 18 | 19 | 19 | 20 | 20 | 18 | 19 | 20 | 20 |
| Sham-Exposed | | | | | | | | | | | | | | | |
| Mean | 0.80 | 0.72 | 0.67 | 0.60 | 0.58 | 0.47 | 0.44 | 0.43 | 0.44 | 0.45 | 0.45 | 0.67 | 0.74 | 0.80 | 0.79 |
| S.E. | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.02 | 0.03 | 0.03 | 0.02 | 0.03 | 0.03 | 0.03 |
| N | 19 | 18 | 17 | 16 | 18 | 17 | 18 | 19 | 19 | 19 | 19 | 16 | 18 | 19 | 19 |
| p | NS* | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

*NS = not significant ($p > 0.05$)

C-T curve is determined by two overlapping phenomena: relative refractory period and recruitment from subliminally excited groups of postsynaptic neurons. Thus, as the interval between the conditioning and test stimuli decreases, increasing numbers of neurons enter their relative refractory periods and fail to elicit a postsynaptic response, causing the amplitude of the test spike to decrease relative to the conditioning spike. At the shorter C-T intervals, however, temporal summation of stimuli permits recruitment of subliminally excited neurons and the test spike amplitude increases, eventually exceeding the amplitude of the conditioning spike. The upward shift in the C-T curve seen in electric-field-exposed animals is consistent with a hypothesis of a persistent field-induced increase in neuronal excitability.

In the original experiment, the exposed and sham-exposed fatigue curves for ganglia that were allowed to remain in the urethan-anesthetized animal for several hours ("second" ganglia) were not significantly different. The times-to-half-amplitude, however, were significantly different (7 exposed = 16.2 $\pm$ 5.5 min; 8 sham-exposed = 2.8 $\pm$ 0.7 min; $p < 0.05$). In the present study, under conditions duplicating those of the original experiment (see interexperiment comparison of standard parameters, Table XI-5), this difference was not confirmed. It is likely that the results from the original second ganglion fatigue experiment were spurious. In that experiment, the fatigue curves for several of the exposed animals, although rapidly decreasing toward half-amplitude in the first few minutes, failed to actually reach half-amplitude for

TABLE XI-5. Mean Values (± S.E.) for Standard Parameters Measured at the Time of the Original Post-Synaptic Compound Action Potential Experiment (I) Compared with the same Parameters Measured During the Replicate Experiment (II). (N shown in parentheses.)

| | I | | II | |
|---|---|---|---|---|
| | Exposed | Sham-Exposed | Exposed | Sham-Exposed |
| Action Potential Amplitude (mV) | 7.6 ± 0.6 (12) | 7.2 ± 0.5 (15) | 8.6 ± 0.3 (40) | 8.2 ± 0.3 (30) |
| Action Potential Area (mV•msec) | 36.7 ± 3.4 (12) | 35.6 ± 3.3 (15) | 46.6 ± 1.7 (40) | 44.3 ± 1.8 (30) |
| Inflection Conduction Velocity (m/sec) | 0.78 ± 0.03 (11) | 0.85 ± 0.08 (12) | 0.69 ± 0.01 (40) | 0.69 ± 0.02 (29) |
| Peak Conduction Velocity (m/sec) (Including synaptic delay) | 0.60 ± 0.02 (11) | 0.63 ± 0.04 (11) | 0.40 ± 0.01 (40) | 0.38 ± 0.01 (29) |
| Chamber Temperature (°C) | 32.1 ± 0.1 (11) | 31.9 ± 0.1 (12) | 32.1 ± 0.1 (40) | 32.1 ± 0.1 (30) |
| Age of Rats (days) | 83.6 ± 0.6 (14) | 84.2 ± 0.6 (15) | 88.8 ± 0.6 (24) | 88.3 ± 0.5 (22) |

30-45 min. Thus, had the time-to-0.4-amplitude been selected for comparison, no difference would have been observed. Results from the present fatigue experiment on "second" ganglia closely resemble those obtained with "first" ganglia reported for the original experiment in the 1978 Annual Report. The fatigue curves for electric-field-exposed and sham-exposed animals are not significantly different.

## C. PERIPHERAL NERVE FUNCTION: ORIGINAL AND REPLICATE STUDIES

1. Methods. Thirty-six male Sprague-Dawley (Hilltop) rats, 77 to 84 days of age, were exposed to a 100-kV/m, 60-Hz electric field for 30 days, while an equal number were maintained in identical housing without the electric field (sham-exposed). The exposure system has been described in detail in previous reports (Phillips and Kaune, 1977; Phillips et al., 1978).

A segment of a cervical vagus nerve was removed from each of 18 electric-field-exposed and 18 sham-exposed rats. The sciatic nerve, including a segment of the tibial nerve, was removed from the remaining 18 electric-field-exposed and 18 sham-exposed rats. Rats were anesthetized with urethan (1.25 g/kg) and the nerves were removed within 2-5 hr after the completion of the 30-day exposure period. Nerves were maintained in vitro in a specially constructed, temperature-controlled ($37.5 \pm 0.3°C$) lucite chamber, where they were continuously superfused with a modified mammalian Ringer's solution equilibrated with 95% $O_2$ and 5% $CO_2$.

Platinum-iridium electrodes were used to stimulate and record activity in sciatic nerves, and suction electrodes were used to stimulate and record activity in vagus nerves. Compound action potentials were recorded photographically for subsequent analyses. In addition, a signal-averaging computer was used to obtain detailed time and amplitude information during the course of individual experiments.

Several parameters were used to compare peripheral nerve function in the exposed and sham-exposed groups:

(1) Characteristics of the compound action potential [including amplitude, area, configuration, rate of rise (dV/dt), and rate of fall (-dV/dt)]

(2) Conduction velocity of the compound action potential, measured at the beginning of the compound action potential (inflection conduction velocity), and at the peak of the compound action potential (peak conduction velocity); the signal-averaging computer was used to obtain an "averaged" (N = 16) peak conduction velocity.

(3) Absolute and relative refractory periods--measured by applying two supramaximal stimuli to the nerve and measuring the height of the second compound action potential as a function of the time interval between the stimuli

(4) Accommodation index--stimulating current was applied as a slowly increasing ramp function with a fixed duration (5 msec for sciatic nerves, 25 msec for vagus nerves); the amplitude

of the stimulus ramp necessary to elicit a threshold response was defined as the accommodation index

(5) Strength-duration curve--the stimulus strength (μA) necessary to elicit a threshold response at fixed stimulus durations

(6) Changes in poststimulus excitability--nerves were repeatedly stimulated with two identical submaximal stimuli. The interval between the stimuli (conditioning-test interval) was adjusted until the first compound action potential (conditioning response) was double its original amplitude. The interval was then increased until the second compound action potential (test response) was at: (1) one-half its control amplitude, (2) lowest amplitude and (3) highest amplitude relative to its control amplitude.

In the replicate study, a group of 14 male, Sprague-Dawley (Hilltop) rats was exposed to a 100-kV/m, 60-Hz electric field for 30 days while an equal number was maintained in identical housing without the electric field (sham-exposed). The exposure system in the replicate was the same as in the original study. Segments of one or both cervical vagus nerves were removed 2-5 hr following the completion of the 30-day exposure period.

In the replicate experiment, the following parameters were examined:

(1) Characteristics of the compound action potential [including amplitude, area, configuration, rate of rise (dV/dt), and rate of fall (-dV/dt)]

(2) Conduction velocity of the compound action potential, measured at the peak of the B component and at the peak of the C component; this differs from the original experiment in that the inflection conduction velocity was not measured. Instead, conduction velocity was measured at the more precisely definable peak of the B component. A signal-averaging computer was used to obtain all conduction velocities.

(3) Strength-duration-curve--the stimulus strength (μA) necessary to elicit a threshold response at fixed stimulus durations was measured.

Unless otherwise noted, differences between means of exposed and sham-exposed animals were compared by the Student's $t$ test (unpaired, unequal or equal variance). Differences were considered to be significant if $p < 0.05$.

2. Results: Original Experiment. Significant differences between electric-field-exposed and sham-exposed animals were observed among the properties used to characterize peripheral nerve function. Table XI-6 contains mean values for the maximum amplitude and area of the compound action potential, as well as relative values for the maximum rates of potential change during the rising and falling phases. The maximum rate at which the compound action potential decreases appears to be reduced by electric-field exposure, although this effect is significant only for vagus (unmyelinated) nerve fibers. Conduction velocity data (Table XI-7)

TABLE XI-6. Properties of the Compound Action Potential in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p |
| | | Vagus Nerve | | | |
| Maximum Amplitude (mV) | 18 | 1.87 ± 0.14 | 15 | 2.12 ± 0.13 | NS* |
| Area (mV•msec) | 18 | 11.56 ± 1.10 | 14 | 11.32 ± 1.12 | NS |
| Maximum dV/dt Rise (V/sec) | 17 | 6.16 ± 0.61 | 16 | 7.62 ± 0.94 | NS |
| Maximum dV/dt Fall (V/sec) | 9 | -1.91 ± 0.21 | 7 | -2.39 ± 0.40 | <0.05 |
| | | Sciatic Nerve | | | |
| Maximum Amplitude (mV) | 14 | 9.78 ± 0.75 | 14 | 8.30 ± 0.84 | NS |
| Area (mV•msec) | 15 | 2.42 ± 0.15 | 15 | 2.06 ± 0.26 | NS |
| Maximum dV/dt Rise (V/sec) | 5 | 17.0 ± 1.8 | 7 | 15.5 ± 1.6 | NS |
| Maximum dV/dt Fall (V/sec) | 5 | -6.70 ± 0.62 | 7 | -8.66 ± 2.87 | NS |

*NS = Not statistically significant ($p > 0.05$, Student's $t$ test)

TABLE XI-7. Conduction Velocities of Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p |
| | | Vagus Nerve | | | |
| Inflection Conduction Velocity (m/sec) | 18 | 0.90 ± 0.02 | 17 | 0.82 ± 0.02 | 0.0097 |
| Peak Conduction Velocity (m/sec) | 18 | 0.74 ± 0.01 | 17 | 0.66 ± 0.02 | 0.0011 |
| Computer Averaged Conduction Velocity (m/sec) | 18 | 0.72 ± 0.01 | 17 | 0.66 ± 0.02 | 0.0021 |
| Nerve Chamber Temperature (°C) | 18 | 37.6 ± 0.04 | 17 | 37.5 ± 0.04 | NS* |
| | | Sciatic Nerve | | | |
| Inflection Conduction Velocity (m/sec) | 13 | 108 ± 15 | 13 | 121 ± 17 | NS |
| Peak Conduction Velocity (m/sec) | 13 | 51 ± 6 | 12 | 50 ± 5 | NS |
| Computer Averaged Conduction Velocity (m/sec) | 12 | 50 ± 4 | 11 | 55 ± 3 | NS |
| Nerve Chamber Temperature (°C) | 17 | 37.4 ± 0.1 | 14 | 37.5 ± 0.1 | NS |

*NS = Not statistically significant ($p > 0.05$, Student's $t$ test)

indicate that electric-field exposure significantly increases conduction velocity in unmyelinated (vagus) nerve fibers but has no effect on myelinated (sciatic) nerve fibers.

In Table XI-8, refractory period and accommodation index measurements [parameters (3) and (4)] from both vagus and sciatic nerves are compared. No significant differences were found between exposed and sham-exposed animals for any of these parameters. The magnitude of the stimulus current necessary for threshold stimulation at fixed stimulus durations [parameter (5)] is shown in Table XI-9. These data were used to plot the strength-duration curves shown in Figure XI-2 for both vagus and sciatic nerves. Preliminary statistical evaluation of these curves using a repeated measures analysis suggests the possibility that chronic electric-field exposure may preferentially affect excitability in nonmyelinated (vagus) nerve fibers. No significant effects of electric-field exposure were observed on poststimulus excitability [parameter (6)] in either vagus or sciatic nerves (Table XI-10).

3. Results: Replicate Experiment. No significant differences were observed between electric-field-exposed and sham-exposed animals in any of the parameters used to characterize unmyelinated nerve fiber (C-fiber) function. Specific properties of the compound action potential are presented in Table XI-11. In the original experiment, the maximum rate at which the action potential returned to its resting level (dV/dt fall, Table XI-6, original experiment) was significantly slower among electric-field-exposed animals. In the replicate experiment (dV/dt C,

TABLE XI-8. Refractory Period and Accommodation Measured in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric Field (or Sham) Exposure

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p |
| | | Vagus Nerve | | | |
| Absolute Refractory Period (msec) | 18 | 1.11 ± 0.02 | 17 | 1.13 ± 0.05 | NS* |
| 50% Relative Refractory Period (msec) | 18 | 1.94 ± 0.26 | 17 | 1.79 ± 0.12 | NS |
| Accommodation Index | 14 | 3.36 ± 0.25 | 12 | 3.23 ± 0.23 | NS |
| | | Sciatic Nerve | | | |
| Absolute Refractory Period, (msec) | 16 | 0.38 ± 0.02 | 16 | 0.35 ± 0.01 | NS |
| 50% Relative Refractory Period (msec) | 16 | 0.51 ± 0.02 | 16 | 0.52 ± 0.02 | NS |
| Accommodation Index | 13 | 2.47 ± 0.15 | 14 | 2.38 ± 0.06 | NS |

*NS = Not statistically significant ($p > 0.05$, Student's $t$ test)

TABLE XI-9. Stimulus Strength Required for Threshold Stimulation at Fixed Stimulus Durations in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure. Data are presented as mean ± S.E. with N shown in parentheses.

| Stimulus Duration (msec) | Stimulus Strength (μA) | | | |
|---|---|---|---|---|
| | Vagus Nerve | | Sciatic Nerve | |
| | Exposed | Sham-Exposed | Exposed | Sham-Exposed |
| 5 | 11.7 ± 1.0 (18) | 12.6 ± 0.8 (17) | 5.0 ± 0.8 (15) | 4.3 ± 0.4 (17) |
| 2 | 12.8 ± 1.0 (18) | 13.8 ± 0.8 (17) | 4.9 ± 0.8 (15) | 4.3 ± 0.4 (17) |
| 1 | 16.8 ± 1.2 (18) | 18.6 ± 1.0 (17) | 5.0 ± 0.8 (15) | 4.3 ± 0.4 (17) |
| 0.5 | 27.4 ± 2.0 (18) | 29.7 ± 1.7 (17) | 5.8 ± 0.8 (15) | 5.1 ± 0.5 (17) |
| 0.2 | 52.0 ± 3.4 (18) | 58.1 ± 3.6 (17) | 8.9 ± 1.1 (15) | 8.5 ± 1.0 (17) |
| 0.1 | 85.8 ± 5.6 (18) | 96.7 ± 5.9 (17) | 14.6 ± 2.1 (15) | 13.7 ± 1.8 *17) |
| 0.08 | 110 ± 10 (18) | 130 ± 12 (17) | 18.4 ± 2.5 (15) | 17.6 ± 2.2 (17) |
| 0.06 | 152 ± 18 (17) | 191 ± 20 (17) | 23.3 ± 3.0 (15) | 22.9 ± 2.6 (17) |
| 0.04 | 261 ± 23 (17) | 307 ± 21 (17) | 32.0 ± 3.8 (15) | 32.5 ± 3.3 (17) |
| 0.02 | 504 ± 33 (17) | 573 ± 34 (17) | 57.8 ± 6.4 (15) | 58.8 ± 5.7 (17) |
| 0.01 | 854 ± 57 (17) | 972 ± 59 (17) | 90.4 ± 9.3 (14) | 97.4 ± 12.6 (16) |
| Chronaxie (msec) | 0.66 ± 0.05 (18) | 0.65 ± 0.04 (17) | 0.25 ± 0.03 (6) | 0.24 ± 0.03 (8) |

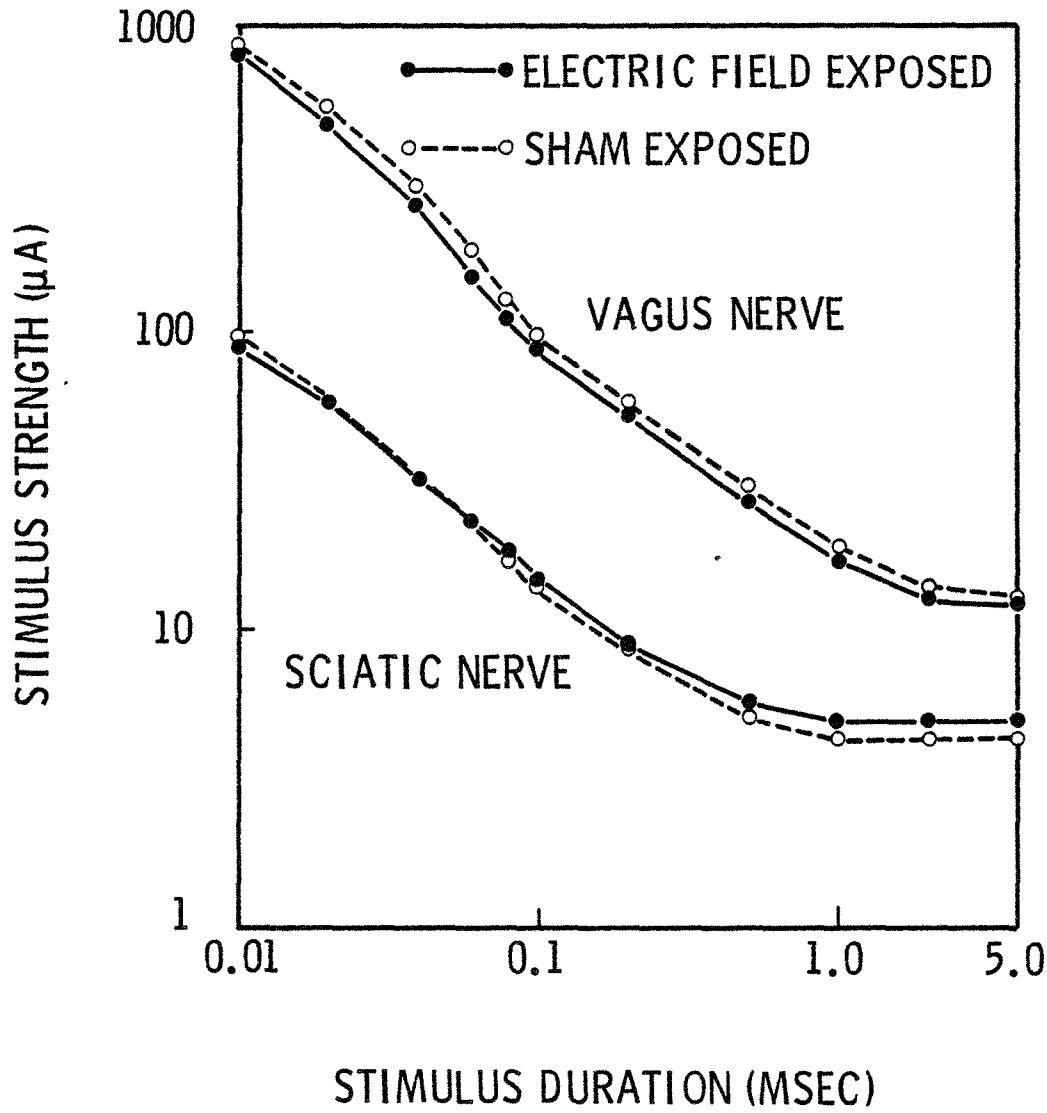



FIGURE XI-2. Stimulus Strength Required for Threshold Stimulation at Fixed Stimulus Durations. Data are presented as means for exposed and sham-exposed vagal and sciatic nerves.

TABLE XI-10. Effects of Poststimulus Excitability in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure

| Conditioning-Test Interval (msec) | Exposed N | Exposed Mean ± S.E. | Sham-Exposed N | Sham-Exposed Mean ± S.E. | p |
|---|---|---|---|---|---|
| **Vagus Nerve** | | | | | |
| 2X Conditioning Response | 18 | 1.26 ± 0.08 | 17 | 1.33 ± 0.06 | NS* |
| 0.5X Test Response | 11 | 11.23 ± 1.59 | 15 | 10.29 ± 0.63 | NS |
| Minimum Test Response | 17 | 6.66 ± 0.47 | 17 | 6.54 ± 0.48 | NS |
| Maximum Test Response | 18 | 47 ± 3 | 17 | 46 ± 2 | NS |
| **Sciatic Nerve** | | | | | |
| 2X Conditioning Response | 13 | 0.30 ± 0.02 | 14 | 0.32 ± 0.02 | NS |
| 0.5X Test Response | 13 | 2.43 ± 0.21 | 14 | 2.69 ± 0.30 | NS |
| Minimum Test Response | 13 | 1.30 ± 0.10 | 14 | 1.30 ± 0.09 | NS |
| Maximum Test Response | 13 | 3.64 ± 0.10 | 14 | 3.79 ± 0.15 | NS |

*NS = Not statistically significant ($p > 0.05$, Student's $t$ test)

TABLE XI-11. Properties of the Compound Action Potential in the Vagus Nerves of Rats Following 30 Days of Exposure or Sham Exposure to a 100-kV/m, 60-Hz Electric Field (Replicate Experiment).

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p |
| Maximum Amplitude: B Component (mV) | 24 | 1.43 ± 0.10 | 26 | 1.68 ± 0.11 | NS* |
| Maximum Amplitude: C Component (mV) | 24 | 1.76 ± 0.14 | 26 | 2.10 ± 0.16 | NS |
| Maximum dV/dt B (V/sec) | 24 | 4.01 ± 0.31 | 27 | 4.20 ± 0.37 | NS |
| Maximum dV/dt B-C (V/sec) | 23 | -6.91 ± 0.49 | 27 | -8.12 ± 0.72 | NS |
| Maximum dV/dt C (V/sec) | 24 | 1.74 ± 0.15 | 27 | 1.99 ± 0.27 | NS |

*NS = not significant; $p > 0.05$

Table XI-11, replicate experiment) we were unable to confirm this effect, although the general relationship was maintained. Other differences between exposed and sham-exposed animals, while not statistically significant, were consistent in both experiments.

No difference was observed in the conduction velocities of the principal components of the C-fiber compound action potential (Table XI-12). This is in marked contrast to observations made in the original study, in which conduction velocities were significantly ($p < 0.01$) increased in electric-field-exposed animals. Animal ages and body weights, as well as nerve-chamber temperatures, were compared for the two experiments. No differences were found that could account for the absence of the effect in the replicate experiment or for the presence of the effect in the original experiment.*

Strength-duration curves for the two principal components of the vagal compound action potential are shown in Figure XI-3. No significant differences between exposed and sham-exposed animals were observed (Table XI-13). As in the original experiment, the strength-duration curve for unmyelinated (vagal) nerve fibers in electric-field-exposed animals is shifted to the left, relative to the sham-exposed controls. The chronaxies are correspondingly shorter for exposed animals than for sham-exposed animals (Table XI-13).

* This experiment was replicated a third time; the results were the same as in the second replicate. Exposure of rats to 100 kV/m for 30 days had no significant effect on conduction velocities of the principal components of the C-fiber compound action potential.

TABLE XI-12. Conduction Velocities of Components of the Compound Action Potential in Vagus Nerves of Rats Following 30 Days of Exposure or Sham Exposure to a 100-kV/m, 60-Hz Electric Field (Replicate Experiment). Conduction velocities were calculated from computer averages of 16 action potentials in each individual nerve.

| | Exposed | | Sham-Exposed | | |
|---|---|---|---|---|---|
| | N | Mean ± S.E. | N | Mean ± S.E. | p |
| Peak Conduction Velocity: B Component (m/sec) | 24 | 0.95 ± 0.01 | 26 | 0.95 ± 0.01 | NS* |
| Peak Conduction Velocity: C Component (m/sec) | 24 | 0.70 ± 0.01 | 26 | 0.70 ± 0.01 | NS |
| Chamber Temperature (°C) | 25 | 37.5 ± 0.1 | 26 | 37.5 ± 0.1 | NS |
| Age of Rats (Days) | 15 | 84.7 ± 0.4 | 14 | 84.9 ± 0.4 | NS |

*NS = not significant; $p > 0.05$

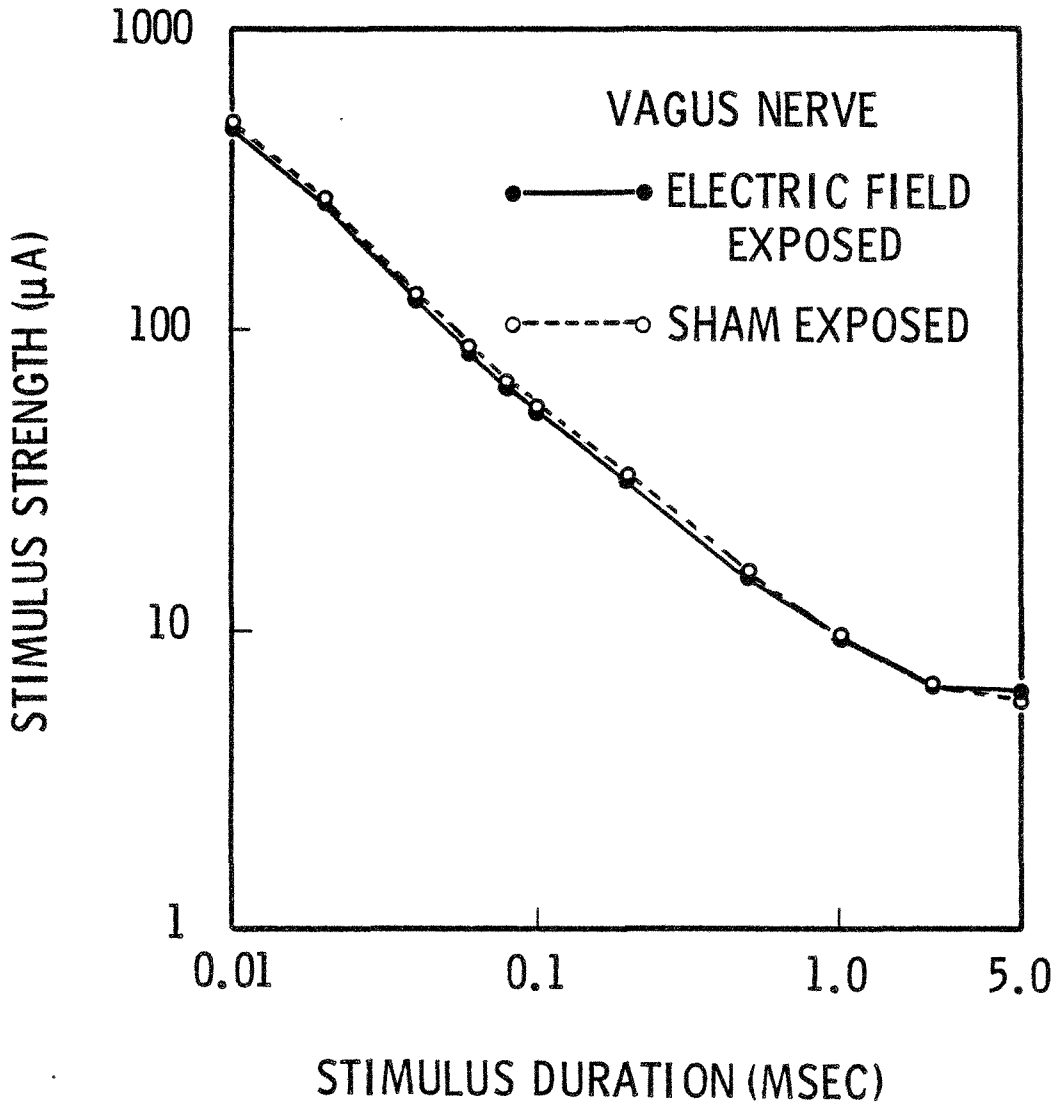



FIGURE XI-3. Strength-Duration Curves for the Two Principal Components of the Vagal Compound Action Potential for Both Exposed and Sham-Exposed Nerves

TABLE XI-13. Stimulus Strength Required for Threshold Stimulation at Fixed Stimulus Durations in the Vagus Nerve of Rats Following 30 Days of 60-Hz Electric Field or Sham Exposure (Replicate Experiment). Data are presented as mean ± standard error with N shown in parentheses.

| Stimulus Duration (msec) | Stimulus Strength (μA) | | | |
|---|---|---|---|---|
| | Exposed: B | Sham-Exposed: B | Exposed: C | Sham-Exposed: C |
| 5 | 7.3 ± 0.3 (23) | 7.4 ± 0.5 (27) | 6.1 ± 0.3 (24) | 5.8 ± 0.4 (27) |
| 2 | 7.7 ± 0.3 (23) | 7.9 ± 0.5 (27) | 6.6 ± 0.3 (24) | 6.5 ± 0.4 (27) |
| 1 | 11.2 ± 0.5 (23) | 11.7 ± 0.7 (27) | 9.4 ± 0.4 (24) | 9.5 ± 0.5 (27) |
| 0.5 | 19.1 ± 0.9 (23) | 20.2 ± 1.2 (27) | 14.9 ± 0.7 (24) | 15.6 ± 1.0 (27) |
| 0.2 | 42.9 ± 2.2 (23) | 45.8 ± 2.9 (27) | 31.0 ± 1.8 (24) | 33.1 ± 2.1 (27) |
| 0.1 | 82.1 ± 4.8 (23) | 85.9 ± 5.6 (27) | 53.6 ± 3.7 (24) | 56.6 ± 4.2 (27) |
| 0.08 | 102.2 ± 5.9 (23) | 109.1 ± 7.3 (27) | 66.0 ± 4.4 (24) | 68.2 ± 4.9 (27) |
| 0.06 | 137.0 ± 8.5 (23) | 143.9 ± 9.8 (27) | 85.1 ± 5.7 (24) | 89.2 ± 6.9 (27) |
| 0.04 | 209.4 ± 12.6 (23) | 217.7 ± 13.9 (27) | 127.3 ± 9.4 (24) | 132.3 ± 10.6 (27) |
| 0.02 | 431.3 ± 24.3 (23) | 450.6 ± 28.1 (27) | 270.8 ± 17.4 (24) | 282.6 ± 19.2 (27) |
| 0.01 | 780.4 ± 43.3 (23) | 828.5 ± 52.1 (27) | 478.5 ± 28.6 (24) | 491.8 ± 30.2 (27) |
| Chronaxie (msec) | 0.80 ± 0.04 (23) | 0.82 ± 0.03 (26) | 0.73 ± 0.04 (24) | 0.81 ± 0.04 (26) |

4. Discussion and Conclusions. The increased conduction velocity in unmyelinated nerve fibers from electric-field-exposed animals reported in the original experiment was not observed in the replicate experiment. This discrepancy cannot be explained on the basis of available information. The conditions of the replicate experiments were designed to closely duplicate those of the original experiment. Improvements in nerve chamber design, instrumentation and technique occurred during the interval between the two experiments. Presumably as a result of these subtle changes, the configuration of the compound action potential differed in the two experiments. Neither procedural improvements nor configurational changes in the action potential could reasonably be used to account for the absence of the effect in the replicate experiment. It is interesting to note that the conduction velocities for exposed and sham-exposed animals measured in the replicate experiment fall exactly in between the corresponding conduction velocities measured in the original experiment.

The hypothesis of increased neuronal excitability continues to be supported by the shift in the strength-duration curves and decreased chronaxie found in electric-field-exposed animals. Trends in other parameters (maximum amplitude, maximum rates of potential change), while consistent with those found in the original experiment, are not obviously compatible with this hypothesis.

The question of whether or not chronic exposure to a 60-Hz electric field can cause persistent changes in peripheral nerve function remains unanswered. Additional experiments, perhaps of longer duration, will be required if we are to answer the question.

D. FUTURE PLANS

Experiments on neuromuscular function are scheduled for completion in December 1979, with the data analysis to be completed in February 1980.

Because of the confirmed effects of electric fields on synaptic transmission (C-T ratio), additional experiments will be conducted to examine the basic mechanisms whereby changes in the C-T ratio are produced, and to determine if the effects are reversible. These experiments will provide some of the additional information necessary to assess the health consequences of exposure to 60-Hz electric fields.

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# XII. REPRODUCTION AND DEVELOPMENT

Principal Investigator: M. R. Sikov

## ABSTRACT

Two experiments were conducted to assess the effects of exposure to uniform 60-Hz electric fields (100 kV/m) on reproduction, and on fetal and postnatal growth and development in the rat. In one experiment (replicate experiment) exposure of the pregnant rat was begun on day 0 of gestation and continued until the resulting offspring reached 8 days of age. It was found that exposure did not affect reproductive performance in either males or females, nor did it affect the survival, size or morphology of the fetuses. In the other experiment, exposure of the pregnant female began at 17 days of gestation and continued through 25 days of postnatal life. These 30-day exposures produced few statistically significant effects on the growth, survival or neuromuscular development of the offspring. Accelerated development of motile behavior was observed in offspring in the first experiment. This difference was not observed in the later experiment. Tests of reproductive integrity of the offspring did not disclose any deficits.

Mice conceived, born, and raised in a 60-Hz (100 kV/m), electric field for three successive generations were compared with an identical sham-exposed group. Since the study is still in progress, no statistical analyses of the data are available. However, the presence of the electric field had no apparent effect on conception, litter size, mortality, or body weights up to 10 wk of age in any of the three generations studied.

## A. INTRODUCTION

The previous Annual Report described two experiments in which we studied the effect of exposure to 100-kV/m electric fields on the growth and development of rats. The first experiment, which involved prenatal exposure and teratologic evaluation, had been completed and was reported in detail (Phillips, et al., 1978). Certain aspects of the second experiment, which involved exposure from the day of conception through 8 days of

postnatal age, also had been completed, and preliminary results were reported (Phillips, et al., 1978). Statistical analyses of these data, as well as additional measurements on the same animals, have now been completed and will be presented in this report. A third experiment, to evaluate the effects of exposure during the perinatal and juvenile period, was also performed during the past year.

Marino et al. (1976) reported a significant effect attributable to exposure to 60-Hz electric fields over three successive generations. The offspring of mice exposed to a vertical electric field at 15 kV/m were reported to show decreased body weights and increased mortality rate for all three generations. Mice exposed to a horizontal electric field at 10 kV/m showed decreased body weights for two successive generations. The authors indicate that the presence of grounding microcurrents may have influenced the results, although they suggest alternative mechanisms.

We designed an experiment to determine whether effects similar to those reported by Marino et al. would be observed under conditions in which such discharges did not occur. The rats in this study were grounded and exposed to 100 kV/m, in contrast to rats of Marino's study which were ungrounded and exposed to 15 kV/m. This study is still in progress, with data from the third generation incomplete at this time; therefore, only preliminary results will be reported here.

## B. METHODS

Rat Experiment 2. The procedures were the same as those described in the 1978 Annual Report. Exposure extended from 0 days of gestation

(d.g.) and continued through 8 days of age. In this experiment, four female offspring (age ∿ 2 mo) from each litter that had been exposed or sham-exposed in utero and for 8 days after birth, together with one randomly selected male (similarly exposed or sham-exposed), were reintroduced into the sham-exposure or exposure field to determine whether deficits in reproductive performance had been induced. At 7 wk of age, one-half of the remaining males from the first two replicates were tested for susceptibility to audiogenic seizures by exposure to a 110- to 114-db white noise for 1 min. The remaining animals in that group were handled in an identical fashion except that they were not subjected to the sound. Blood samples, obtained after completion of the seizure test, were furnished to other investigators to determine whether changes in corticosteroid levels had been produced by the stress of the seizure test, and whether these changes differed between sham-exposed and exposed groups. The animals which were not subjected to the seizure test were killed and necropsied, which included weighing several organs; tissues were preserved for histopathologic examination. Blood samples were taken from other animals for more extensive hematologic and hormonal analyses by other investigators.

The males of the third replicate were given Metrazol prior to testing to heighten their susceptibility to audiogenic seizures. The males from the fourth replicate were killed at 28 days of age to determine whether transient early alterations in organ weights had been induced.

Rat Experiment 3. This experiment, which was performed to evaluate the effects of exposure during late gestation and during postnatal development, was performed in five replicates. Within each replicate, one male and four female rats were caged together; vaginal smears from females were examined daily. The day of a sperm-positive vaginal smear was defined as day 0 of gestation. At 17 d.g., females were transferred to littering cages [which were identical to the mating cages used in Experiment 1 and contained Antron-III (an electrical conductor) for nesting (Phillips and Kaune, 1977)] and randomly selected for placement in the electric field or sham field. The dams delivered and reared their litters during exposure, and were removed from the field when the pups reached 21 days of age. The offspring were removed from the field 4 days later, at 25 days of age.

At delivery, the interval since mating was recorded, as well as the number of living and stillborn offspring. At 1 day of age, litters were reduced to eight pups (evenly divided between the sexes when possible); litters of fewer than six were eliminated. At that time, as well as at several subsequent times, pups were weighed and examined for gross abnormalities. Offspring were subjected (at various times) to a battery of tests to evaluate reflex development and general neurological status. The measures, which are commonly employed in our laboratory (Sikov et al., 1977), were described in the previous Annual Report.

Mouse Multigeneration Experiment. The exposure system consisted of two identical units: one for vertical field exposure and the other

for sham exposure (Phillips and Kaune, 1977). The exposure system is designed to minimize microcurrents through the watering system when the animals drink. Preconceived bias was eliminated from the studies because the exposed and sham-exposed groups were not identified to the investigators until all measurements had been completed. Exposure to the 60-Hz (100-kV/m) electric field was continuous except for an approximately 4-hr daily shutdown period for animal care and data collection. The exposure room was on a 12-hr light/12-hr dark cycle, and had an average temperature of 21°C and a relative humidity of 30% during this study.

The experimental design may be seen in Table XII-5. Swiss-Webster mice, obtained from Hilltop Laboratories, were used for the $F_0$ generation. Male and female animals were acclimated to the housing system in the exposure room for 5 days before the system was energized. Subsequently, all mice were conceived, born and raised with the exposure system energized.

The study involved two replicates (A and B), with an exposed group and a sham-exposed group in each replicate. Each replicate started with 16 exposed and 16 sham-exposed female mice ($F_0$). Two female mice were housed with one male for mating for a period of 1 wk; females were checked daily for evidence of mating. A few days before delivery, eight pregnant females were randomly selected from each group and housed individually with a small amount of conductive nesting material (Antron-III).

After delivery, the pups ($F_1$) were counted, sexed, weighed and marked with an identification code. Each litter was reduced to eight pups (four females and four males, when possible) and the pups were weighed

weekly for 10 wk. They were checked for tooth eruption, eye-opening, and testes descent or vaginal opening at appropriate ages, and weaned at 21 days of age.

At 10 weeks of age, 16 exposed and 16 sham-exposed females (two from each litter, if possible) were randomly selected from among the $F_1$ animals. Breeding males were selected (one from each litter, if possible) so that there was a maximum of genetic mixing. There were no sibling matings, and reciprocal crosses were avoided; males and females from each litter were represented whenever possible. After selection of the breeding animals, the remaining mice were provided to another investigator for hematologic evaluation (see Section IV, Hematology and Serum Chemistry).

The breeding and evaluation procedures were successively repeated until a total of three offspring generations had been obtained. A fourth generation was used for detailed neuromuscular evaluations. Statistical tests on the data have been deferred until completion of the study.

## C. RESULTS

Experiment 2. As was reported previously (Phillips et al., 1978), there were no differences between exposed and sham-exposed groups in litter sizes, birth weights, and subsequent growth and survival. The data obtained when the animals were bred showed no detectable differences attributable to the original or subsequent exposure, or to interactions between them.

Most results obtained from tests of neuromuscular and neurological development did not differ between the sham-exposed and exposed groups. The only notable difference was the greater percentage of exposed offspring showing motile behaviors (movement, grooming, standing), and a greater percentage of the sham-exposed showing the righting reflex at 14 days of age. The results in the two groups were indistinguishable upon retesting at 21 days of age. These differences have now been analyzed and found to be statistically significant at the 0.05 level by an analysis of variance using an arc sine transformation.

Organ weights were obtained at sacrifice from the rats not subjected to audiogenic seizure testing (Table XII-1), and showed no remarkable differences attributable to the experimental treatment.

Experiment 3. The only birth parameter which differed between the two groups was the number of stillbirths (Table XII-2). This also differed from the results of Experiment 2, which are shown for comparison. The larger number in the sham-exposed group was partially due to one litter of 20, in which 8 were stillborn. A total of 12 sham-exposed litters included stillborn pups, as compared to a single litter in which there were three stillborns in the exposed group. Only a few offspring died throughout the remainder of the experiment; these also occurred primarily in sham-exposed litters (Table XII-2). Body weight at 1 day of age, and at subsequent ages, was similar in the two groups. The exposed females were slightly heavier than the sham-exposed at each interval, but the differences were not statistically significant (Table XII-3).

TABLE XII-1. Organ Weights of Rats Exposed to a 60-Hz, 100-kV/m Electric Field Throughout Prenatal Development and the First 8 Postnatal Days (Mean % Body Weight ± S.E.)

| Age (Sex): | 28 Days (Males) | | 53-56 Days (Males) | | 74-77 Days (Females) | |
|---|---|---|---|---|---|---|
| Organ | Exposed | Sham-Exposed | Exposed | Sham-Exposed | Exposed | Sham-Exposed |
| Liver | 5.1 ± 0.1 | 5.1 ± 0.1 | 4.4 ± 0.1 | 4.4 ± 0.2 | 4.2 ± 0.1 | 4.1 ± 0.1 |
| Spleen | 0.43 ± 0.01 | 0.44 ± 0.04 | 0.30 ± 0.02 | 0.31 ± 0.01 | --- | --- |
| Adrenal | 0.02 ± 0.001 | 0.02 ± 0.001 | 0.02 ± 0.001 | 0.02 ± 0.002 | 0.03 ± 0.001 | 0.03 ± .001 |
| Testes | 0.61 ± 0.02 | 0.59 ± 0.02 | 0.95 ± 0.02 | 0.94 ± 0.01 | --- | --- |
| Ovaries | --- | --- | --- | --- | 0.05 ± 0.002 | 0.05 ± 0.002 |
| Epididymides | 0.071 ± 0.003 | 0.071 ± 0.003 | 0.14 ± 0.010 | 0.15 ± 0.01 | --- | --- |
| Seminal Vesicles | --- | --- | 0.14 ± 0.01 | 0.11 ± 0.01 | --- | --- |
| Uterus | --- | --- | --- | --- | 0.17 ± 0.01 | 0.18 ± 0.01 |
| Kidney (Left) | --- | --- | --- | --- | 0.36 ± 0.01 | 0.37 ± 0.01 |
| Thymus | --- | --- | --- | --- | 0.17 ± 0.01 | 0.17 ± 0.01 |
| Bladder & Prostate | 0.18 ± 0.01 | 0.17 ± 0.01 | 0.13 ± 0.01 | 0.14 ± 0.01 | --- | --- |

TABLE XII-2. Litter Size and Survival of Offspring of Pregnant Rats Exposed to a 60-Hz Electric Field at 100 kV/m

| | Experiment 2** | | Experiment 3*** | |
|---|---|---|---|---|
| | Exposed | Sham-Exposed | Exposed | Sham-Exposed |
| Total Females in Group | 24 | 20 | 29 | 29 |
| Mean Live Births/Litter ± S.E. | 9.9 ± 0.8 | 11.2 ± 0.8 | 10.2 ± 0.9 | 10.8 ± 0.7 |
| Number of Stillbirths | 8 | 3 | 3 | 24 |
| Number Discarded* | 5 | 2 | 7 | 5 |
| Number of Litters Evaluated | 19 | 18 | 22 | 24 |
| Number of Postnatal Deaths | 3 | 4 | 2 | 8 |

*Less than 6 offspring for continuing postnatal evaluation
**Exposure: 0 d.g. through 8 days postnatally
***Exposure: 17 d.g. through 25 days postnatally

TABLE XII-3. Body Weights of Rats Exposed to a 60-Hz Electric Field at 100 kV/m from 17 Days of Gestation Through 25 Days Postnatally

| Age (Days) | Mean Weight* ± S.E. (g) | | | |
|---|---|---|---|---|
| | Males | | Females | |
| | Exposed | Sham-Exposed | Exposed | Sham-Exposed |
| 1 | 7.5 ± 0.1 | 7.3 ± 0.2 | 7.1 ± 0.1 | 6.8 ± 0.2 |
| 7 | 16.4 ± 0.3 | 16.0 ± 0.3 | 15.9 ± 0.3 | 15.1 ± 0.4 |
| 14 | 32.6 ± 1.0 | 32.9 ± 0.9 | 32.0 ± 0.9 | 31.6 ± 0.8 |
| 21 | 51.8 ± 1.5 | 50.8 ± 1.6 | 50.4 ± 1.4 | 48.8 ± 1.5 |
| 28 | 90.2 ± 1.5 | 89.0 ± 2.1 | 83.8 ± 1.2 | 81.6 ± 2.2 |
| 35 | 150.6 ± 1.6 | 147.8 ± 1.6 | 128.6 ± 2.3 | 126.8 ± 1.3 |
| 42 | 214.4 ± 2.7 | 210.6 ± 3.2 | 170.0 ± 1.8 | 169.8 ± 1.9 |

*Calculated as means of individual litter means

The various measures of physical maturation were obtained at the same times as in Experiment 2, and again showed no consistent differences between the two experimental groups. The data obtained from a number of the measures of neuromuscular development, pooled across replicates (Table XII-4), suggested differences between groups, but they were not statistically significant at $p < 0.05$. These included the grasp reflex at 1 day ($p = 0.08$), head lift at 7 days ($p = 0.09$) and movement at 1 and 7 days ($p = 0.2$). The effects of electric-field exposure on "grooming" "standing alone," and "righting" that were seen in Experiment 2 were not observed in Experiment 3. The only measure which was significantly affected was negative geotropism ($p = 0.03$).

Mouse Multigeneration Experiment. The number of pregnancies among $F_0$, $F_1$ and $F_2$ mice resulting from 1-wk breeding periods are shown in Table XII-5. On an overall basis, 83 of 96 (86.5%) exposed mice and 88.5% of sham-exposed females became pregnant, with little difference between generations or replicates. There was no consistent pattern of difference between the two groups, and it is unlikely that the 2% difference will be statistically significant.

The total number of mice born, average litter size and mortality data are shown in Table XII-6. Although there were slightly fewer births in the exposed group, there appear to be no real differences between the two groups in average litter size or total number of mice born. The number of litters born was identical for each group.

TABLE XII-4. Percent of Rat Offspring Showing Criterion or Normal Response to Several Measures of Neuromuscular Development (Approximately 180 Offspring per Group). Offspring were exposed from 17 d.g. through 25 days of age to a 60-Hz electric field at 100 kV/m.

| | | Percent of Offspring Showing Normal Response | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | | | | Sham-Exposed | | | |
| Measure of Development | Age: | 1 | 7 | 14 | 21 | 1 | 7 | 14 | 21 |
| Grasp | | 76 | 65 | 99 | 92 | 91 | 65 | 97 | 90 |
| Righting | | -- | 21 | 64 | 95 | -- | 17 | 61 | 95 |
| Movement | | 32 | 27 | 79 | 92 | 18 | 16 | 74 | 94 |
| Head Lift | | 67 | 34 | 79 | 95 | 70 | 26 | 63 | 93 |
| Grooming | | -- | -- | 44 | 87 | -- | -- | 45 | 89 |
| Standing Alone | | -- | -- | 25 | 82 | -- | -- | 22 | 86 |
| Negative Geotropism | | -- | -- | 32 | 59 | -- | -- | 22 | 66 |

TABLE XII-5. Experimental Design and Pregnancy Data from Multigeneration Mouse Experiment

| | | Rack 1 (Exposed) | | | | | Rack 2 (Sham-Exposed) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number | Number | Pregnancies | | Number | Number | Number | Pregnancies | | Number |
| Replicate | Generation | Females Bred | Males Used | Number Females | | Females Kept | Females Bred | Males Used | Number Females | | Females Kept |
| A | $F_0$ | 16 | 8 | 13 | 81.2 | 8 | 16 | 8 | 13 | 81.2 | 8 |
| B | $F_0$ | 16 | 8 | 13 | 81.2 | 8 | 16 | 8 | 15 | 93.8 | 8 |
| A | $F_1$ | 16 | 8 | 14 | 87.5 | 8 | 16 | 8 | 16 | 100 | 8 |
| B | $F_1$ | 16 | 8 | 14 | 87.5 | 8 | 16 | 8 | 12 | 75 | 8 |
| A | $F_2$ | 16 | 8 | 14 | 87.5 | 8 | 16 | 8 | 14 | 87.5 | 8 |
| B | $F_2$ | 16 | 8 | 15 | 93.8 | 8 | 16 | 8 | 15 | 93.8 | 9 |
| Total | | 96 | 48 | 83 | 86.5 | | 96 | 48 | 85 | 88.5 | |

TABLE XII-6. Summary of Mice Born, Litter Size and Mortality Data in Multigeneration Mouse Experiment

| Replicate | Generation | Total Pups Born* | Average Litter Size ($\bar{x} \pm SD$) | Day 1 Mortality | | Mortality of Pups Selected** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Day 7 | | Day 70 | |
| | | | | Number Dead | % | (N) | Number Dead | % | Number Dead | % |
| **Rack 1 (Exposed)** | | | | | | | | | | |
| A | $F_1$ | 83 | 10.4 ± 1.7 | 0 | 0 | (64) | 0 | 0 | 3 | 4.7 |
| B | $F_1$ | 97 | 12.1 ± 1.0 | 0 | 0 | (64) | 7 | 10.9 | 18 | 28.1 |
| A | $F_2$ | 89 | 11.1 ± 2.8 | 16 | 18.0 | (53) | 1 | 1.9 | 9 | 17.0 |
| B | $F_2$ | 90 | 11.2 ± 1.9 | 1 | 1.1 | (64) | 0 | 0 | 0 | 0 |
| A | $F_3$ | 83 | 10.4 ± 2.8 | 8 | 9.6 | (57) | 0 | 0 | 1 | 1.8 |
| B | $F_3$ | 84 | 10.5 ± 1.4 | 0 | 0 | (64) | 0 | 0 | 4 | 6.2 |
| Total A | | 255 | 10.6 ± 2.4 | 24 | 9.4 | (174) | 1 | 0.6 | 13 | 7.5 |
| Total B | | 271 | 11.3 ± 1.6 | 1 | 0.4 | (192) | 7 | 3.6 | 22 | 11.5 |
| Total A&B | | 526 | 11.0 ± 2.0 | 25 | 4.8 | (366) | 8 | 2.2 | 35 | 9.6 |
| **Rack 2 (Sham-Exposed)** | | | | | | | | | | |
| A | $F_1$ | 88 | 11.0 ± 2.2 | 0 | 0 | (64) | 8 | 12.5 | 11 | 17.2 |
| B | $F_1$ | 99 | 12.4 ± 1.8 | 7 | 7.1 | (64) | 1 | 1.6 | 11 | 17.2 |
| A | $F_2$ | 96 | 12.0 ± 2.1 | 1 | 1.0 | (64) | 0 | 0 | 7 | 10.9 |
| B | $F_2$ | 82 | 10.2 ± 2.4 | 3 | 3.7 | (61) | 9 | 14.8 | 13 | 21.3 |
| A | $F_3$ | 98 | 12.2 ± 2.0 | 1 | 1.0 | (64) | 0 | 0 | 2 | 3.1 |
| B | $F_3$ | 87 | 10.9 ± 2.0 | 0 | 0 | (64) | 0 | 0 | 0 | 0 |
| Total A | | 282 | 11.8 ± 2.0 | 2 | 0.7 | (192) | 8 | 4.2 | 20 | 10.4 |
| Total B | | 268 | 11.2 ± 2.2 | 10 | 3.7 | (189) | 10 | 5.3 | 24 | 12.7 |
| Total A&B | | 550 | 11.5 ± 2.1 | 12 | 2.2 | (381) | 18 | 4.7 | 44 | 11.6 |

*The total number of pups born is probably an underestimate because of frequent cannibalism of stillborn or weak offspring by dams.

**All litters were reduced to 8 pups on day 1 to reduce the influence of litter size on the growth rate.

Evidently, the presence of the electric field had no influence on mortality in the three generations of mice. No real differences in mortality between the two groups were observed, nor were any trends indicated. There were no deaths among the dams, and causes for most of the deaths among the young mice appeared to be unrelated to treatment.

In the sham-exposed groups, two litters were lost because of malfunctions in the watering system, with subsequent dehydration and agalactia in the dams. Two other litters were lost because the dams escaped from their cages and could not return to their young. A few mice died accidentally.

Of the 48 litters in the exposed groups, five were lost. Lactation apparently became insufficient between 7 and 14 days postpartum in three dams, with subsequent loss of those litters. A temporary malfunction of the water system is suspected in these cases. Two other litters were lost because the dams exhibited very little maternal instinct and failed to take care of their young.

Irrespective of treatment, animals from several litters failed to gain weight properly between 7 and 14 days, or between 14 and 21 days of age; these mice later gained weight rapidly. Most of the problems and litter losses noted above occurred early in the experiment; the incidence declined as the investigators recognized and corrected the problems. The only deaths without apparent cause were four offspring (1.1%) from the exposed group, and seven (1.8%) from the sham-exposed group.

A comparison of weights between exposed and sham-exposed female mice on selected days of age may be seen in Table XII-7; corresponding

TABLE XII-7. Average Weights of Three Generations of Female Mice Exposed to a 60-Hz Electric Field at 100 kV/m

| Age: | | Weights (grams) Day 1 | | Day 14 | | Day 28 | | Day 35 | | Day 70 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Generation | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ |
| | | | | | RACK 1 EXPOSED | | | | | | |
| A | $F_1$ | (34) | 1.8 ± 0.2 | (34) | 7.1 ± 0.6 | (34) | 16.4 ± 1.9 | (34) | 20.3 ± 1.6 | (34) | 28.9 ± 1.4 |
| A | $F_2$ | (22) | 1.8 ± 0.2 | (22) | 7.6 ± 0.4 | (22) | 19.0 ± 1.7 | (11) | 23.6 ± 1.6 | (22) | 29.2 ± 1.8 |
| A | $F_3$ | (24) | 1.8 ± 0.1 | (24) | 7.0 ± 0.8 | (24) | 16.7 ± 2.4 | (24) | 22.1 ± 1.8 | (23) | 28.6 ± 1.1 |
| Total A | $F_1 - F_3$ | (80) | 1.8 ± 0.2 | (80) | 7.2 ± 0.6 | (80) | 17.2 ± 2.0 | (69) | 21.4 ± 1.7 | (79) | 28.9 ± 1.4 |
| B | $F_1$ | (23) | 1.8 ± 0.2 | (23) | 7.3 ± 1.0 | (23) | 16.5 ± 2.1 | (23) | 22.8 ± 1.5 | (23) | 29.5 ± 2.5 |
| B | $F_2$ | (36) | 1.9 ± 0.2 | (36) | 7.1 ± 1.1 | (36) | 18.1 ± 2.3 | (36) | 23.2 ± 1.9 | (36) | 29.4 ± 1.3 |
| B | $F_3$ | (29) | 2.0 ± 0.1 | (29) | 7.8 ± 0.6 | (27) | 18.0 ± 2.8 | (27) | 22.3 ± 1.9 | (27) | 29.9 ± 1.8 |
| Total B | $F_1 - F_3$ | (88) | 1.9 ± 0.2 | (88) | 7.4 ± 0.9 | (86) | 17.6 ± 2.4 | (86) | 22.8 ± 1.8 | (86) | 29.6 ± 1.8 |
| A & B | $F_1 - F_3$ | (168) | 1.9 ± 0.2 | (68) | 7.3 ± 0.8 | (166) | 17.4 ± 2.2 | (155) | 22.2 ± 1.8 | (165) | 29.3 ± 1.6 |
| | | | | | RACK 2 SHAM-EXPOSED | | | | | | |
| A | $F_1$ | (28) | 1.9 ± 0.2 | (28) | 7.4 ± 0.6 | (27) | 17.1 ± 1.8 | (27) | 21.3 ± 1.5 | (26) | 29.9 ± 1.6 |
| A | $F_2$ | (28) | 1.9 ± 0.1 | (28) | 7.2 ± 0.9 | (27) | 17.2 ± 2.8 | (23) | 22.9 ± 2.0 | (27) | 30.7 ± 1.9 |
| A | $F_3$ | (30) | 1.8 ± 0.1 | (30) | 7.2 ± 0.8 | (29) | 17.5 ± 1.9 | (29) | 23.0 ± 1.4 | (28) | 29.9 ± 1.9 |
| Total A | $F_1 - F_3$ | (86) | 1.9 ± 0.1 | (86) | 7.3 ± 0.8 | (83) | 17.3 ± 2.2 | (79) | 22.4 ± 1.6 | (81) | 30.2 ± 1.8 |
| B | $F_1$ | (27) | 1.9 ± 0.2 | (27) | 7.6 ± 0.6 | (25) | 18.8 ± 1.8 | (25) | 23.7 ± 1.0 | (24) | 29.0 ± 1.6 |
| B | $F_2$ | (30) | 2.0 ± 0.1 | (19) | 7.5 ± 0.9 | (29) | 17.9 ± 1.7 | (29) | 23.2 ± 1.9 | (28) | 29.3 ± 1.9 |
| B | $F_3$ | (34) | 1.8 ± 0.2 | (34) | 7.3 ± 0.5 | (34) | 18.0 ± 1.5 | (34) | 23.3 ± 1.3 | (34) | 28.5 ± 1.8 |
| Total B | $F_1 - F_3$ | (91) | 1.9 ± 0.2 | (80) | 7.4 ± 0.6 | (88) | 18.2 ± 1.6 | (88) | 23.4 ± 1.4 | (86) | 28.9 ± 1.8 |
| A & B | $F_1 - F_3$ | (177) | 1.9 ± 0.2 | (66) | 7.4 ± 0.7 | (171) | 17.8 ± 1.9 | (167) | 22.9 ± 1.5 | (167) | 29.5 ± 1.8 |

data for the males are presented in Table XII-8. No weight differences were observed from one generation to the next; weights from all three generations within replicates were pooled and are presented graphically in Figure XII-1. There appear to be no weight differences between exposed and sham-exposed groups. Gender had the only obvious effect on weight, with males consistently outgaining females after 21 days of age.

This experiment is still in progress and the data have not yet been subjected to statistical analyses. However, there appear to be no differences in weights between the exposed and sham-exposed groups. Apparently, under the conditions of this experiment, the presence of a 60-Hz (100-kV/m) vertical electric field had no effect on mice conceived, born, and raised in the field for three successive generations with regard to body weight, breeding capacity, litter size, or mortality.

## TABLE XII-8. Average Weights of Three Generations of Male Mice Exposed to a 60-Hz Electric Field at 100 kV/m

| Age: | | Weights (grams) Day 1 | | Day 14 | | Day 28 | | Day 35 | | Day 70 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate | Generation | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ | (N) | $\bar{x} \pm SD$ |
| | | | | | RACK 1 EXPOSED | | | | | | |
| A | $F_1$ | (30) | 1.8 ± 0.2 | (30) | 7.0 ± 0.8 | (27) | 16.4 ± 2.4 | (27) | 23.8 ± 2.4 | (27) | 34.6 ± 2.1 |
| A | $F_2$ | (23) | 1.8 ± 0.2 | (22) | 7.5 ± 0.6 | (22) | 20.4 ± 2.2 | (11) | 26.2 ± 1.6 | (22) | 35.9 ± 1.6 |
| A | $F_3$ | (33) | 1.9 ± 0.2 | (33) | 7.2 ± 0.7 | (33) | 19.0 ± 2.2 | (31) | 25.0 ± 2.1 | (32) | 34.2 ± 1.8 |
| Total A | $F_1 - F_3$ | (86) | 1.8 ± 0.2 | (85) | 7.2 ± 0.7 | (82) | 19.2 ± 2.3 | (69) | 24.7 ± 2.1 | (81) | 34.8 ± 1.8 |
| B | $F_1$ | (17) | 1.9 ± 0.2 | (17) | 7.4 ± 0.9 | (17) | 20.3 ± 1.8 | (17) | 27.3 ± 1.4 | (17) | 37.0 ± 2.1 |
| B | $F_2$ | (28) | 2.0 ± 0.2 | (28) | 7.2 ± 1.2 | (28) | 19.3 ± 3.7 | (28) | 26.2 ± 2.8 | (28) | 37.0 ± 2.3 |
| B | $F_3$ | (35) | 2.0 ± 0.1 | (35) | 8.0 ± 0.7 | (34) | 19.8 ± 3.2 | (34) | 26.8 ± 2.6 | (33) | 38.9 ± 2.3 |
| Total B | $F_1 - F_3$ | (80) | 2.0 ± 0.2 | (80) | 7.6 ± 0.9 | (79) | 19.7 ± 3.1 | (79) | 26.7 ± 2.4 | (78) | 37.8 ± 2.3 |
| A & B | $F_1 - F_3$ | (166) | 1.9 ± 0.2 | (165) | 7.4 ± 0.8 | (161) | 19.4 ± 2.7 | (148) | 25.8 ± 2.3 | (159) | 36.3 ± 2.0 |
| | | | | | RACK 2 SHAM-EXPOSED | | | | | | |
| A | $F_1$ | (28) | 2.0 ± 0.2 | (28) | 7.5 ± 0.7 | (27) | 19.7 ± 1.9 | (27) | 24.8 ± 2.4 | (26) | 36.9 ± 2.1 |
| A | $F_2$ | (28) | 2.0 ± 0.2 | (28) | 7.3 ± 0.8 | (28) | 18.6 ± 3.0 | (24) | 25.8 ± 2.4 | (28) | 36.0 ± 2.1 |
| A | $F_3$ | (34) | 1.9 ± 0.2 | (32) | 7.4 ± 0.8 | (34) | 18.4 ± 2.6 | (32) | 26.2 ± 2.5 | (32) | 36.9 ± 2.7 |
| Total A | $F_1 - F_3$ | (90) | 2.0 ± 0.2 | (88) | 7.4 ± 0.8 | (87) | 18.9 ± 2.5 | (83) | 25.6 ± 2.4 | (86) | 36.6 ± 2.3 |
| B | $F_1$ | (28) | 1.9 ± 0.2 | (28) | 7.6 ± 0.7 | (28) | 20.4 ± 2.4 | (28) | 27.5 ± 1.7 | (28) | 36.7 ± 2.1 |
| B | $F_2$ | (23) | 2.1 ± 0.2 | (18) | 7.1 ± 0.6 | (21) | 19.5 ± 1.9 | (21) | 26.4 ± 2.0 | (20) | 35.4 ± 2.4 |
| B | $F_3$ | (30) | 2.0 ± 0.2 | (30) | 7.5 ± 0.6 | (30) | 19.6 ± 1.9 | (30) | 26.5 ± 1.6 | (30) | 36.4 ± 2.3 |
| Total B | $F_1 - F_3$ | (81) | 2.0 ± 0.2 | (76) | 7.4 ± 0.6 | (79) | 19.9 ± 2.1 | (79) | 26.8 ± 1.7 | (78) | 36.2 ± 2.2 |
| A & B | $F_1 - F_3$ | (171) | 2.0 ± 0.2 | (164) | 7.4 ± 0.8 | (166) | 19.4 ± 2.3 | (162) | 26.2 ± 2.1 | (164) | 36.4 ± 2.3 |

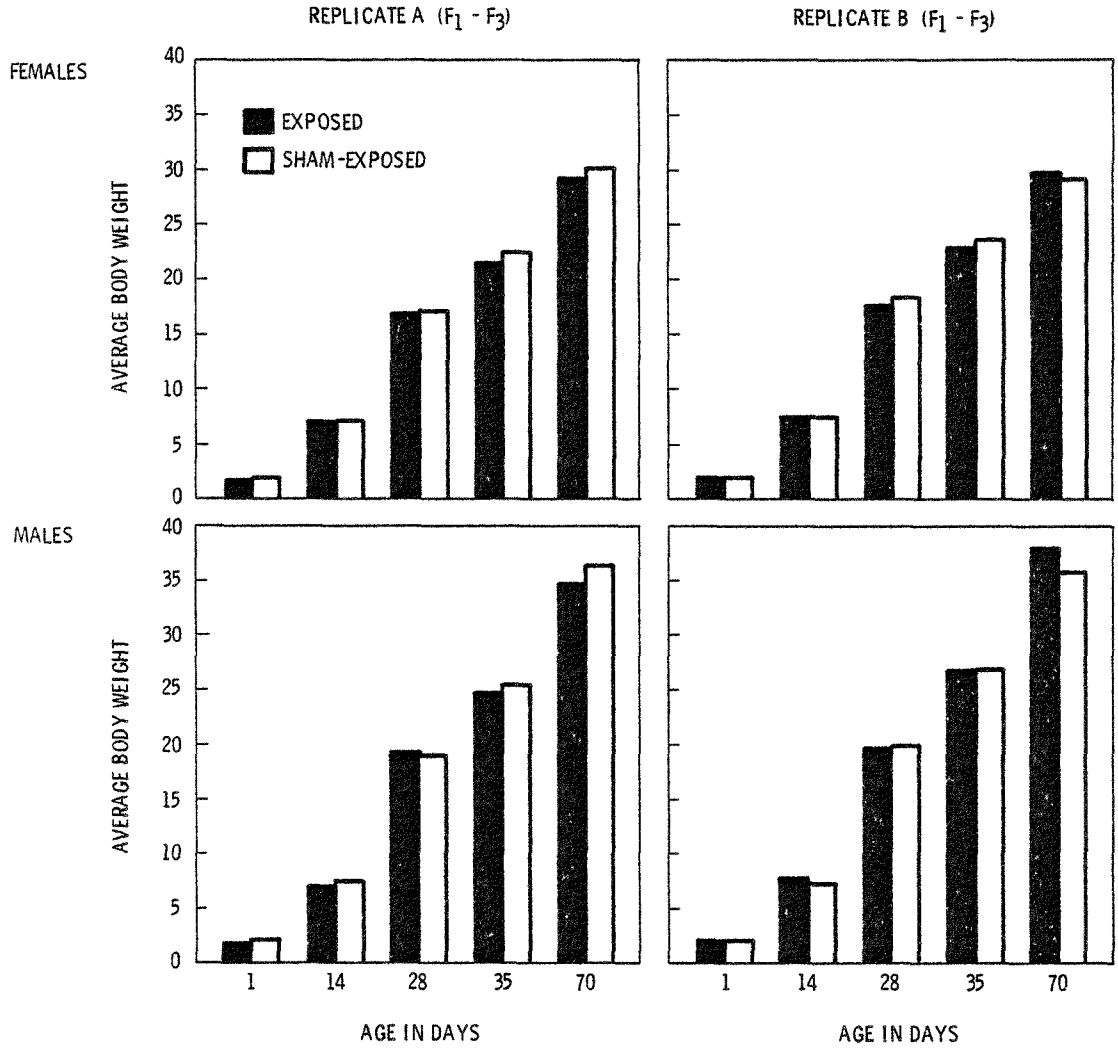



FIGURE XII-1. Comparison of Growth in Mice Exposed or Sham Exposed to 100 kV/m Electric Field over Three Generations. Weights pooled for three generations; data for two independent replicates are shown.

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# XIII. ANIMAL BEHAVIOR

Principal Investigators: D. L. Hjeresen and R. Sheldon

## ABSTRACT

Behavioral effects of high-strength, 60-Hz electric fields were studied in several experiments. Using a shuttlebox in which one end was shielded from a variety of electric-field strengths while the other was exposed, we determined that during short-term (45-min) tests rats avoided exposure at $\geq$ 90 kV/m; this response was not significantly attenuated or enhanced over four test sessions made at weekly intervals. Over a longer-duration test, rats avoided exposure at $\geq$ 75 kV/m, although they preferred exposure to fields less than 50 kV/m, but only during the light part of the daily cycle. In both the short- and longer-duration exposures, traverses from one end of the shuttlebox to the other end were transiently increased among exposed rats. Thirty days of exposure to a 100-kV/m electric field significantly attenuated subsequent avoidance performance in the shuttlebox, indicating a possible adaptation to the electric field.

Mice were tested to determine the effects of electric-field exposure on sound-induced seizure sensitivity. Results indicated no effect of electric-field exposure at 100 kV/m for 14 or 30 days.

## A. INTRODUCTION

The perception of chronic stimulation by an animal can result in activation of the pituitary-adrenocortical axis, causing numerous changes in systemic function, such as increased blood pressure and heart rate, and alterations in blood chemistry (Selye, 1956). Perception of 32-kV/m, 60-Hz electric fields by pigeons has been reported (Graves, et al., 1977) using the presence of the field as a cue in a conditioned suppression paradigm. Avoidance of 100-kV/m, 50-Hz electric fields by rats has

been reported by Bayer et al. (1977). The last Annual Report (Phillips, et al., 1978) described research we have conducted which shows that rats avoid electric fields of 90 kV/m and greater during a single 45-min exposure session.

This report describes three perception and avoidance experiments which substantiate and extend the earlier findings. In the first experiment, we studied avoidance of 60-Hz electric fields by rats during four sequential 45-min tests, spaced 1 wk apart. In the second experiment (and the subsequent replicate), perception and avoidance of electric fields by rats were examined over a 23.5-hr session. In the third set of experiments, we investigated the effects of chronic exposure of rats to a 100-kV/m field prior to the perception and avoidance tests.

Experiments were also conducted to assess the possible effect of electric-field exposure for 14 or 28 days on the sensitivity of mice to sound-induced seizures.

Additionally, several engineering and dosimetric tests were performed to make sure that the behavioral results were not the result of factors other than the electric field per se. These tests measured corona and ozone formation, electric-field strength, and vibration within the shuttlebox.

## B. METHODS

1. Shuttlebox Experiments. The Special Studies Exposure System described in the Second Interim Progress Report (Phillips and Kaune, 1977) was used to generate the electric fields for the behavioral experiments.

Apparatus. Rats were tested for electric field avoidance in a Lexan* shuttlebox (Figure XIII-1) described in the Second Interim Progress Report (Phillips and Kaune, 1977). Briefly, this shuttlebox, 103 cm long by 11 cm wide by 10 cm deep, had 44.5 cm of one end shielded from the electric field by covering the surface with aluminum window screen. The opposite end was covered with visually identical plastic mesh screen which did not attenuate the electric field. In a center (transition) region, two light beams allowed us to monitor the position and traverse activity of the rat.

In all shuttlebox experiments water was supplied from a manifold located in the field-free region below the shuttlebox. Seven demand-activated valves, 12.7 cm apart, protruded approximately 0.5 cm above the mesh floor, three on either end and one in the center of the shuttlebox. These valves were at ground potential, as was the water. This system has been described in detail elsewhere (Phillips et al., 1976a).

Food was supplied to the rats during exposure sessions that exceeded 45 min. Approximately 25 g of pelleted food was available, ad libitum, at either end of the shuttlebox from Lexan* hoppers that had a low profile to minimize any perturbation of the electric field.

The shuttlebox was ventilated at the top (136 holes; 0.7-cm-diameter, 2.5 cm apart).

Electric Field Measurements. Measurements inside the shuttlebox at floor level were made with a flat, nonperturbing 5-cm-diameter circular probe (Kaune, 1978).

*General Electric type MR 4000, polycarbonate plastic

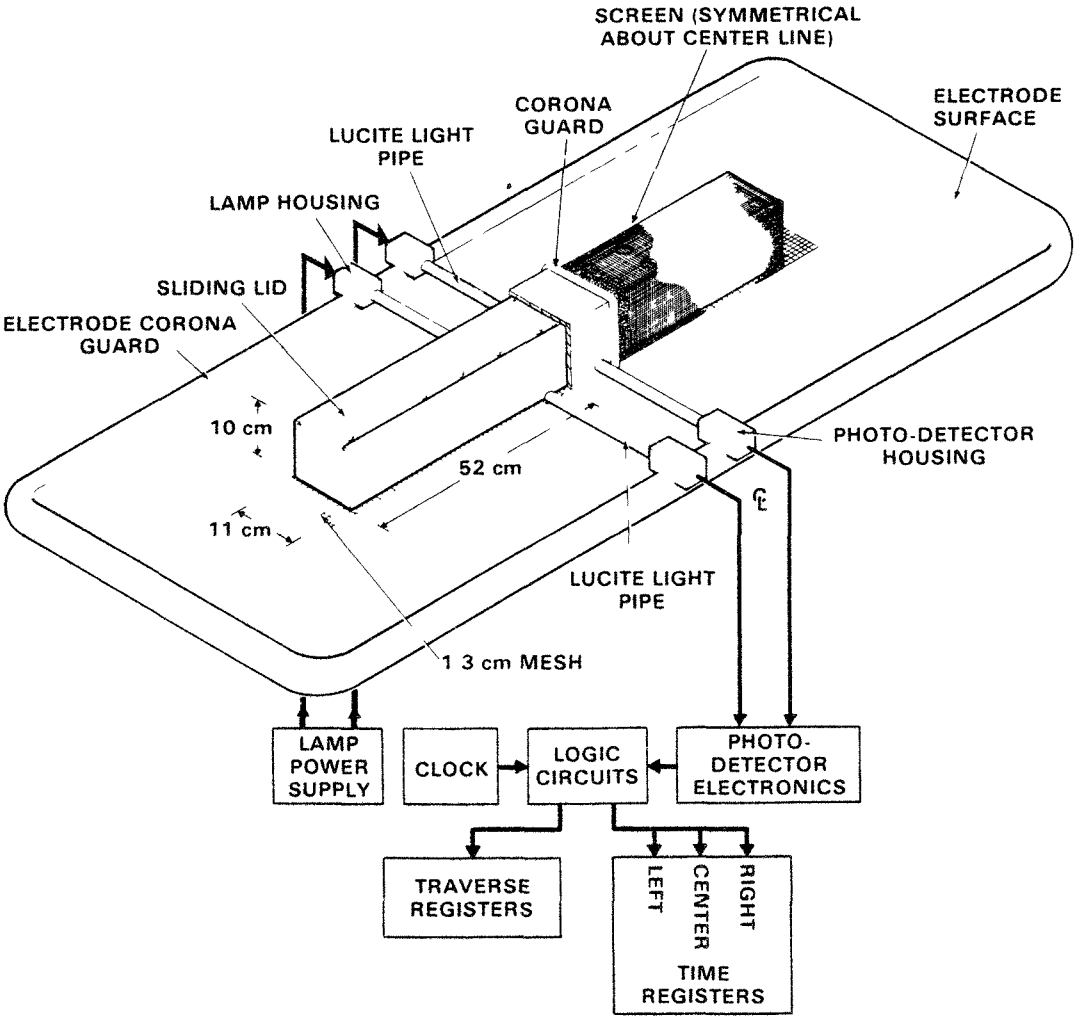



FIGURE XIII-1. Diagram of Shuttlebox System Used to Test Perception and Avoidance of Electric Fields

Corona and Ozone Measurements. We tested for corona discharge by audible noise, emission of visible light, and ozone generation. We checked audible noise by listening, which experience has shown to be a sensitive method. Visible light emission was monitored using extended-exposure photography (Beamer, 1978). Ozone levels were measured with a chemiluminescent ozone analyzer, a series of potassium-iodide impingers and an ozone reaction tube-type sampler.* The $O_3$ sensitivity level of the analyzer is 1 ppb, while the impinger and sampler methods are sensitive to $O_3$ concentrations of 5 ppb.** The techniques used, and many of the results, have been described by Phillips et al. (1978).

Vibration Measurements. The amplitude and frequency of floor vibrations in the shuttlebox were measured with two piezoelectric accelerometers.*** Electrically isolated magnetic mounts were used to attach the two accelerometers to the underside of the floor of the shuttlebox. Typically, one accelerometer was placed in the shielded end of the shuttlebox and one in the unshielded end. The sensitivity of these accelerometers is about 1 $m/sec^2$; their frequency is from 2 Hz to 12 kHz.

Procedures for Behavioral Experiments. Two experiments were conducted to investigate the behavioral response of rats to a range of electric field strengths. The parameters evaluated were avoidance behavior, traverse activity, food consumption and serum corticosterone levels. Avoidance of electric fields was defined as a statistically significant increase in

*Bendix Gas/Tec pump with Dräger Detection Tubes Drägerwerk, A. G. Lubek, F. R. of Germany
**Bendix Corp., Model 8002, Lewisburg, WV
***BBN Instruments Co., Model 508, Cambridge, MA

the time that exposed rats spent in the shielded end of the shuttlebox apparatus. Traverse activity is defined as the number of traverses made between the two ends of the shuttlebox during test sessions.

Forty naive male Sprague-Dawley rats (Hilltop Labs), approximately 90 days old, were distributed into five groups that had equivalent body weight distributions. In the first experiment, the groups (N = 8/group) were randomly designated for behavioral tests at field strengths of 0, 60, 75, 90 and 105 kV/m, with a single field-strength value used for animals within each group throughout the experiment. These particular field strengths were selected on the basis of preliminary experiments, which suggested that rats avoid electric fields of 100 and 133 kV/m but not 75-kV/m fields (Phillips and Kaune, 1977). Rats were subjected to one test (45 min) per week for 4 wk. The amount of time spent in exposed, center and shielded regions, the number of traverses to each region, and the duration (time on each side of the box per traverse) were recorded.

Prior to and between sessions, rats were housed individually in standard stainless steel cages where they were given free access to food and water. The light:dark cycle was 12 hr:12 hr (lights on at 7 a.m. and off at 7 p.m.). The order of testing experimental and control groups was balanced across time of day to avoid potential diurnal cycle effects, and to insure that a minimum of one subject from each group was tested on a given day. Prior to each session, the wire-mesh screen and shuttlebox were sponged and dried, and the shuttlebox and cover were rotated 180° to alternate the side of exposure, thus balancing side of exposure within and across groups. During a given day, half the rats were tested

with the right side of the box exposed to the field, while the remainder were exposed on the opposite side. This procedure was followed to avoid possible confounding due to side bias or spatial association.

In the second experiment, the groups (N = 8/group) were tested at field strengths of 0, 25, 50, 75, and 100 kV/m for 23.5 hr. (Because of an electric power failure, it was necessary to eliminate one rat in the 0-kV/m group from the experiment.) The 25- and 50-kV/m field strengths were used to test the possibility that a behavioral response may occur at a lower field strength with a longer duration of exposure.

The longer exposure duration of the second experiment allowed a comparison of avoidance and traverse activity over the light and dark periods of a 24-hr cycle. In addition to avoidance and traverse activity measures, food consumption at each end of the shuttlebox was measured. Within 2 min after the end of each session, a blood sample was taken by cardiac puncture while the rat was anesthetized with fluothane gas (Halothane). Serum samples were analyzed fluorometrically for corticosterone concentration.

In the 23.5-hr tests, the shuttlebox was rotated 180° daily to reduce possible side bias. Both food containers were filled with 25 g of fresh food before each session. Sessions began at 11:00 a.m. and ended at 10:30 a.m. the following morning. The two light periods (8 hr and 3.5 hr), with one dark period (12 hr) between them, allowed us to examine changes of side preference during the light and dark cycles under the influence of electric fields.

2. Chronic Exposure Experiments. A series of experiments were conducted in which rats were either exposed or sham exposed to a 100-kV/m, 60-Hz electric field for 30 days prior to testing in the shuttlebox at field strengths of 0, 25, 50, 75 and 100 kV/m (N = 12 exposed and 12 sham-exposed per field strength/group).

Due to space limitations within the exposure system, three replicate experiments were conducted and the results were combined. In each experiment, 40 male Sprague-Dawley rats (55-60 days of age) were exposed ($\sim$ 20 hr/day) for 30 days prior to behavioral testing. The shuttlebox apparatus (Figure XIII-1) was the same as that used in previous experiments. In the first of the three experiments the emphasis was on those field strengths where effects had been seen in other avoidance studies, i.e., 50, 75, and 100 kV/m. After the first experiment we decided that information from a 25-kV/m exposure was necessary; the designs of experiments 2 and 3 were therefore altered to include exposure of rats at 25 kV/m.

Each test session lasted 45 min. The order in which rats were tested was balanced to avoid possible bias by diurnal effects. No rat was tested more than once. Records were kept of the traverse activity and the amount of time rats spent in the exposed, center and shielded regions of the shuttlebox.

3. Audiogenic Seizure Study

Electric-Field Exposures. Mice in these experiments were exposed to a 100-kV/m field for 14 or 28 days. The exposures were conducted in the Mouse Exposure System (Phillips et al., 1978).

Apparatus. Mice were tested for audiogenic seizure susceptibility and latency in a 30-gal. galvanized steel garbage can. Two alarm bells that produced a "white" noise output of 110 db were mounted inside the can. The can was located inside a sound-deadened room. A mirror, angled over the can, allowed investigators to time latency to seizures from outside the room through a window in the door of the sound-deadened enclosure.

Procedure. Sixty male SJL/J mice, received at 14 days of age, were housed in groups with ad libitum access to food and water until they were 21 days of age. The SJL/J mouse (Jackson Laboratories) is genetically susceptible to sound-induced central nervous system seizures; however, for maximum sensitivity, the mice must be "primed" at 21 days of age. This priming consisted of a 60-sec exposure to the 110-db noise. No mice exhibited seizures during this priming. At 28 days of age all mice were pretested for seizure latency. The SJL/J mice demonstrate two sequential forms of seizures when exposed to loud noise: first, they go into a rapid running behavior, followed by a clonic-tonic seizure. The susceptibility of mice to such seizures is dependent upon several factors, including the general excitability state of the central nervous system.

Based on mean latencies to both types of seizure at 28 days of age, two homogeneous groups were selected for subsequent exposure to the electric field. These mice were exposed or sham exposed for 14 or 28 days (from day 29 of age to day 42, or from day 29 to day 57), then retested for seizure latency. All seizure testing was conducted between 10:30 a.m. and 12:00 p.m. to avoid possible effects of circadian rhythm on seizure sensitivity.

C. EXPERIMENTAL RESULTS

1. Environmental Measurements

Shuttlebox Electric Field Measurements. Field-strength measurements made inside the outer box which incorporated the metal and plastic screens indicated a very small field beneath the metal end, and 95% of the applied field beneath the plastic end. However, when measurements were made with the inner box in place, an erratic instability in field strength was observed in the unshielded end of the system. Sudden random shifts of as much as 25% in field strength were observed, but only with both boxes in place. The nature of this instability is not well understood but may be related to charge migration through the air between the tops of the two boxes.

In replicate experiments of our early work, and for the subsequent behavioral experiments, a new shuttlebox system was built incorporating only one Lexan box (Figure XIII-1) in which field strength measurements were stable. The measured, floor-level field strength along the length of the box is shown in Figure XIII-2. Field-strength scans across the width of the box in the unshielded region showed the field to be quite uniform ($\pm$ 1%) in the exposed end of the box and fairly uniform ($\pm$ 10%) in the transition region between the shielded and unshielded ends.

Shuttlebox Vibration Measurements. The ambient levels of acceleration of the floor of the shuttlebox without the electric field were typically in the range of 0.05 m/sec$^2$, peak-to-peak, over a wide frequency range. Occasionally, much stronger vibrations ($\sim$ 0.5 m/sec$^2$, peak-to-

THIS FIGURE INDICATES THE % OF THE APPLIED ELECTRIC FIELD ATTENUATED BY THE METAL AND PLASTIC COVERINGS OVER THE SHUTTLEBOX.

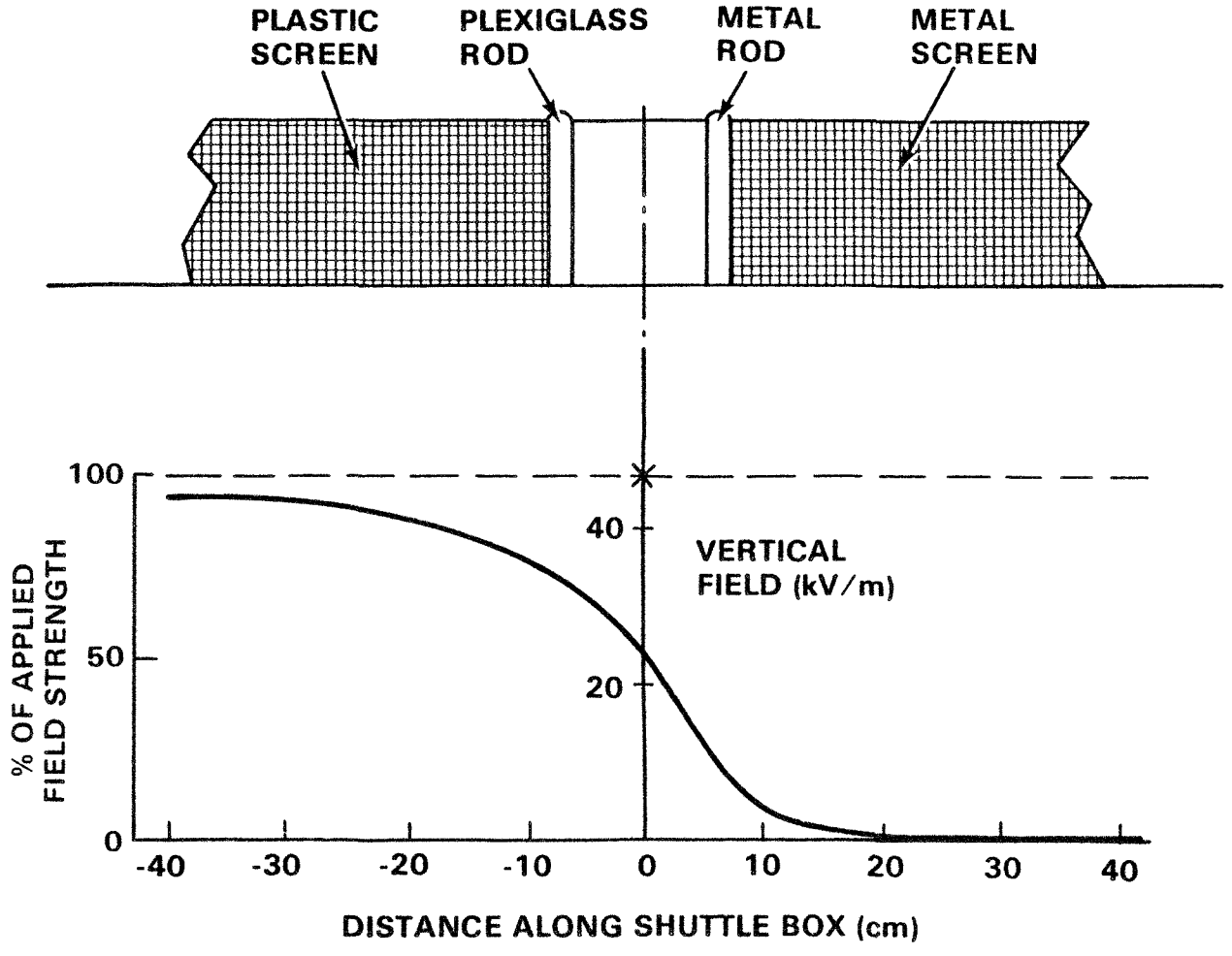



FIGURE XIII-2. Field Strength (kV/m) Along Long Axis of the Perception and Avoidance Shuttlebox

peak) with frequencies in the range 30-100 Hz were observed, evidently associated with the operation of electric motors in the building's heating and ventilation system.

With the shuttlebox removed and the system energized to a field strength of 130 kV/m, the level of vibration was unchanged from the 0-field case. With the shuttlebox in place, however, a 120-Hz vibration of 0.15 $m/sec^2$, peak-to-peak, was observed in the unshielded end of the shuttlebox. When the shuttlebox was reversed, vibration was observed in the shielded end of the shuttlebox. The two accelerometers were also interchanged, and the same results were obtained. Rotating the entire lower electrode unit 180° resulted in the vibration also moving to the other side of the exposure system. The conclusion was that one end of the electrode was vibrating while the other was not. The amplitude of this vibration, measured as a function of field strength, was found to depend on the square of the field strength. These data, and the observation that the vibration frequency is 120 Hz, indicate that this vibration is the result of electrical attraction between the two exposure electrodes (Reitz and Milford, 1960). A 120-Hz vibration with a peak-to-peak acceleration of 0.15 $m/sec^2$ corresponds to a peak-to-peak physical displacement of the shuttlebox floor of 0.25 μm.

Corona and Ozone Testing. The results of photographic room mapping for visible light emissions were negative, as were tests for audible noise. The results of ozone testing were also negative at numerous locations within the shuttlebox and exposure room. Ozone concentrations did not exceed ambient levels present without the electric field.

2. Behavorial Tests

Shuttlebox Side Preference Studies. The results of the repeated, short (45-min) sessions (Figure XIII-3, Table XIII-1) indicate a significant avoidance response by rats to electric-field exposure. In the first 45-min session, rats exposed to field strengths of 90 or 105 kV/m spent significantly less time in the exposed end of the shuttlebox than did 0-kV/m controls (Student's $t$ test = 3.24 and 4.28, respectively, both, $p < 0.01$; 15 df). When data were analyzed by a repeated measures analysis of variance (Winer, 1962; Satterthwaite, 1946), it was found that exposed rats spent significantly more of the 45-min session in the unexposed region of the shuttlebox, both across sessions [$F(4,35) = 3.64$, $p < 0.05$] and within three of the individual sessions:

Session 1 - $F(4,35) = 3.29$, $p < 0.05$
Session 2 - $F(4,35) = 0.63$, NS
Session 3 - $F(4,35) = 3.70$, $p < 0.05$
Session 4 - $F(4,35) = 7.28$, $p < 0.01$

The source of the variance differences among groups is primarily from the 90 and 105 kV/m groups [Dunnett's $t$ test (Winer, 1972)]. The $t$ values were computed on the means of the four sessions for each group with the following results:

0 kV/m vs. 60 kV/m: $F(4,35) = 1.95$, NS
0 kV/m vs. 75 kV/m: $F(4,35) = 0.78$, NS
0 kV/m vs. 90 kV/m: $F(4,35) = 2.56$, $p < 0.05$
0 kV/m vs. 105 kV/m: $F(4,35) = 3.70$, $p < 0.01$

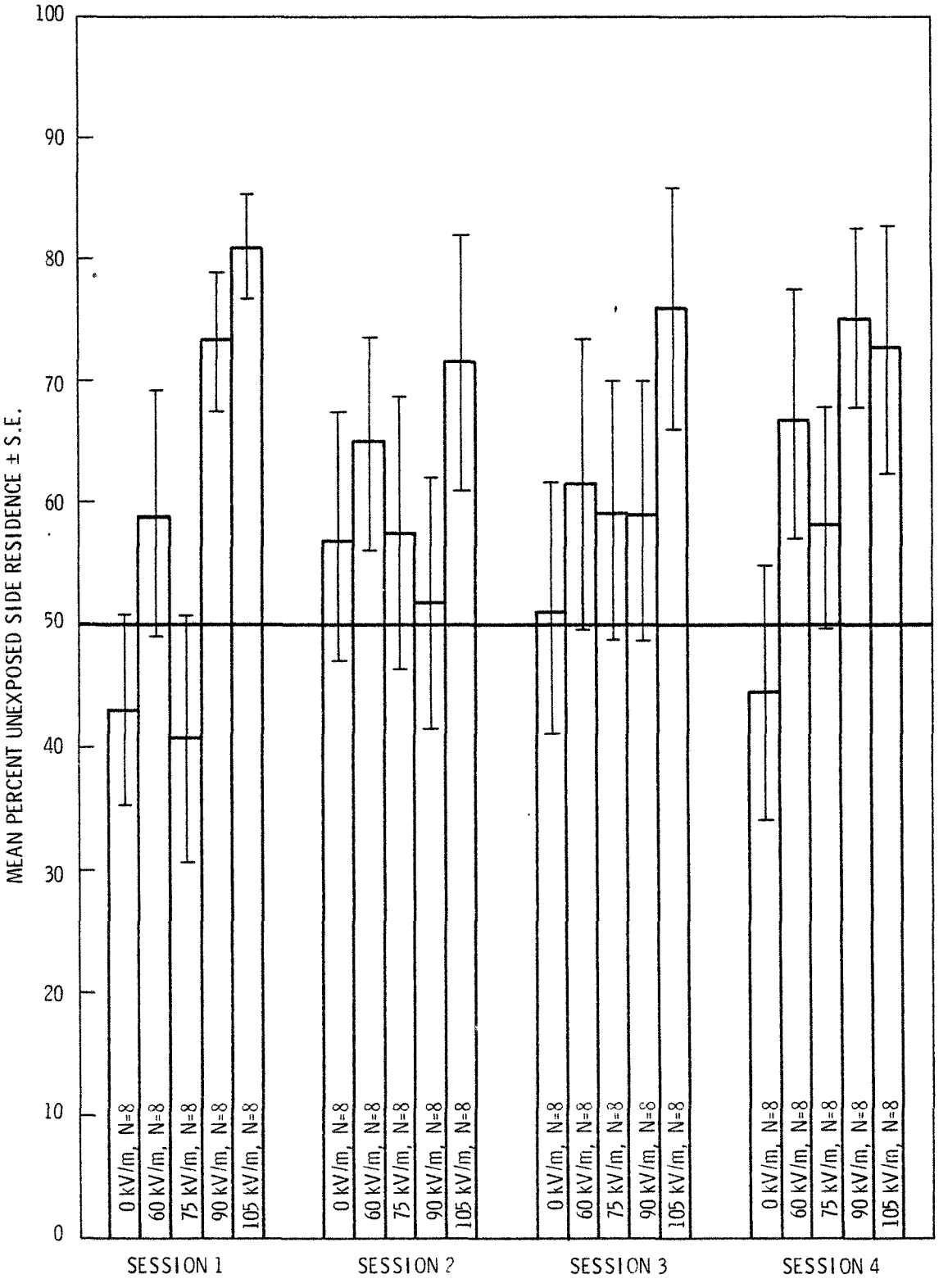



FIGURE XIII-3. Mean Percent of Time (± S.E.) Rats Spent on the Unexposed Side of the Shuttlebox as a Function of Field Strength and Test Session. Data are normalized to eliminate time spent in the center.

TABLE XIII-1. Mean (± S.E.*) Percentage of Time Rats Spent in the Exposed, Center and Unexposed (Shielded) Regions of a Shuttlebox During Four 45-Min Sessions of Exposure to 60-Hz Electric Fields

| Field Strength (kV/m) | N | Mean % of Residence Time (± S.E.) Exposed | Center | Unexposed | Significance** |
|---|---|---|---|---|---|
| | | SESSION 1 | | | |
| 0 | 8 | 51.1 ± 7.9 | 10.3 ± 1.7 | 38.5 ± 7.8 | NS |
| 60 | 8 | 35.6 ± 2.3 | 13.5 ± 2.3 | 50.8 ± 9.6 | NS |
| 75 | 8 | 53.9 ± 10.2 | 9.4 ± 1.6 | 36.9 ± 10.0 | NS |
| 90 | 8 | 24.3 ± 3.9 | 9.9 ± 2.4 | 66.0 ± 5.8 | <0.01 |
| 105 | 8 | 17.4 ± 2.8 | 10.1 ± 1.8 | 72.4 ± 4.3 | <0.01 |
| | | SESSION 2 | | | |
| 0 | 8 | 39.1 ± 9.8 | 6.7 ± 1.8 | 53.1 ± 10.7 | NS |
| 60 | 8 | 33.0 ± 8.4 | 6.2 ± 1.0 | 60.7 ± 8.7 | NS |
| 75 | 8 | 40.0 ± 5.9 | 5.9 ± 1.9 | 54.1 ± 11.2 | NS |
| 90 | 8 | 43.6 ± 9.9 | 9.5 ± 1.5 | 46.9 ± 10.2 | NS |
| 105 | 8 | 25.6 ± 9.7 | 5.6 ± 1.4 | 67.5 ± 10.6 | NS |
| | | SESSION 3 | | | |
| 0 | 8 | 45.7 ± 10.2 | 6.7 ± 1.7 | 47.5 ± 9.8 | NS |
| 60 | 8 | 36.6 ± 11.9 | 4.7 ± 1.3 | 58.6 ± 12.0 | NS |
| 75 | 8 | 38.5 ± 11.1 | 5.5 ± 1.3 | 56.0 ± 10.6 | NS |
| 90 | 8 | 38.6 ± 10.7 | 6.1 ± 1.5 | 55.2 ± 11.1 | NS |
| 105 | 8 | 22.5 ± 10.5 | 5.7 ± 1.1 | 71.5 ± 10.1 | <0.05 |
| | | SESSION 4 | | | |
| 0 | 8 | 52.7 ± 10.8 | 5.1 ± 0.8 | 42.1 ± 10.4 | NS |
| 60 | 8 | 31.5 ± 10.0 | 5.5 ± 0.8 | 63.0 ± 9.7 | NS |
| 75 | 8 | 39.5 ± 9.4 | 5.2 ± 0.8 | 55.1 ± 9.5 | NS |
| 90 | 8 | 23.0 ± 7.2 | 8.5 ± 1.3 | 68.7 ± 7.3 | NS |
| 105 | 8 | 26.0 ± 10.2 | 5.9 ± 1.1 | 68.1 ± 10.2 | NS |

* Values have been rounded off.

** Two-tailed Mann-Whitney U Test, comparing values of 0-kV/m group with those of experimental group

Traverse activity, as determined by the number of end-to-end traverses in the shuttlebox during repeated 45-min sessions, was significantly affected by the electric field [$F(4,35) = 8.36$, $p < 0.05$]; (Table XIII-2). However, while these data indicate a trend toward higher traverse activity among exposed animals over repeated sessions, analysis within sessions does not reveal a statistically significant difference between groups.

Subsequent to the experiment, two possible problems were identified: 1) the electric field was erratic when the inner/outer shuttlebox was used, as already discussed; 2) a 4% fifth harmonic and 3% seventh harmonic were observed in the electric field (Phillips et al., 1978). Both of these problems were eliminated, as discussed earlier. To test whether the observed behavioral effects could have resulted from either of these problems, a replicate experiment (40 naive rats tested in a single 45-min session) was performed. The results of the first study were substantiated (Figure XIII-4). Rats exposed to fields of 90 or 105 kV/m spent significantly more time in the shielded end of the shuttlebox than sham-exposed controls [Student's $t$ test = 2.54 (13 df) and 1.82 (14 df), respectively; $p < 0.05$]. Neither the electric-field instability nor the harmonic distortion present in the first experiment was apparently the factor mediating the avoidance response.

In 23.5-hr tests, rats exposed to 75 or 100 kV/m spent significantly more time [$F(4,34) = 10.06$, $p < 0.001$] in the region shielded from the electric field than 0-kV/m controls (Figure XIII-5). Rats tested at 25 or 50 kV/m spent significantly more time [$F(4,34) = 9.58$,

TABLE XIII-2. Number of Traverses (± S.E.) Through the Center Region of the Shuttlebox Made by Rats Exposed to Various Electric Field Strengths During Four 45-Min Exposure Sessions

| | | Number of Traverses | | | |
|---|---|---|---|---|---|
| kV/m | N | Week 1 | Week 2 | Week 3 | Week 4 |
| 0 | 8 | 51.38 ± 9.01 | 41.50 ± 10.37 | 34.25 ± 5.52 | 25.75 ± 3.28 |
| 60 | 8 | 58.63 ± 13.43 | 41.25 ± 8.66 | 38.13 ± 8.87 | 36.13 ± 4.05 |
| 75 | 8 | 57.63 ± 9.61 | 37.75 ± 12.00 | 33.38 ± 10.53 | 42.13 ± 9.79 |
| 90 | 8 | 47.25 ± 8.67 | 49.75 ± 7.96 | 39.25 ± 11.25 | 44.13 ± 6.68 |
| 105 | 8 | 56.38 ± 9.50 | 34.38 ± 6.01 | 35.13 ± 6.78 | 40.38 ± 9.25 |

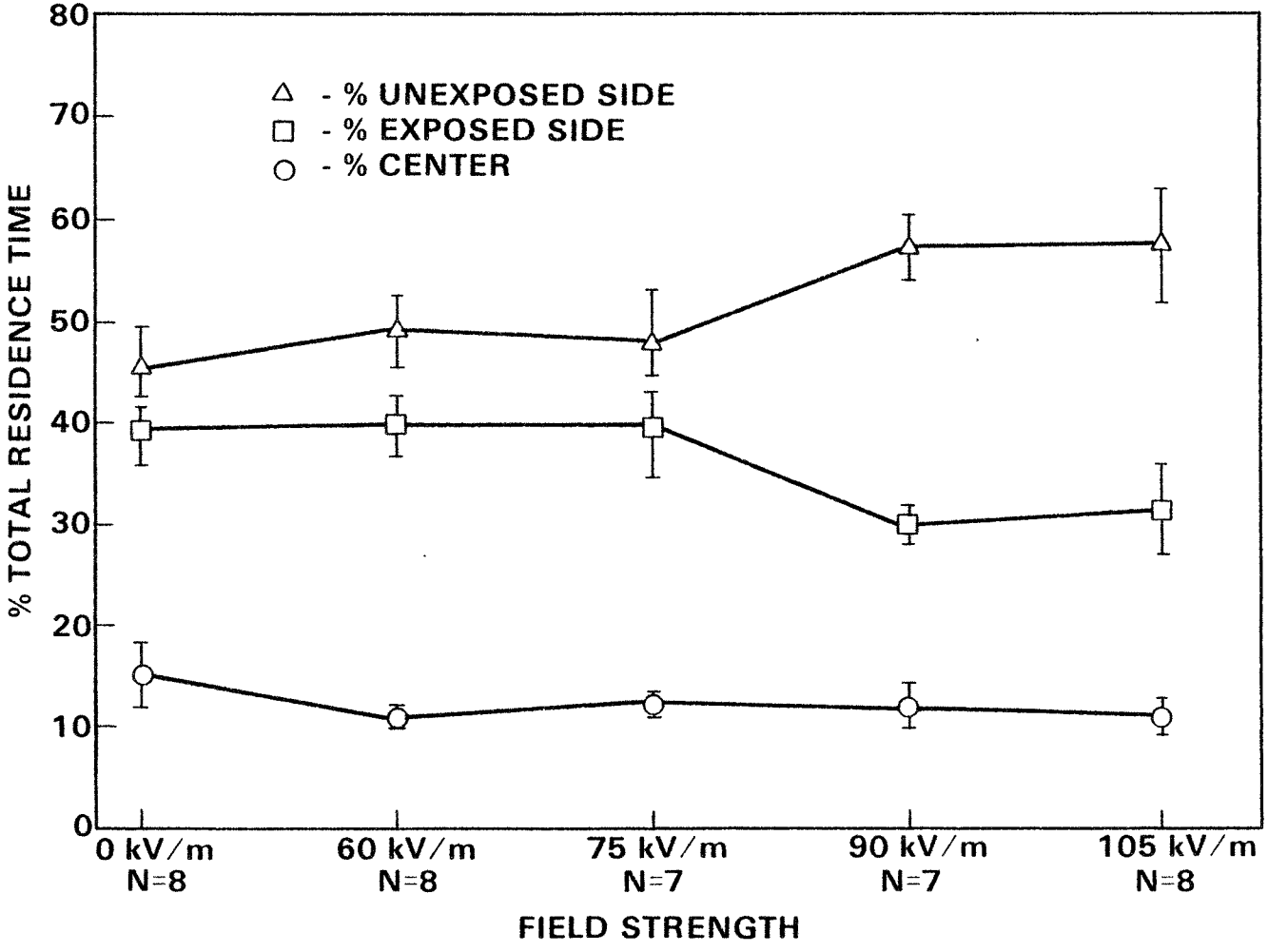



FIGURE XIII-4. Mean Percent of Time (± S.E.) Rats Spent in Exposed, Center and Unexposed Regions of the Shuttlebox During a Single, 45-min Test Session. Data are presented in hourly increments during the light and dark periods of the session.

UNEXPOSED SIDE PREFERENCE OF 40 NAIVE RATS TESTED FOR ELECTRIC FIELD AVOIDANCE DURING A SINGLE 23.5 HOUR SESSION. NOTE THE EXPOSED SIDE PREFERENCE AT 25 AND 50 kV/m.

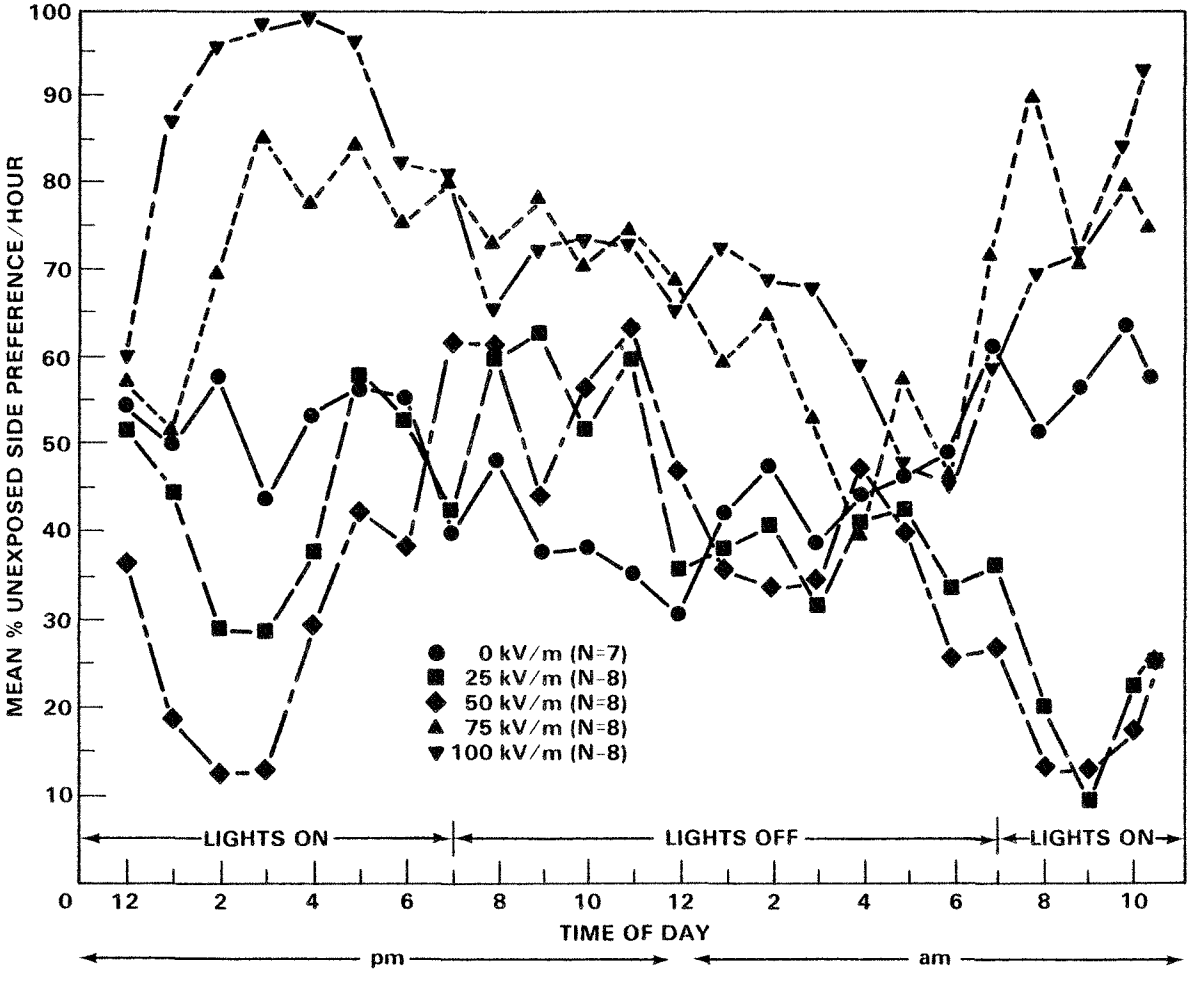



FIGURE XIII-5. Mean Percent of Time Rats Spent in the Unexposed Region During a 23.5-hr Test Session. Data are presented in hourly increments during the light and dark periods of the session.

$p < 0.05$] in the exposed region of the shuttlebox than controls during the light portion of the daily cycle, but not during the dark portion. Avoidance of the exposed side among rats tested at 75 or 100 kV/m decreased steadily during the dark period, then increased at the onset of the second light period. The opposite was the case for rats tested at 25 or 50 kV/m.

Traverse activity (Figure XIII-6) over the 23.5-hr sessions was significantly affected [$F(4,34) = 79.28$, $p < 0.001$] by the light cycle, as expected. All rats were initially active in the novel shuttlebox environment. This traverse activity decreased to low levels within 2 hr after the start of the session. During the first hour of exposure there was a significant [$F(4,34) = 7.16$, $p < 0.05$] effect of exposure on traverse activity. Control rats made fewer traverses than exposed rats, while the group exposed at 100 kV/m made the greatest number of traverses during the first hour. Traverse activity in all groups increased slightly with the start of the dark period, continued to rise during the period, then decreased at the onset of the next light period (Figure XIII-6).

Over the 23.5-hr sessions, rats exposed at 75 or 100 kV/m spent significantly [$F(4,34) = 10,06$, $p < 0.05$] less time on the exposed side per traverse than rats tested at 0 kV/m. During these sessions, rats exposed at 25 or 50 kV/m did not differ significantly from 0-kV/m rats in residence time per traverse on the exposed side.

There were no differences among groups in the amount of total food consumed on the exposed or shielded side of the shuttlebox, nor were there any differences in total food consumption among groups.

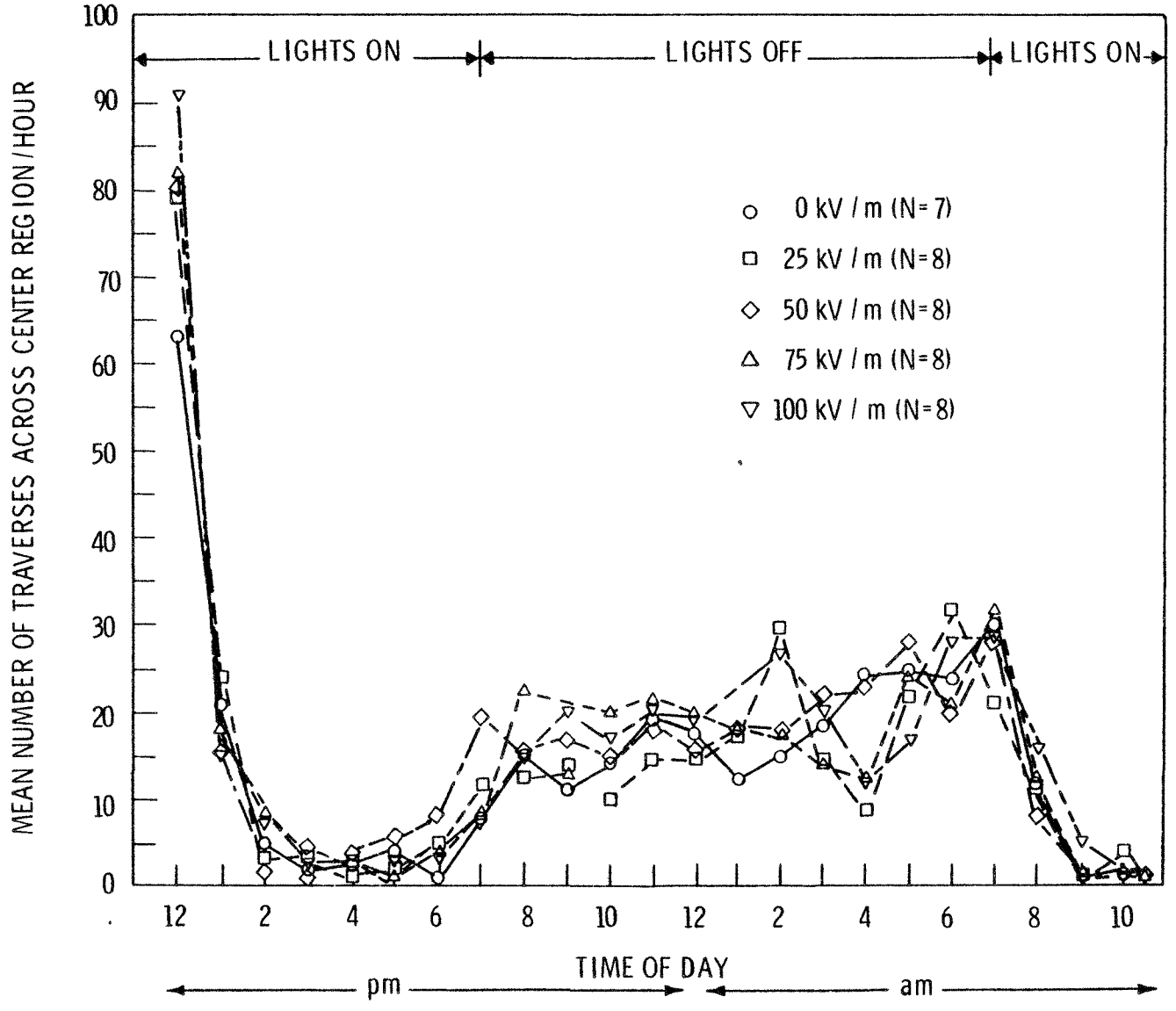



FIGURE XIII-6. Mean Number of Traverses Through the Center Region of the Shuttlebox by Rats During a 23.5-hr Test Session. Data are presented in hourly increments during the light and dark periods of the session.

The results of fluorometric analysis of serum corticosterone levels indicated no statistically significant differences among groups (Table XIII-3).

The results of a subsequent replication of the second (23.5-hr) experiment did not differ significantly from the results reported here, with one exception: rats tested at 25 kV/m did not differ significantly from control (0-kV/m) rats. Rats tested at 75 or 100 kV/m avoided the electric field, while rats tested at 50 kV/m spent significantly more time exposed to the field during the light period than 0-kV/m controls. There were no significant differences among groups in traverse activity after the first hour of exposure. The results of the initial experiment and the replicate are compared in Tables XIII-4 to -8 by light and dark periods within the 23.5-hr session.

Chronic Exposure Experiments. The results indicate that rats exposed to 100 kV/m for 20 hr/day for 30 days prior to the behavorial test spent a significantly higher percentage of the 45-min session in the exposed region when tested at 25 kV/m, than did previously sham-exposed controls (Student's $t$ test = 2.10 $p < 0.05$, 22 df). Within the pre-exposed group, there was significantly greater exposed-side residence time across field strengths [$F(4,55) = 6.44$, $p < 0.05$], while within the previously sham-exposed group there was significantly greater unexposed-side residence across field strengths [$F(4,54) = 6.84$, $p < 0.05$].

The behavior of rats pre-exposed to 100 kV/m for 30 days differed from that of previously sham-exposed rats at two field strengths: 25 and 100 kV/m (Figure XIII-7). Preference for the exposed side of the

TABLE XIII-3. Plasma Corticosterone Levels (Fluorometric Assay) in Rats at the End of 24-Hr Shuttlebox Test

| Field Strength (kV/m) | N | Corticosterone Levels (Mean ± S.E.) |
|---|---|---|
| 0 | 6 | 155 ± 17 |
| 25 | 6 | 140 ± 16 |
| 50 | 6 | 129 ± 30 |
| 75 | 6 | 140 ± 32 |
| 100 | 5 | 171 ± 40 |

TABLE XIII-4. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of the Shuttlebox During a 23.5-hr Exposure Session to Electric Fields at 0-100 kV/m. Values shown are means ± S.E.

| Field Strength (kV/m) | Initial Experiment (N = 39) | | | Replicate Experiment (N = 40) | | |
|---|---|---|---|---|---|---|
| | Exposed | Center | Unexposed | Exposed | Center | Unexposed |
| 0 | 47.2 ± 1.7 | 4.5 ± 0.8 | 48.3 ± 1.8 | 49 ± 2.6 | 9 ± 1.1 | 42 ± 2.5 |
| 25 | 51.6 ± 7.9 | 8.6 ± 3.8 | 39.0 ± 6.7 | 43 ± 2.7 | 9 ± 0.9 | 48 ± 2.6 |
| 50 | 59.3 ± 3.5 | 5.9 ± 1.2 | 34.6 ± 3.4 | 55 ± 2.7 | 15 ± 1.7 | 30 ± 2.6 |
| 75 | 27.6 ± 2.6 | 3.1 ± 0.5 | 69.0 ± 2.7 | 32 ± 2.7 | 11 ± 1.5 | 57 ± 2.8 |
| 100 | 19.8 ± 2.4 | 5.6 ± 1.1 | 76.1 ± 1.0 | 29 ± 2.5 | 5 ± 0.6 | 66 ± 2.5 |

TABLE XIII-5. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of the Shuttlebox During First Light Period (Hr 1-8) With Electric Field Exposure at 0-100 kV/m. Values shown are means ± S.E.

| Field Strength (kV/m) | Initial Experiment (N = 39) | | | Replicate Experiment (N = 40) | | |
|---|---|---|---|---|---|---|
| | Exposed | Center | Unexposed | Exposed | Center | Unexposed |
| 0 | 47.3 ± 2.5 | 1.4 ± 0.6 | 51.3 ± 2.4 | 50.1 ± 4.8 | 9.4 ± 2.1 | 39.1 ± 4.7 |
| 25 | 53.7 ± 4.6 | 3.3 ± 1.5 | 42.9 ± 4.1 | 42.9 ± 5.1 | 9.1 ± 2.0 | 46.1 ± 4.8 |
| 50 | 67.0 ± 6.6 | 1.6 ± 0.4 | 31.5 ± 6.3 | 67.8 ± 4.7 | 9.2 ± 2.5 | 23.1 ± 4.2 |
| 75 | 24.0 ± 4.4 | 2.1 ± 0.5 | 72.6 ± 4.8 | 43.5 ± 5.3 | 9.2 ± 2.7 | 47.9 ± 5.2 |
| 100 | 10.9 ± 4.5 | 1.7 ± 0.6 | 87.4 ± 5.0 | 41.9 ± 5.2 | 4.2 ± 1.4 | 54.0 ± 5.2 |

TABLE XIII-6. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of a Shuttlebox During the Second Light Period with Electric Field Exposure at 0-100 kV/m. Values shown are means ± S.E.

| Field Strength (kV/m) | Initial Experiment (N = 39) | | | Replicate Experiment (N = 40) | | |
|---|---|---|---|---|---|---|
| | Exposed | Center | Unexposed | Exposed | Center | Unexposed |
| 0 | 36.2 ± 4.6 | 6.3 ± 3.2 | 57.4 ± 2.9 | 64.2 ± 7.3 | 1.4 ± 0.4 | 31.6 ± 7.1 |
| 25 | 71.7 ± 3.2 | 8.9 ± 3.5 | 19.1 ± 4.0 | 34.0 ± 6.9 | 3.6 ± 1.3 | 62.6 ± 6.6 |
| 50 | 77.5 ± 6.3 | 6.6 ± 4.4 | 15.8 ± 1.9 | 58.7 ± 7.7 | 7.8 ± 3.7 | 33.6 ± 7.7 |
| 75 | 20.2 ± 5.4 | 0.7 ± 0.7 | 79.0 ± 4.7 | 33.9 ± 7.6 | 10.7 ± 4.4 | 55.5 ± 7.9 |
| 100 | 13.6 ± 4.3 | 6.2 ± 3.4 | 80.1 ± 6.3 | 24.5 ± 7.1 | 2.6 ± 1.1 | 73.3 ± 7.0 |

TABLE XIII-7. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of a Shuttlebox During Light Periods (Hr 1-8 and 23.5) with Electric Field Exposure at 0-100 kV/m. Values shown are means ± S.E.

| Field Strength (kV/m) | Initial Experiment (N = 39) | | | Replicate Experiment (N = 40) | | |
|---|---|---|---|---|---|---|
| | Exposed | Center | Unexposed | Exposed | Center | Unexposed |
| 0 | 43.6 ± 2.6 | 3.0 ± 1.2 | 53.3 ± 1.9 | 55.5 ± 4.1 | 7.6 ± 1.7 | 37.0 ± 3.9 |
| 25 | 59.7 ± 4.0 | 5.2 ± 1.6 | 35.0 ± 4.5 | 41.4 ± 4.1 | 7.2 ± 1.4 | 51.[illegible] ± 4.0 |
| 50 | 70.5 ± 4.8 | 3.3 ± 1.4 | 26.3 ± 4.7 | 64.8 ± 4.0 | 8.7 ± 2.1 | 26.6 ± 3.8 |
| 75 | 22.8 ± 3.2 | 1.6 ± 0.5 | 74.8 ± 3.4 | 40.0 ± 4.3 | 9.7 ± 2.3 | 50.4 ± 4.3 |
| 100 | 11.8 ± 3.1 | 3.2 ± 1.2 | 85.0 ± 3.8 | 36.1 ± 4.2 | 3.7 ± 1.0 | 60.4 ± 4.2 |

TABLE XIII-8. Percent of Time Rats Spent in the Exposed, Center and Shielded Regions of a Shuttlebox During the 12-Hr Dark Period with Electric Field Exposure at 0-100 kV/m. Values shown are means ± S.E.

| Field Strength (kV/m) | Initial Experiment (N = 39) | | | Replicate Experiment (N = 40) | | |
|---|---|---|---|---|---|---|
| | Exposed | Center | Unexposed | Exposed | Center | Unexposed |
| 0 | 50.8 ± 2.0 | 5.9 ± 1.1 | 43.3 ± 2.4 | 42.1 ± 3.0 | 11.2 ± 1.3 | 46.9 ± 3.1 |
| 25 | 44.3 ± 3.4 | 11.9 ± 1.6 | 44.5 ± 3.3 | 43.9 ± 3.5 | 11.0 ± 1.2 | 45.3 ± 3.3 |
| 50 | 48.1 ± 2.6 | 8.6 ± 1.7 | 43.0 ± 3.8 | 44.7 ± 3.3 | 21.1 ± 2.5 | 34.3 ± 3.4 |
| 75 | 32.4 ± 3.7 | 4.6 ± 0.7 | 63.3 ± 3.7 | 23.7 ± 2.9 | 12.3 ± 1.9 | 64.2 ± 3.5 |
| 100 | 27.8 ± 2.0 | 8.0 ± 1.6 | 64.3 ± 2.9 | 22.8 ± 2.6 | 6.6 ± 0.7 | 70.6 ± 2.7 |

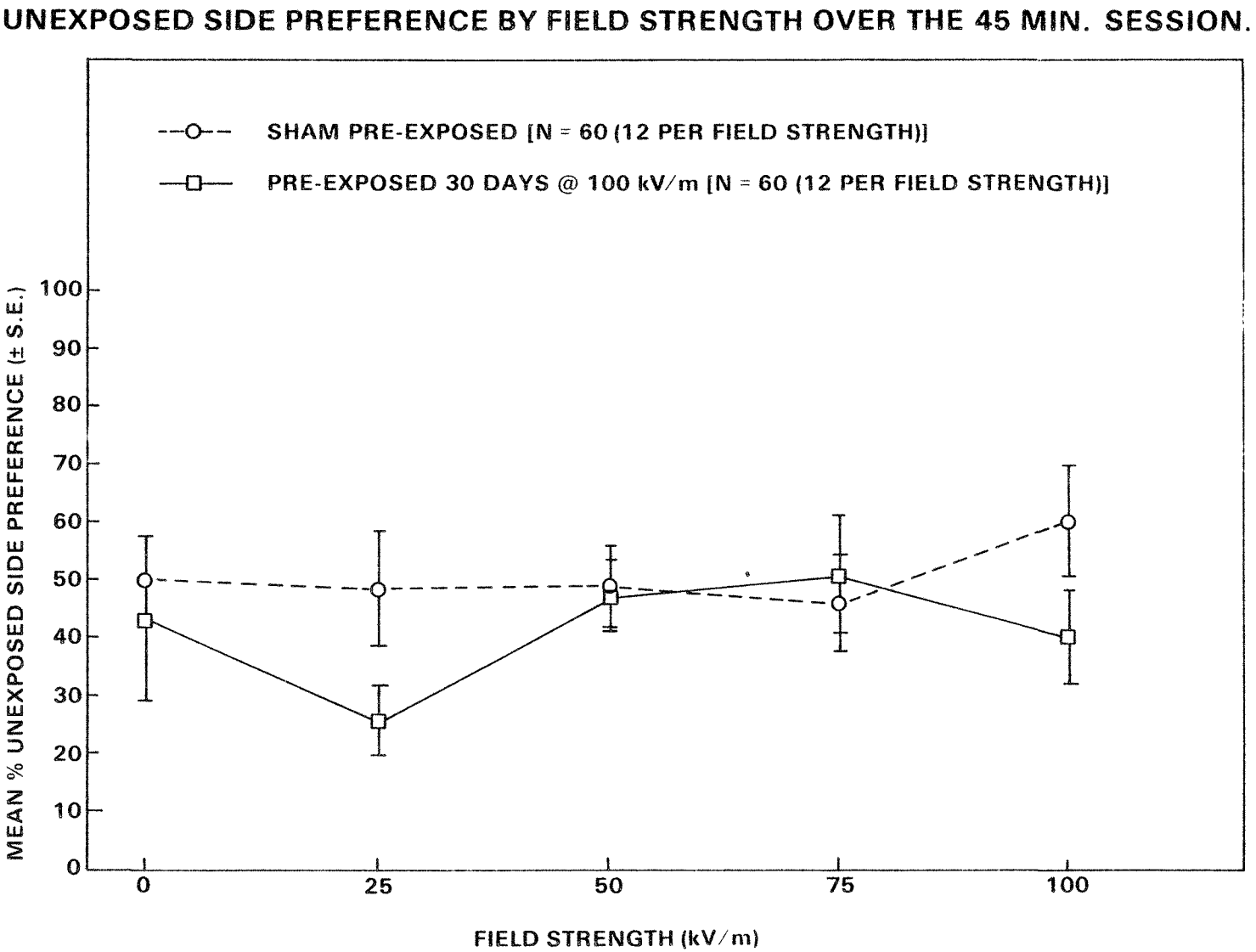



FIGURE XIII-7. Mean Number of Traverses Through the Center Region of the Shuttlebox by Rats During a 23.5-hr Test Session. Data are presented in hourly increments during the light and dark periods of the session.

shuttlebox by the 25-kV/m group was significantly increased over controls during the last 15 min of the session (Student's $t$ test = 2.18, $p < 0.05$, 22 df). The apparent preference, among pre-exposed rats, for the exposed side at 100 kV/m is not statistically significant.

During the 45-min behavorial tests there were no significant differences between groups (pre-exposed or sham-exposed) in the number of traverses made to any region at any of the field strengths tested. At 0 kV/m, the sham-exposed rats made more traverses to the center and unexposed regions of the shuttlebox than exposed rats during the 15-min to 30-min period of the session (Student's $t$ test = 2.24 and 2.49, respectively, $p < 0.05$, 22 df), but overall (0-45 min), there was no significant difference between groups.

3. Audiogenic Seizure Experiments. The latencies to running fits and clonic-tonic seizures did not differ significantly between control and exposed mice (Table XIII-9 and -10).

## D. DISCUSSION

1. Shuttlebox Side-Preference Experiments. These experiments demonstrate behavioral effects of 60-Hz electric fields and substantiate earlier reports of avoidance (Bayer et al., 1977) and increased activity (Moos, 1964) during exposure. The results do not support previous findings of lowered serum corticosterone levels (Marino et al., 1976) due to exposure to a 60-Hz electric field, although this may be due to the different exposure durations in the two experiments.

TABLE XIII-9. Audiogenic Seizure Sensitivity of Male SJL/J Mice After 14 Days Exposure to 60-Hz Electric Fields

BEFORE EXPOSURE

| Group | Number Seized/ Number Tested | Latency to Running Fit Mean ± S.E. | Latency to Clonic-Tonic Seizure Mean ± S.E. |
|---|---|---|---|
| Exposed | 13/13 | 4.66 ± 0.23 | 7.98 ± 0.25 |
| Sham-Exposed | 13/13 | 4.65 ± 0.24 | 7.77 ± 0.24 |

AFTER EXPOSURE

| Group | Number Seized/ Number Tested | Latency to Running Fit Mean ± S.E. | Latency to Clonic-Tonic Seizure Mean ± S.E. |
|---|---|---|---|
| Exposed | 11/11 | 4.02 ± 0.22 | 8.05 ± 0.30 |
| Sham-Exposed | 9/11 | 4.13 ± 0.17 | 8.78 ± 0.37 |

TABLE XIII-10. Audiogenic Seizure Sensitivity of Male SJL/J Mice After 28 Days Exposure to 60-Hz Electric Fields

BEFORE EXPOSURE

| Group | Number Seized/ Number Tested | Latency to Running Fit Mean ± S.E. | Latency to Clonic-Tonic Seizure Mean ± S.E. |
|---|---|---|---|
| Exposed | 16/16 | 4.77 ± 0.19 | 7.87 ± 0.23 |
| Sham-Exposed | 16/16 | 4.98 ± 0.21 | 7.88 ± 0.24 |

AFTER EXPOSURE

| Group | Number Seized/ Number Tested | Latency to Running Fit Mean ± S.E. | Latency to Clonic-Tonic Seizure Mean ± S.E. |
|---|---|---|---|
| Exposed | 12/16 | 5.08 ± 0.27 | 7.57 ± 0.19 |
| Sham-Exposed | 9/16 | 5.09 ± 0.25 | 7.60 ± 0.28 |

It is important to note that the results in this study were obtained after secondary field factors (e.g., corona, ozone and noise) were eliminated as possible sources of biological effects or psychological cues. Preference and avoidance experiments were conducted with exposed and shielded regions of the shuttlebox each oriented toward the vibrating end of the exposure system 50% of the time. Orientation of the shuttlebox made no difference in results, and responses cannot be ascribed to field-induced cage vibration.

The avoidance response demonstrates that rats can discriminate between exposed and shielded regions of the shuttlebox, and that the threshold for this avoidance behavior is related to the duration of exposure. In the short-duration test (45 min), statistically significant avoidance of electric fields of 90 kV/m or higher was observed, while rats exposed at 60 or 75 kV/m had a similar but not statistically significant response. In a longer test (23.5 hr), the minimum field strength for avoidance was 75 kV/m, while rats exposed at 25 or 50 kV/m had an apparent preference for exposure during the light period. While bimodal behavioral responses mediated by different stimulus intensities have been noted previously (Schneirla, 1959), these results provide the first evidence of such a bimodal response to a 60-Hz electric field.

Our experiments demonstrate an increased traverse activity in exposed animals. In 45-min tests, both exposed and control rats showed equal traverse activity during the first test session. However, in subsequent sessions, exposed rats had more traverses than control rats, the latter showing a greater decline in traverses over sessions. Over

longer exposure durations, exposed rats initially had more traverses than controls, but this higher traverse activity was not maintained.

Analysis of serum corticosterone levels does not indicate sustained arousal due to field exposure. However, since blood samples were drawn at the end of a 23.5-hr exposure session, our analysis would not reflect early, transitory effects of electric fields on corticosterone levels such as those described by Graves and Poznaniak (in press).

We conclude that rats perceive and respond to electric fields due to their direct interaction with the electric field. One possible explanation for the observed findings is perception of field-induced movement of the skin or hair of the rat. The threshold for electric-field-induced hair oscillation has been determined in swine (Phillips, et al., 1976b; Kaune et al., 1977); we plan to conduct similar research with rats.

2. Audiogenic Seizure Experiment. Exposure of mice to a 100-kV/m electric field for 14 or 30 days did not affect their sensitivity to sound-induced seizures. These results suggest that exposure to electric fields does not affect the intrinsic level of the central nervous system excitability.

## E. FUTURE PLANS

Two studies of avoidance learning in rats exposed to various electric-field strengths are currently in progress. One experiment is examining effects of acute exposure (24 hr) on learning. The other is

studying avoidance learning of rats exposed in utero and raised in the field; groups of exposed and control rats are being tested at 30, 60, and 90 days of age. All electric-field exposures are at 100 kV/m.

Our plans for the coming year include a study on the perception threshold of rats in an electric field. This study will use a conditioned suppression paradigm. These experiments will provide a basis for other studies to investigate the modality of electric-field perception. An experiment has also been planned to investigate whether exposure to a 60-Hz electric field will produce malaise/illness in rats.

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# XIV. MUTAGENESIS

Principal Investigators: F. P. Hungate and S. C. Causey

## ABSTRACT

Studies of mutagenic effects of high-strength dc fields using a wide variety of microbial test systems has failed to provide evidence of increased frequency of induction of either forward or reverse mutation events. Because of this, earlier studies with Salmonella strain TA100, which had indicated that dc fields are mutagenic, are being repeated.

Tests of mutagenicity of dc fields are continuing with Drosophila, using a repair-deficient strain.

## A. INTRODUCTION

Little is known about the mutagenic effects of electric fields. Studies from the U.S. Navy Project Sanguine suggest that small electromagnetic fields do not produce significantly increased frequencies of recessive lethals in Drosophila. However, the fields tested were smaller than those encountered under high-voltage transmission lines, and the data provide no basis for confidence that larger electric fields are not mutagenic. The present study is directed toward testing for mutagenic effects of very large fields. It is assumed that negative data will provide confidence that lesser fields to which people are exposed are nonmutagenic. If the data indicate a mutagenic potential, a test system will then be available to examine the nature of dose-effect relationships and to study the mechanisms involved. Data reported earlier (Hungate,

et al., in press) indicated significantly increased mutation frequencies following exposure of the Ames Salmonella strain TA100 and Photobacterium fisheri, MAV to dc electric fields of 800 kV/m. Inconclusive data were obtained from tests for sex-linked recessive lethal mutation in Drosophila. Major effort was devoted this year to identify alternative biological test systems useful for establishing the nature of the dose-response relationship and the possible mechanisms involved in mutation induction.

## B. MATERIALS AND METHODS

The exposure apparatus was designed to provide large uniform fields suitable for exposing organisms contained in a petri dish. The paired electrodes are 30-cm-diameter copper plates with their edges rolled to avoid corona. The electrodes are separated by a Lucite spacer, 25 cm in diameter, 3 mm thick and, typically, 2 cm high, although heights of 1.5 to 6 cm have also been used. A small hole in the Lucite spacer permits insertion of a polyethylene tube, which delivers moisture-saturated air into the chambers during exposures. This air leaks from the chambers through the small interface between the Lucite spacer and the electrodes. An Associated Research DC HYPOT, Model 5270, is used to energize the upper copper plate to obtain the desired field strengths for bacterial exposures. The lower plate is connected to ground. A battery pack applying 3.7 kV dc has been used for the Drosophila tests, and a second, similar pack is now available to provide 7.4 kV dc for the bacterial studies. The applied voltage is measured at the start and end of each

test with a static voltmeter. Simultaneous control specimens are maintained in an identical system. The controls are supplied with the same air source as exposed specimens, but with no applied voltage.

Bacteria are cultured in 125-ml flasks, with shaking, in a water bath held at 37°C. When a predetermined cell density (in the exponential growth phase) is achieved, the culture is diluted with fresh medium and a 20-ml aliquot is placed in each 10 x 1.5-cm plastic petri dish, to be exposed or used as a control culture. During the exposure period, the lids of the petri dishes are removed to prevent shielding by the lid. Following exposure, typically for 16-24 hr, the bacteria are resuspended in the medium, transferred to capped centrifuge tubes, pelleted, the medium removed, and the cells resuspended in a small volume of fresh medium. Approximately $10^8$ cells are surface-plated on test medium to identify the frequency of mutants appearing on medium permitting growth of only mutated cells. The total cell numbers are determined by serial dilution and plating on nonselective medium.

Drosophila Canton-S strain pupae (0-24 hr postpupation) were exposed for 3 days to the dc field. A control was run simultaneously and was handled comparably. It received the same washed air as supplied to the exposed pupae. Following removal from the field and hatching of the flies, the males were individually mated to Muller-5 strain virgin females to test for the presence of sex-linked recessive lethals (Abramson and Lewis, 1971).

C. RESULTS AND DISCUSSION

Biological Tests. Three E. coli strains carrying plasmids (extrachromosomal DNA) which code for resistance to specific drugs or antibiotics were studied for evidence of changes induced in the plasmids by electric fields. Plasmid RP4, in E. coli strain J53, confers resistance to ampicillin, kanamycin and tetracycline. In three tests, these cells were exposed for 21 hr and evaluated for expression of drug resistance. An average of 29% reduction of resistant cells was observed following exposure to a 500-kV/m dc field, compared to an average of 5.5% reduction in the unexposed controls. In all exposures, the air within the exposure chamber, as well as in the control chamber, was continually flushed with washed sterile air. In one similar test of E. coli strain C600, containing the plasmid R6K (which codes for resistance to ampicillin and streptomycin), there was a reduction of 17.8% in colonies resistant to the combined antibiotics, while unexposed controls showed no change. E. coli J53, containing the plasmid R388 (which codes for resistance to trimethoprim and sulfonamides), has been tested twice, with an average reduction of 25%, while controls were unchanged. While factors associated with resistance to ampicillin appear to be the most sensitive to the dc fields, we have not been able to isolate stable mutants, nor is there evidence of plasmid loss (curing).

Three tests of E. coli CSH23, carrying an F'lac pro A,B plasmid, which enables the host to utilize lactose as a carbon source, showed a slight (0.1%) loss of ability to grow on lactose in one test, and no

change in the other two tests. While it was determined that plasmid curing had occurred in one test, this system appears to be relatively unresponsive to dc fields.

It was well known that a variety of physical agents induce lysogeny in lysogenic strains of λ-phage. Two strains have been tested for response to a 500-kV/m dc field. E. coli CSH49, which contains the φ80hD type virus, showed a 35-40% greater yield of virus particles in two 21-hr exposures and no increased yield in a third, compared to controls. A 4-hr exposure of this same system gave a yield 20% over the control. Similarly, E. coli CSH45, containing λ-phage, yielded a 37% increase of virus particles following a 21-hr exposure to the same field strength. Thus, at this time, there is a slight indication that lysogeny is induced by exposure to a dc field. This effect could be due to an inactivation of the genes that maintain the lysogenic state or to a change in their product, a repressor protein.

E. coli CSH23 has been tested for mutation of the Nal-A gene. Mutation at this locus enables cells to grow on otherwise restrictive concentrations of nalidixic acid. In four tests we observed no enhancement of mutation frequency over that in controls, following growth overnight in a 500-kV/m dc field.

One additional test was completed for induction of sex-linked lethal recessive mutants in Drosophila, giving 5 mutants per 2140 tested from male pupae exposed for 3 days to 185 kV/m, as compared to 6 mutants per 1919 tested in controls. This is the first test of males that gave fewer mutants in exposed pupae than in controls. Combining these results

with the data previously reported indicates 27 mutants per 7929 (0.34%) exposed male pupae and 17 per 6934 (0.25%) in controls. Female pupae similarly exposed gave 3 mutants per 2751 exposed and 1 per 2792 in the controls. Combined with previous data, the total is 12 mutants per 11,889 exposed (0.10%) and 7 mutants per 9347 controls (0.07%). While suggestive, these data fail to show a significant difference between the control and experimental groups.

We are currently testing a repair-deficient strain of Drosophila obtained from Dr. Melvin Green, University of California, Davis to determine if its response to electric fields is greater than that observed using the Canton-S strain.

Physical Tests. In the absence of recognized models to account for direct effects of dc fields, we have examined possible indirect causes of the observed biological effects. Since ozone is a well-recognized byproduct of electric fields, particularly if corona is present, we examined the exposure system photographically, using sensitive film and darkroom conditions. With fields up to 900 kV/m there was no evidence of corona. In addition, a Bendix Ozone Analyzer (Model 8002), capable of measuring ozone in concentrations down to 1 ppb, was used to test for ozone production. This unit detected no ozone with field strengths up to 500 kV/m, and barely detectable concentrations (1 ppb) with fields through 800 kV/m. When the washed air supply was disconnected, the unit immediately indicated ozone concentrations typical of the room air, around 15-30 ppb. These data indicate that the exposure system is

unusually free of ozone due to bubbling the incoming air through water, and that ozone is therefore not a causative factor for tne biological effects observed.

The possibility of X-ray production in the field was investigated by placing three lithium fluoride dosimeter chips (Harshaw Chemical Co., TLD-700), wrapped in a single layer of paper, in a 1000-kV/m field for up to 3 days. Tests involved both flowing wet air and normal room air (60-70% RH). The initial test indicated some dose accumulation ($27 \pm 3$ mR, compared to $14 \pm 0$ on control chips), but in two replicates there was no evidence of X-ray production ($20 \pm 3$ mR in exposed vs. $19 \pm 4$ in controls; $19 \pm 0$ in exposed vs. $17 \pm 0$ in controls).

Attempts to evaluate air ions as possible factors are in a formative state. Since available equipment for measuring air ion concentrations requires much more air than is available from our chambers, we must now resort to calculated levels based upon measurement of current flow. Other techniques for direct measurement are being studied but none have yet been tested.

## D. FUTURE PLANS

The experiments with Salmonella TA100 will be repeated. If positive effects are again found, several sources of possible artifacts can be evaluated: 1) replace polystyrene petri dishes with glass dishes to eliminate the possibility that high dc fields interacted with the plastic to produce toxic byproducts; 2) replace the dc power supply (transformer)

with a better power supply to eliminate ac ripple; 3) add a second control group, one which is kept in an energized exposure system but shielded from the dc field by a metal screen (Faraday) cage. No further experiments on dc electric fields are contemplated in FY 1980. Experiments will be initiated to assess possible mutagenetic effects of ac electric fields using test systems developed in the course of the dc experiments.

The analysis of recessive sex-linked lethal induction in Drosophila will continue until it is clear that such mutational events are, or are not, induced by the electric field. In this effort, we are now using the repair-deficient strain of Drosophila obtained from Dr. M. Green, and are also testing 370-kV, as well as 185-kV, dc fields. These tests will also be done in ac fields as soon as a continuous ozone-monitoring system is in place.

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# XV. DOSIMETRY

Principal Investigator: W. T. Kaune

## ABSTRACT

Interactive effects in electric-field exposure systems can significantly alter the fields experienced by an experimental subject. We have made measurements with a hemi-ellipsoidal rat model which show: (1) interactions between grounded rats and overhead electrodes in exposure systems can enhance exposure to electric fields, with the enhancement being considerably greater in multiple-animal than in single-animal systems; (2) plastic rat cages will decrease slightly the overall exposure of grounded rats to electric fields, but will increase by as much as (or more than) 20% the electric fields at the surface of those parts of the subject's body close to the walls of the cage; (3) animals will be significantly shielded from electric fields in systems where several animals are simultaneously exposed in close proximity to one another.

The short-circuit current, $I_{SC}$ ($\mu$A), induced in a rat of weight W(g) by an unperturbed electric field, $E_O$ (kV/m), is $I_{SC} = \alpha W^n E_O$, where $\alpha = 0.0033$ and $n = 0.63$.

Measurements with a prone rat model show that 15% of the short-circuit current originates in the head, 16% in the portion of the body between transverse sections through the neck and chest, 23% between transverse sections through the chest and abdomen, and 43% in the body posterior to a section through the abdomen. When the rat rears, these values change to 41%, 30%, 18%, and 11%, respectively, and the short-circuit current is doubled.

## A. HEMI-ELLIPSOID MEASUREMENTS

1. Introduction. Electric-field exposure is conventionally specified in terms of the unperturbed field strength; that is, the field strength measured or calculated before a living organism is introduced into the field. Living tissues are conductors (Geddes and Baker, 1967) and the applied

field will be perturbed by the body of an exposed organism. Therefore, the actual fields experienced by the organism--the fields at the outer surface and inside the body--will be different from the unperturbed electric field.

Estimates show that the fields induced inside the body of a living organism exposed to a 60-Hz electric field are very small (Barnes et al., 1967; Phillips et al., 1976a; Sheppard and Eisenbud, 1977). Therefore, to a good approximation, the perturbation in the applied field outside the body depends only on the nature of the applied field, the body shape, and the location and orientation of the body relative to ground. It therefore follows that the field outside and at the outer surface of the body of a living organism can be explored using a conducting model of the same shape.

The currents induced inside the body of an exposed organism arise as the result of a time-varying, induced electric charge on the surface of the body. Since the induced field inside the body is very small, the surface-charge density will be almost exactly the same as that induced on the surface of a conducting model (Reitz and Milford, 1960).

These considerations show that the electric field at the outer surface of the body or, alternatively, the induced charge on the surface of the body, is a key parameter in evaluating the interaction of the body with an applied 60-Hz electric field.

A previous progress report (Phillips et al., 1978) described a conducting hemi-ellipsoid model which was instrumented for the measurement of short-circuit currents and electric fields at 11 points on the surface

of the model. The hemi-ellipsoidal shape was selected because: (1) the fields at the surface can be calculated for uniform field exposure, and can thus be used as a check on the measurement technique; and (2) this shape is a rough approximation to the shape of a prone (resting) rat.

Using this model, we have studied the dependence of the surface electric fields on the following parameters of typical parallel-plate exposure system: electrode spacing; presence or absence of animal cages; and presence or absence of other animals.

## 2. Experimental Apparatus and Methods

*Hemi-Ellipsoid Model*. The hemi-ellipsoid model was made from balsa wood and was painted with a silver-base conducting paint. The principal axes of the model measure 8.13, 6.30, and 3.18 cm, respectively. Eleven 1.3-cm x 1.3-cm copper patches were set flush in the surface of the ellipsoid along the $x = 0$ and $z = 0$ planes, as shown in Figure XV-1. Each patch was electrically isolated from the remainder of the conductive surface of the model and was equipped with a wire for direct readout of its induced current. These wires were routed through holes drilled from the base of the ellipsoid to the patch location.

A total of 12 wires were attached to the ellipsoid, 11 to the patches, and one to the conducting surface of the body. A switch was constructed which either connected one selected input to the output while grounding all the other inputs, or connected all inputs to the output. The output lead from the switch was connected to a low-impedance, current-to-voltage converter, which has been described elsewhere (Kaune, *in press*).

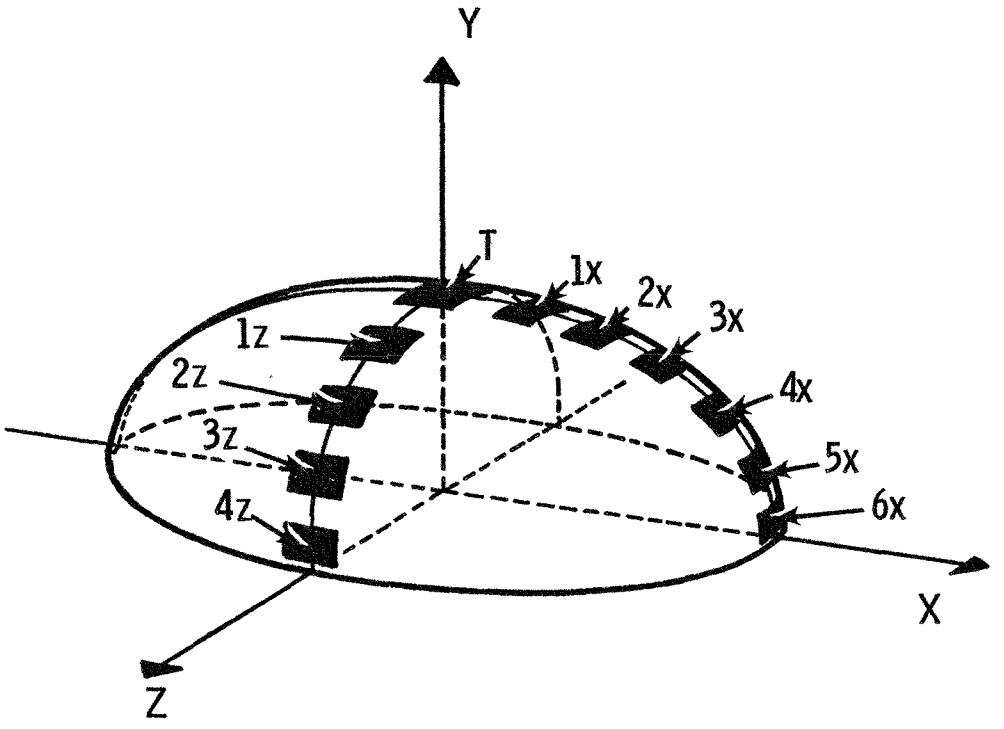



FIGURE XV-1. Hemi-Ellipsoid Model Instrumented for Measurement of Electric Fields at 11 Places on Its Conducting Surface.

*Exposure System*. The Special Studies Exposure System was used to generate vertical, uniform, 60-Hz electric fields for use during the tests described here. This system consisted of two planar 1.0-m x 1.8-m parallel metal electrodes, separated by a gap of 0.55 m (Phillips et al., 1976a; Kaune, in press). The upper electrode was energized and the lower one was grounded.

Some of the tests required that the electrode separation be varied. Rather than move the relatively bulky electrodes described above, a separate 0.76-m x 1.68-m electrode was fabricated from thin aluminum sheet attached to a frame made of 2.5-cm-diameter steel tubing. This electrode was inserted between the two permanent electrodes of the Special Studies System, and positioned at a given height above the lower electrode, using cylindrical plexiglass spacers at the four corners. A wire was connected to the upper electrode to complete the high-voltage circuit. Considerable care was taken to always place the spacers and the movable electrode in the same horizontal position relative to the permanent electrodes.

*Rat Cage Modules*. Lexan modules for rat housing during electric-field exposure (Phillips and Kaune, 1977) were used in these measurements. A cage module has overall dimensions of 52 cm wide x 51 cm long x 10.7 cm high, and is subdivided into eight individual rat cages (Figure XV-2). Drinking water is supplied from four nozzles whose tips protrude slightly above the floor into notches cut into the central wall of the module. The inside dimensions of the rat cage are 12.4 cm wide x 25.1 cm long x 10.0 cm

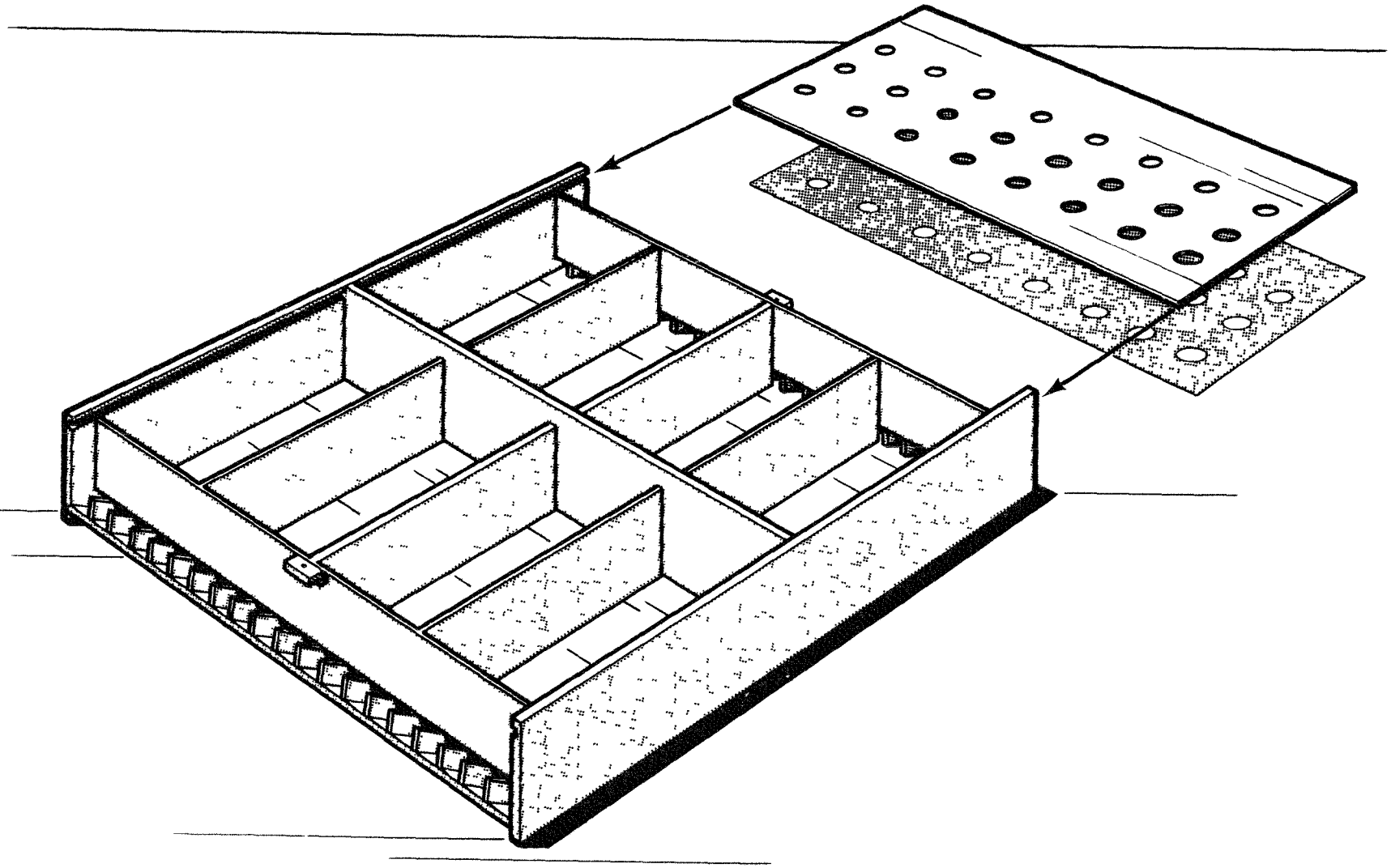


FIGURE XV-2. Rat Cage Module is Made of Lexan and Houses Eight Rats for Exposure to 60-Hz Electric Fields.

high. Each cage has two 0.32-cm-thick side walls and two 0.64-cm-thick side walls, as shown in Figure XV-2; the roof thickness is 0.47 cm. Food pellets are supplied from an inclined holder outside the outer wall (Figure XV-2).

*Unperturbed Electric-Field Measurements*. The unperturbed electric field between the exposure electrodes was measured on the surface of the lower electrode using a flat disc probe, which has been described elsewhere (Phillips et al., 1976b; Kaune, in press). This probe provided a measurement of the average surface field over a 5-cm-diameter circle.

3. Results

*Comparison Between Measurement and Theory*. In a previous progress report (Phillips et al., 1978), patch and short-circuit currents of the hemi-ellipsoid model were measured during exposure to a uniform electric field and were found to agree well with theoretical predictions.

*Effect of Electrode Separation: Single Animal System*. An important consideration in the design of electric-field exposure systems is the spacing between electrodes. For parallel-plate systems meant to generate unperturbed, uniform electric fields, larger electrode spacings require (1) that larger voltages be used for a given electric field strength, and (2) that larger electrodes be used to minimize fringing

effects on field uniformity. Smaller electrode spacings are, therefore, desirable, but are limited by possible interactions between the exposed animal and the upper electrode. Such interactions would alter the charge distribution on the upper electrode and would enhance the electric field at the surface of the body of the exposed subject.

To investigate the effect of electrode spacing, the hemi-ellipsoid was placed at the center of the lower electrode (for these tests, the surface of the lower electrode was aluminum plate). The long axis (i.e., the x-axis) of the ellipsoid was lined up with the long axis of the exposure system. Measurements of the patch currents and the model short-circuit currents were made for electrode spacings of 8.7, 4.9, 3.6, 2.4, 2.0, 1.6, and 1.2, where the spacings are expressed as numbers of hemi-ellipsoid heights (6.30 cm). As the electrode spacing was varied, the high voltage was adjusted to maintain a constant, unperturbed field strength, as determined using the flat probe located on the x-axis 27 cm from the hemi-ellipsoid model (the field perturbation from the hemi-ellipsoid perturbed the field probe reading by only 1.2% [Phillips et al., 1978]).

To detect any changes as the electrode spacing was decreased, all measured currents were divided by values for an electrode spacing of 8.7. The results are given in Table XV-1; in this table, the patch locations are identified using the same notations as in Figure XV-1. The estimated uncertainty in any ratio is 0.01, resulting from round-off errors in the meters used to monitor the field probe and patch currents.

TABLE XV-1. Surface Electric Fields and Short-Circuit Currents for a Grounded Hemi-Ellipsoidal Model Exposed to a Vertical, 60-Hz, Electric Field. Data are presented for various electrode separations; at each separation, the voltage was adjusted to give a constant unperturbed electric field. Measured values are expressed as fractions of the values measured for an electrode separation of 8.7 hemi-ellipsoid heights.

| Location of Measurement | Electrode Separation in Units of Hemi-Ellipsoid Height | | | | | |
|---|---|---|---|---|---|---|
| | 4.9 | 3.6 | 2.4 | 2.0 | 1.6 | 1.2 |
| 6X | 1.01 | 1.01 | 1.03 | 1.04 | 1.08 | 1.14 |
| 5X | 1.01 | 1.01 | 1.03 | 1.04 | 1.08 | 1.17 |
| 4X | 1.01 | 1.01 | 1.03 | 1.05 | 1.10 | 1.24 |
| 3X | 1.00 | 1.00 | 1.02 | 1.05 | 1.12 | 1.37 |
| 2X | 1.00 | 1.00 | 1.03 | 1.06 | 1.15 | 1.58 |
| 1X | 1.00 | 1.00 | 1.03 | 1.06 | 1.18 | 1.80 |
| Top | 1.00 | 1.00 | 1.03 | 1.07 | 1.19 | 1.92 |
| 1Z | 1.00 | 1.00 | 1.03 | 1.06 | 1.17 | 1.66 |
| 2Z | 1.00 | 1.00 | 1.02 | 1.05 | 1.12 | 1.38 |
| 3Z | 1.00 | 1.00 | 1.02 | 1.04 | 1.10 | 1.26 |
| 4Z | 1.00 | 1.00 | 1.02 | 1.03 | 1.08 | 1.20 |
| Short-Circuit Current | 1.00 | 1.00 | 1.02 | 1.05 | 1.13 | 1.39 |

These data show field enhancements for the smaller electrode spacings. It is remarkable, however, how small these enhancements are; for example, at the very top of the body, the field is only increased by 7% when the electrode spacing is reduced from 8.7 to just 2.0 hemi-ellipsoid heights.

*Effect of Electrode Separation: Multiple-Animal Systems.* The Special Studies Exposure System was fitted with two rat cage modules and the hemi-ellipsoid model was placed in the cage shown in Figure XV-3. Measurements of the patch and short-circuit currents of the model were taken for various electrode spacings, and with the cages surrounding the model either empty or loaded with rats.

High-voltage settings were determined for the various electrode spacings by replacing the model with the flat field probe and requiring that the unperturbed electric field be constant. This procedure was performed before and after a series of measurements with the hemi-ellipsoid model, and the resulting high-voltage settings were found to vary by not more than 1%.

Preliminary tests showed that the variability resulting from movement by live animals was sufficient to confuse effects associated with electrode spacing. Dead rats were therefore used to test the effects of electrode spacing in multiple animal systems. The bodies of nine animals were placed in an approximately normal position, lined up with the long axes of the nine cages surrounding the hemi-ellipsoid; needle electrodes were inserted in the abdomens of the animals and connected to ground.

Measurements were taken for electrode separations of 8.7, 4.9, 3.6, 3.0, 2.4, and 1.8 hemi-ellipsoid heights. The hemi-ellipsoidal model was oriented both with the patches that are labeled 1x-6x in Figure XV-1 toward the center of the cage, and away from the center of the cage (in this latter orientation, the patches will be referred to as 1x'-6x'). The measurements for patches 1z-4z, the top patch, and the short-circuit current of the model were averaged for the two orientations of the model, since by symmetry considerations, they should be (and were, within experimental error) unchanged by a 180° rotation. Measurements with the cages surrounding the model either empty or loaded with nine rats are presented in Table XV-2; as in Table XV-1, the data are presented in the form of ratios to the values measured with an electrode spacing of 8.7. Uncertainties in the ratios given in Table XV-2 are 0.02, resulting from uncertainties in high-voltage normalization and from round-off errors in the digital meters used to measure the field probe and model currents.

Comparison of the "empty-cage" data in Table XV-2 to the data in Table XV-1 (with the cages removed) suggests that interaction with overhead electrodes is slightly enhanced by cages; however, differences are slight, and may result from experimental errors. Of more significance is the empty/full cage comparison (Table XV-2), which shows that interaction with overhead electrodes is significantly enhanced by the proximity of other animals. For example, to limit enhancement effects to less than 5% requires that the electrode spacing be at least 2.4 animal heights for single subjects and at least 3.6 animal heights for multiple subjects.

TABLE XV-2. Surface Electric Fields and Short-Circuit Currents in a Hemi-Ellipsoidal Model Placed in a Rat Cage Module and Exposed to a Vertical 60-Hz Electric Field. Data are presented for various electrode separations and with the other cages in the module empty or full of rats. Electric field and current data are expressed as ratios relative to the values with an electrode separation of 8.9 hemi-ellipsoid heights. The model was oriented so that patch 6x was toward the center of the cage, and 6x' was away from the center of the cage.

| Location of Measurement | Electrode Separation in Units of Hemi-Ellipsoid Height | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 4.8 | | 3.6 | | 3.0 | | 2.4 | | 1.8 | |
| | Empty | Full | Empty | Full | Empty | Full | Empty | Full | Empty | Full |
| 6x | 1.00 | 1.02 | 1.00 | 1.05 | 1.01 | 1.07 | 1.02 | 1.13 | 1.07 | 1.24 |
| 5x | 1.00 | 1.02 | 1.01 | 1.05 | 1.01 | 1.08 | 1.03 | 1.13 | 1.08 | 1.24 |
| 4x | 1.00 | 1.02 | 1.01 | 1.05 | 1.02 | 1.07 | 1.03 | 1.13 | 1.09 | 1.24 |
| 3x | 1.00 | 1.03 | 1.01 | 1.05 | 1.01 | 1.08 | 1.03 | 1.13 | 1.10 | 1.25 |
| 2x | 1.01 | 1.02 | 1.01 | 1.05 | 1.02 | 1.08 | 1.04 | 1.13 | 1.11 | 1.25 |
| 1x | 1.01 | 1.02 | 1.01 | 1.05 | 1.02 | 1.08 | 1.04 | 1.13 | 1.12 | 1.25 |
| Top | 1.00 | 1.02 | 1.01 | 1.04 | 1.02 | 1.07 | 1.04 | 1.12 | 1.13 | 1.25 |
| 1x' | 1.01 | 1.02 | 1.02 | 1.05 | 1.02 | 1.07 | 1.05 | 1.11 | 1.12 | 1.23 |
| 2x' | 1.01 | 1.02 | 1.02 | 1.05 | 1.02 | 1.07 | 1.05 | 1.11 | 1.11 | 1.22 |
| 3x' | 1.01 | 1.02 | 1.02 | 1.04 | 1.02 | 1.06 | 1.05 | 1.09 | 1.10 | 1.19 |
| 4x' | 1.01 | 1.02 | 1.02 | 1.05 | 1.02 | 1.06 | 1.05 | 1.09 | 1.08 | 1.17 |
| 5x' | 1.01 | 1.01 | 1.02 | 1.04 | 1.02 | 1.05 | 1.04 | 1.09 | 1.07 | 1.15 |
| 5x' | 1.01 | 1.02 | 1.02 | 1.04 | 1.02 | 1.05 | 1.04 | 1.08 | 1.07 | 1.15 |
| 1z | 1.00 | 1.03 | 1.02 | 1.05 | 1.02 | 1.08 | 1.04 | 1.13 | 1.12 | 1.25 |
| 2z | 1.00 | 1.02 | 1.01 | 1.06 | 1.02 | 1.08 | 1.04 | 1.12 | 1.10 | 1.24 |
| 3z | 1.01 | 1.03 | 1.02 | 1.05 | 1.02 | 1.07 | 1.04 | 1.12 | 1.09 | 1.24 |
| 4z | 1.00 | 1.03 | 1.02 | 1.05 | 1.02 | 1.07 | 1.04 | 1.12 | 1.09 | 1.23 |
| Short-Circuit Current | 1.01 | 1.03 | 1.02 | 1.05 | 1.02 | 1.07 | 1.04 | 1.12 | 1.10 | 1.23 |

*Effects of Plastic Animal Cages.* The Special Studies Exposure System was arranged as shown in Figure XV-3, except that the plastic cage modules were removed for some tests. Patch and short-circuit currents of the hemi-ellipsoid model were measured both with the animal cages (including roofs) in place, and removed; ratios between these two sets of data were calculated. These data are given in Figures XV-4, XV-5 and XV-6 for three positions of the model within a rat cage.

Figure XV-4 shows the effects of the cage module on the surface electric fields and on the short-circuit current of the hemi-ellipsoid model placed in the center of a rat cage; reductions of 1-6% in all values were observed. With the roof of the cage removed, additional reductions of 1-2% were observed.

Figure XV-5 presents data with the model against the edge of the cage. A significant enhancement in the surface field was observed near the cage wall. Figure XV-6 presents data with the model in the corner of the cage; again, enhancements were observed in surface field strength near the walls.

*Effect of Rats in Other Cages.* The Special Studies Exposure System was arranged as shown in Figure XV-3. Measurements of the patch and short-circuit currents of the model were made with the nine cages closest to the model filled with live rats (weight range, 400-500 g), and empty. Multiple observations were made, covering: (1) 15 min of the exploratory period, immediately after placing the animals in the cages;

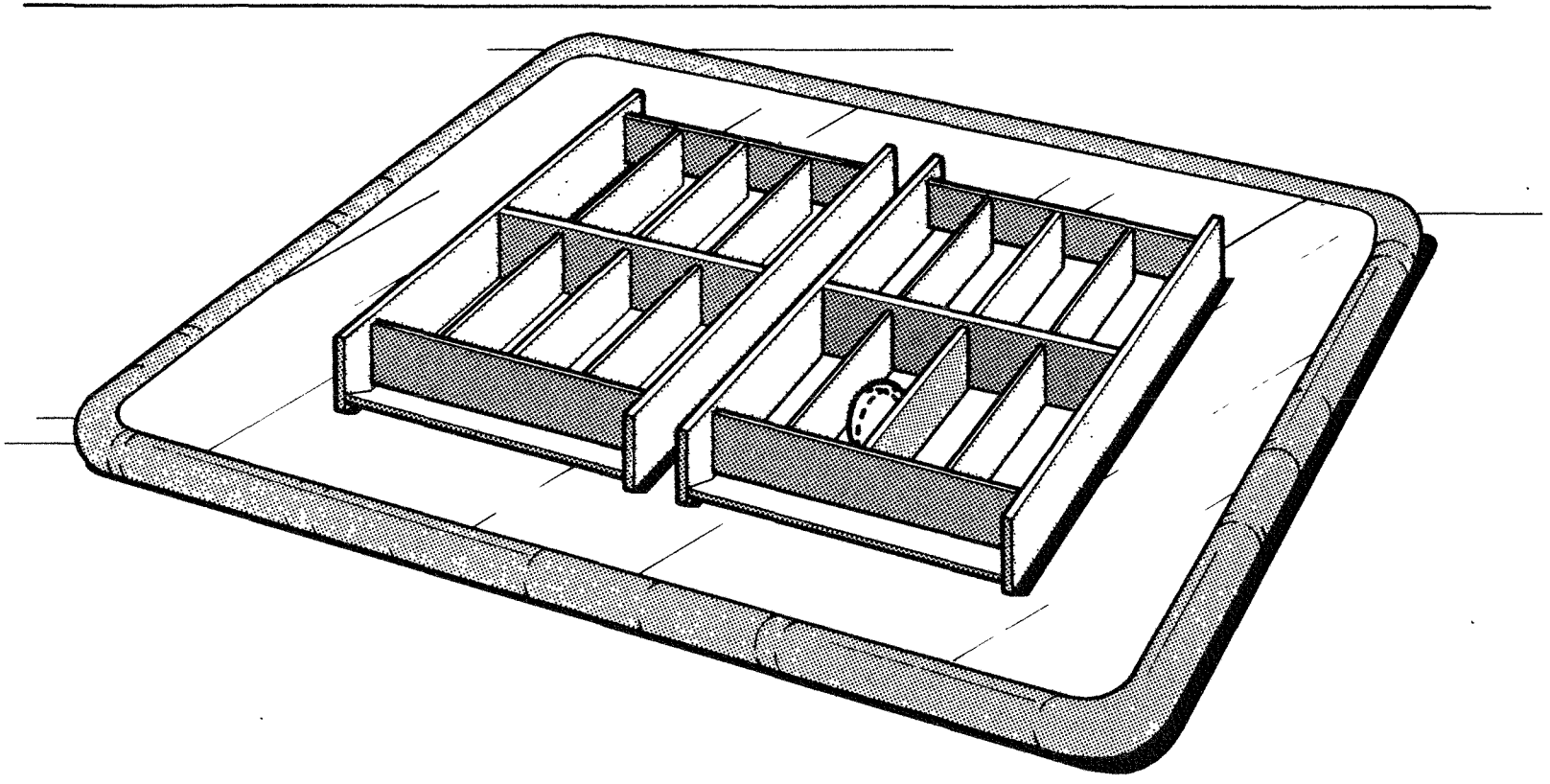


FIGURE XV-3. Two Rat Cage Modules Mounted on Lower Electrode of Special Studies Exposure System. Hemi-ellipsoid rat model is shown in cage used for most of measurements reported in text.

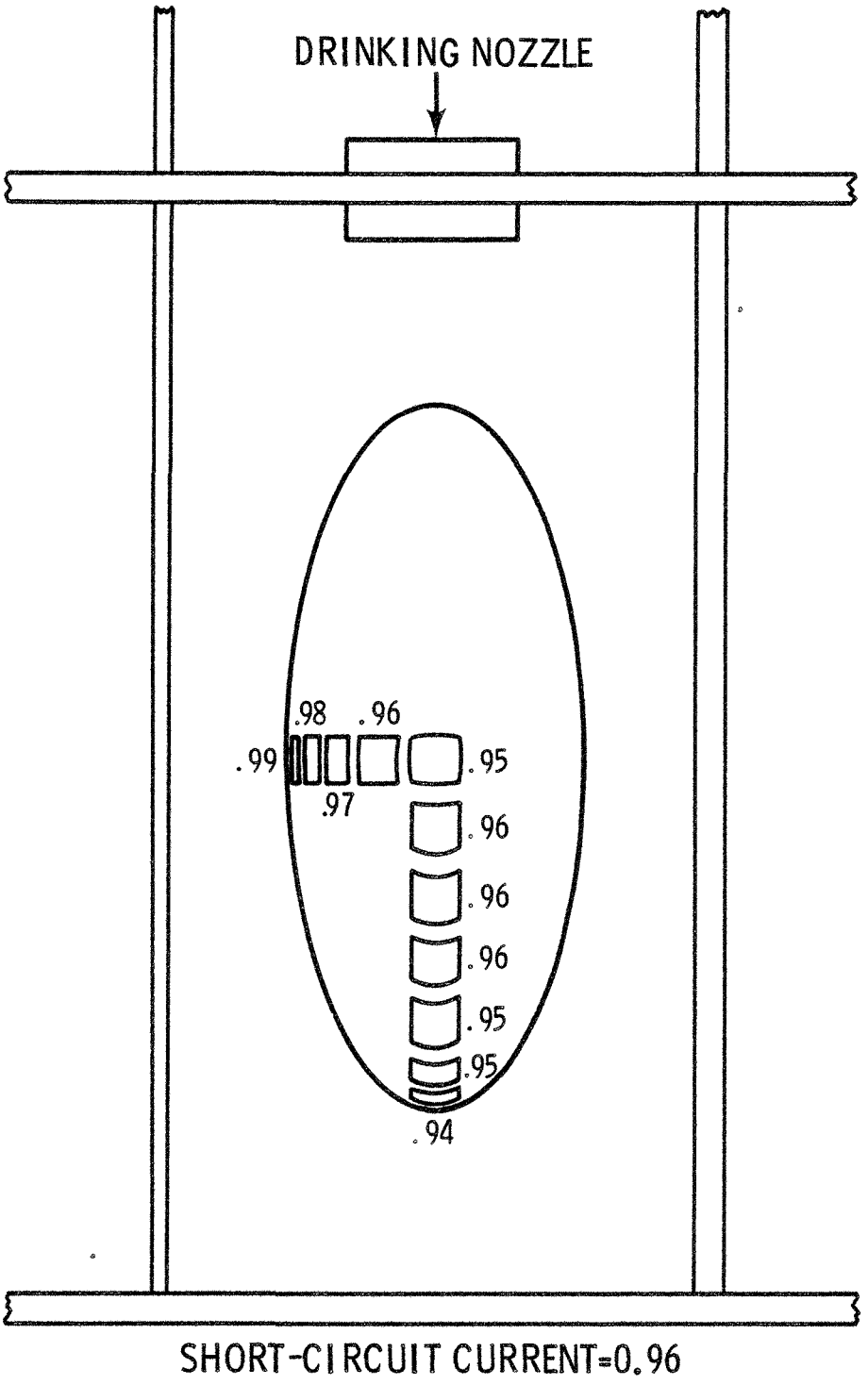



FIGURE XV-4. Top View of Hemi-Ellipsoid Model in Center of Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Cage in Place to Those Measured with Cage Removed.

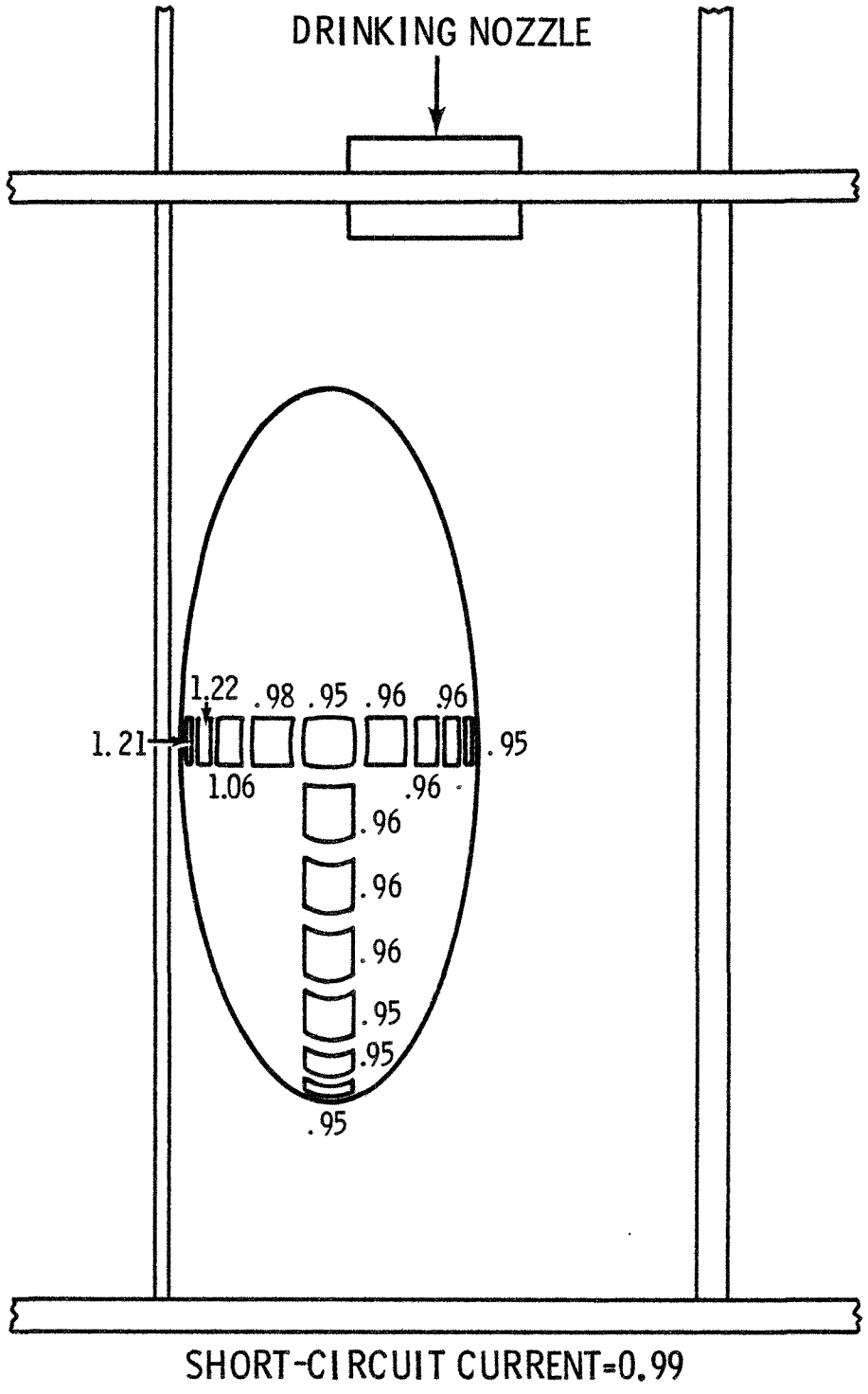



FIGURE XV-5. Top View of Hemi-Ellipsoid Model Nest to Edge of Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Cage in Place to Those Measured with Cage Removed.

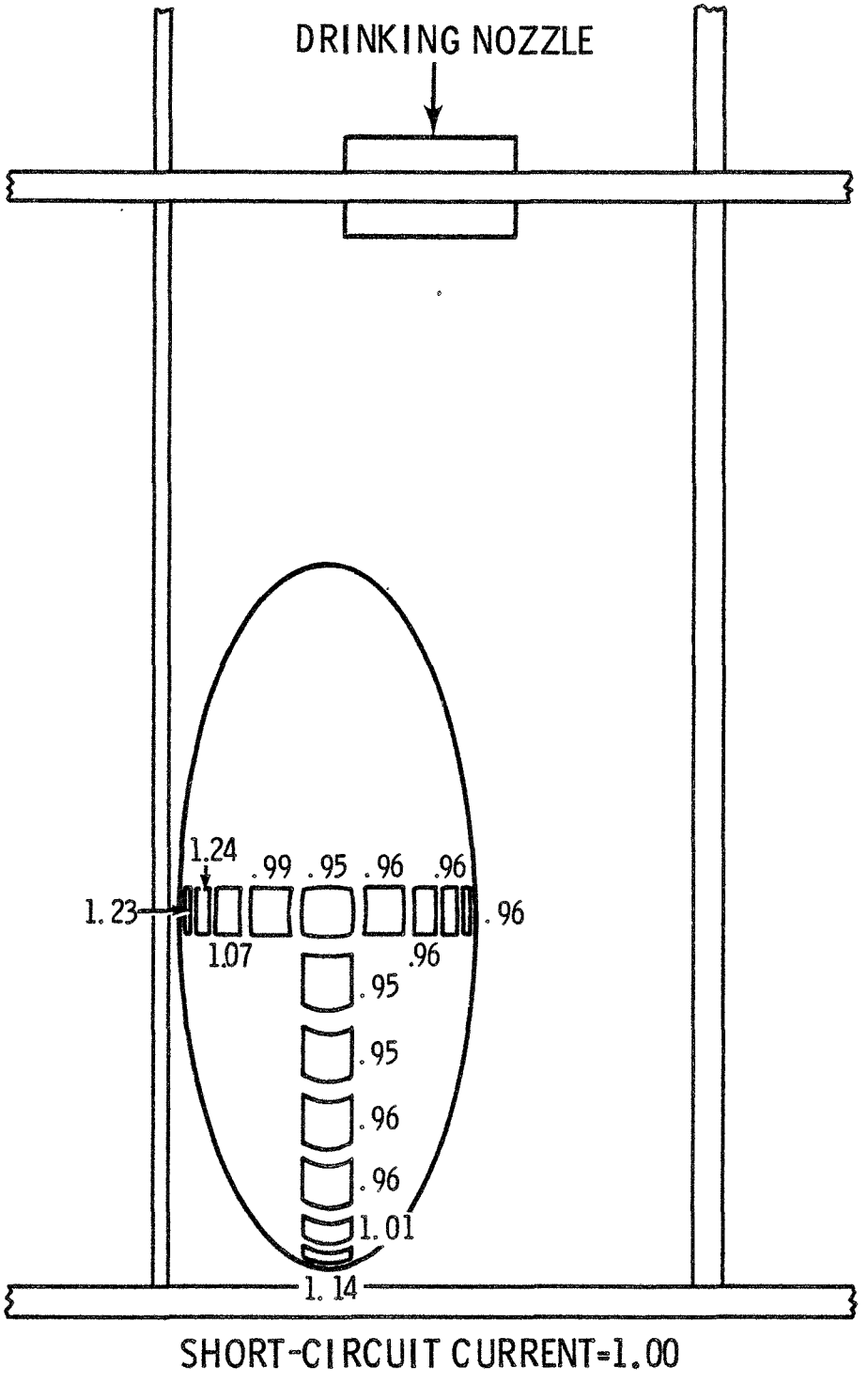



FIGURE XV-6. Top View of Hemi-Ellipsoid Model in Corner of Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Cage in Place to Those Measured with Cage Removed.

and (2) 1 hr during which the rats were asleep. Ratios between data taken during the active period and empty-cage data are given in Figure XV-7. These ratios show clearly that interactions in multiple-animal systems have the effect of significantly reducing surface electric fields and short-circuit currents. The measured 31% reduction in short-circuit current is consistent with earlier reported measurements, which showed a 35% reduction (Phillips and Kaune, 1977).

Since multiple measurements were made, estimates of variability were obtained. During the active period, variabilities in individual patch currents were relatively large (standard deviations up to 35% of means) for those patches close to the floor (and, thus, most strongly shielded by the bodies of neighboring rats); variabilities were small (standard deviations less than 10% of means) for those patches close to the top of the body, and for the short-circuit current.

Comparison of active and sleeping-rat data showed systematic differences: active rats more strongly shielded the model than did sleeping rats. The short-circuit current of the model was about 10% lower when the neighboring rats were active than when they were sleeping.

4. Conclusions. Electric fields to which humans, animals, or other living organisms are exposed are typically specified in terms of the field strengths present before introduction of the subjects. In parallel-plate exposure systems, the exposure field strength is often calculated by dividing the interelectrode voltage by the separation

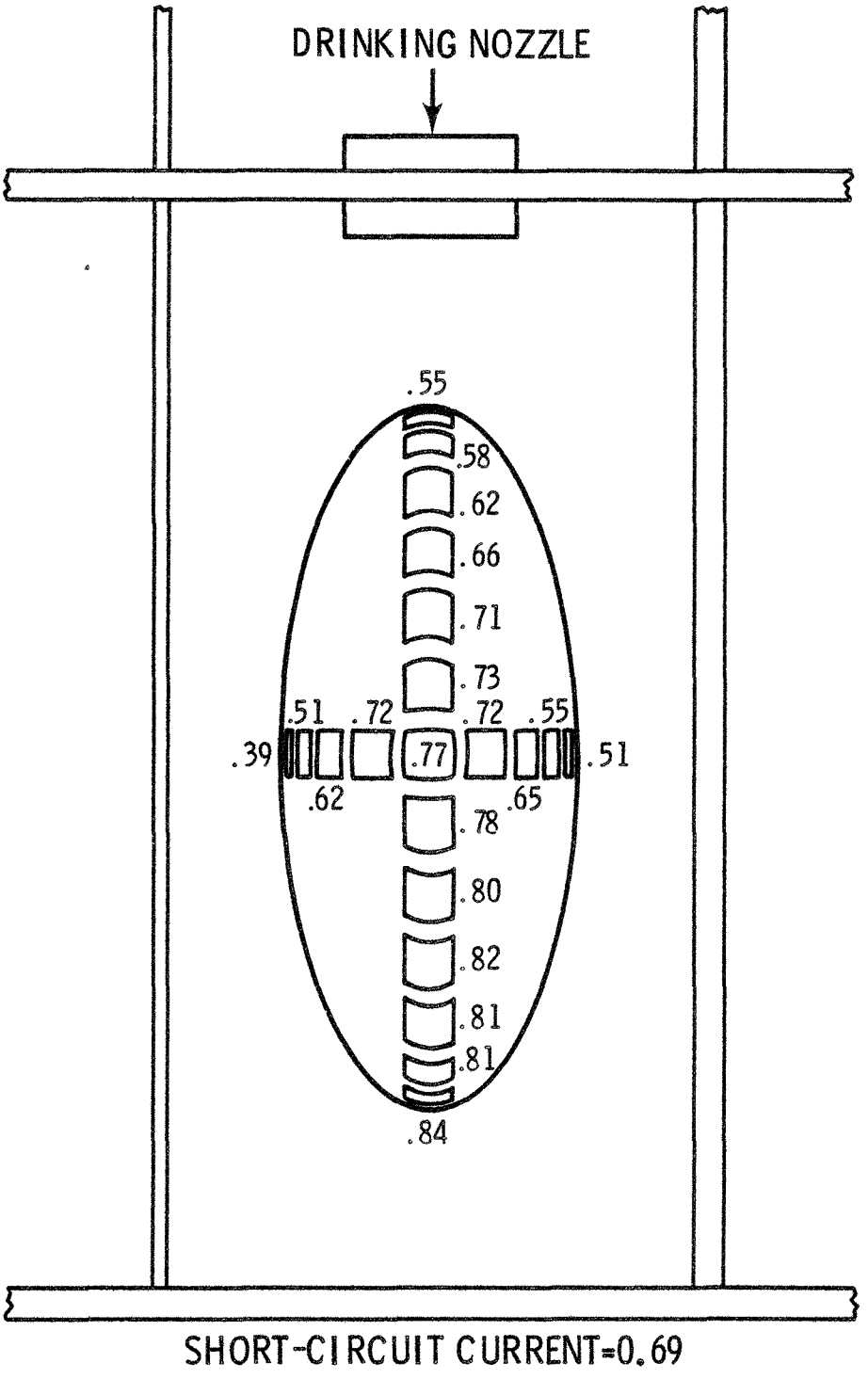



FIGURE XV-7. Top View of Hemi-Ellipsoidal Model in Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Nine Surrounding Cages Filled with Live, Active Rats to Those Measured with Surrounding Cages Empty.

between electrodes, thus neglecting any effects on field strength due to the presence of the animal cages. Measurements have been presented here which prove that interactions among exposed subjects, electrodes, and animal cages can significantly alter the fields the subjects actually experience. These are, specifically: (1) interactions between grounded rats and overhead electrodes in exposure systems can enhance exposure to electric fields, with the enhancement being considerably greater in multiple-animal than in single-animal systems; (2) plastic rat cages will decrease slightly the overall exposure of grounded rats to electric fields, but will increase by as much as (or more than) 20% of the electric fields at the surface of those parts of the subject's body close to the walls of the cage; (3) animals will be significantly shielded from electric fields in systems where several animals are simultaneously exposed in close proximity to one another.

## B. RAT SHORT-CIRCUIT CURRENTS

1. Introduction. The current which passes between a grounded animal exposed to an electric field and ground is called the short-circuit current. To complement published short-circuit currents for humans (EPRI, 1975; Deno, 1977) and swine (Phillips et al., 1976a; Kaune et al., 1978), measurements with rats were undertaken.

2. Methods. Seven Sprague-Dawley rats (weight range, 143-500 g) were killed and individually exposed to a 60-Hz, 44-kV/m electric field produced by the Special Studies Exposure System. Short-circuit current measurements were made as follows: (1) Each rat was placed in an approximately normal resting posture, tail outstretched, on the surface of the lower exposure electrode. (2) The body was insulated from the electrode with a sheet of Mylar. (3) A needle electrode was inserted in the abdomen and connected to a current-to-voltage converter [sensitivity, 1 μA = 0.1 V; input impedance, < 100 Ω at 60 Hz (Kaune, in press)].

3. Results. The data are shown in Figure XV-8. The estimated reliability of these measurements is ±6%, based on measurements of the effect of minor changes in body shape (±3%) and measurements of the variability in short-circuit current among rats of the same weight (±5%). The data are described by the formula,

$$I_{sc} = \alpha W^{n} E_{o}, \qquad (1)$$

where $I_{sc}$ is short-circuit current in μA, W is weight in g, $E_o$ is the unperturbed field strength in kV/m, $\alpha = 0.0033 \pm 0.0013$, and $n = 0.63 \pm 0.06$.

## C. INDUCED CURRENT DISTRIBUTION IN RATS

1. Introduction. The measurements reported here were made to determine the distribution of short-circuit current within the grounded bodies of prone and rearing rats exposed to a 60-Hz electric field.

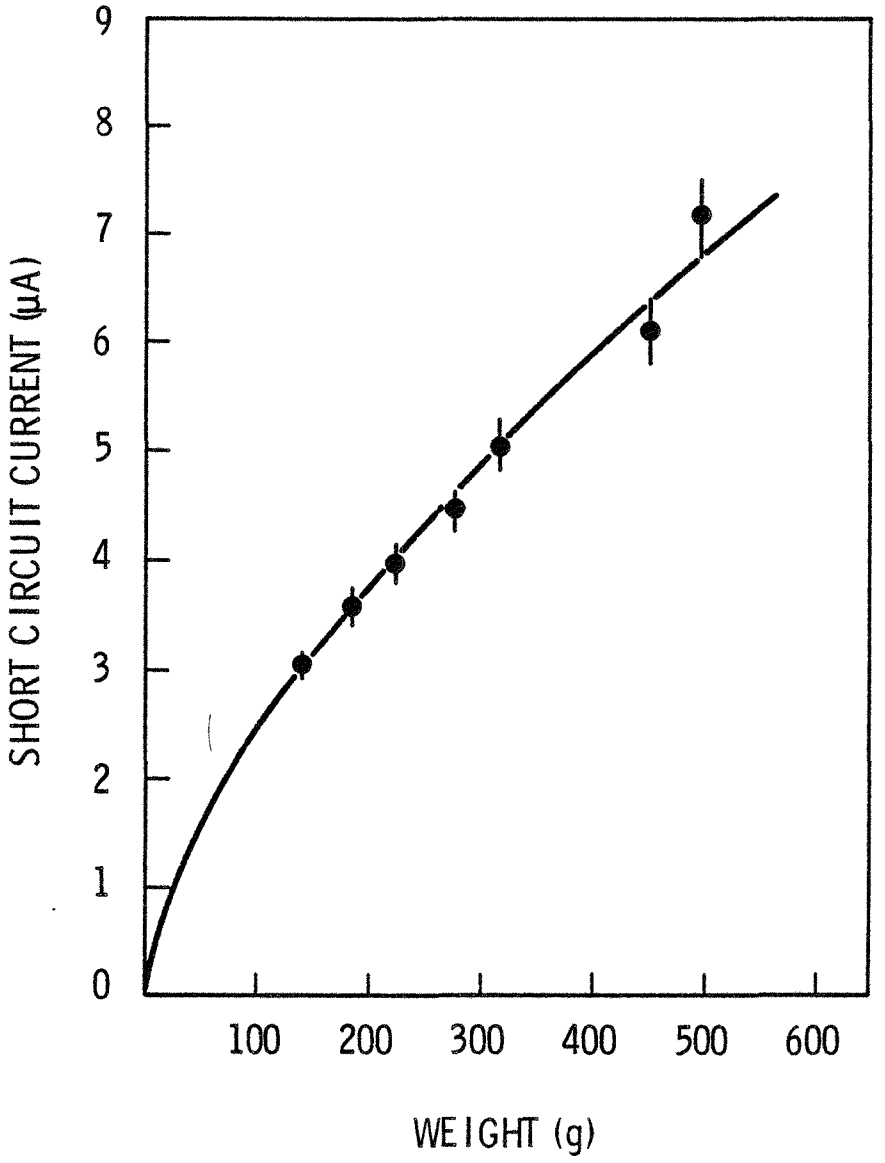



FIGURE XV-8. Short-Circuit Current Induced in Resting Rats of Various Weights Exposed to Vertical, Uniform, 60-Hz, 44-kV/m Electric Field. Solid curve is theoretical fit to data described in text.

2. Methods. A dead, 500-g, Sprague-Dawley rat was frozen and sectioned into five pieces through the neck, chest, abdomen, and base of the tail (Figure XV-9). Wires were soldered to thumbtacks, which were then coated with ECG electrode paste and inserted in the interior faces of the five body sections. The frozen body pieces were sewn back together, with Mylar sheets inserted between the various segments for electrical insulation.

For induced-current measurements, each of the leads from the five body sections was, in turn, connected by coaxial cable to the current-to-voltage converter described earlier, while the other four leads were directly grounded.

3. Results. Measurements were made with the partially frozen rat in the normal resting posture, and also propped up at angles of 70° and 90° to the horizontal, to simulate a rearing rat. The data, expressed as fractions of short-circuit current, are presented in Figure XV-9. For the rearing posture, the 70° and 90° measurements (which differed by no more than 15%) were averaged. The short-circuit current in the normal resting posture (obtained by adding the induced currents for the five body sections) was compared to the prediction of Eq. (1), with good agreement. We thereby proved, experimentally, that a partially frozen rat is a conducting model (this was also verified by direct 60-Hz impedance measurements, which showed sufficient conduction between two needle electrodes placed in the neck and rump of a partially frozen rat).

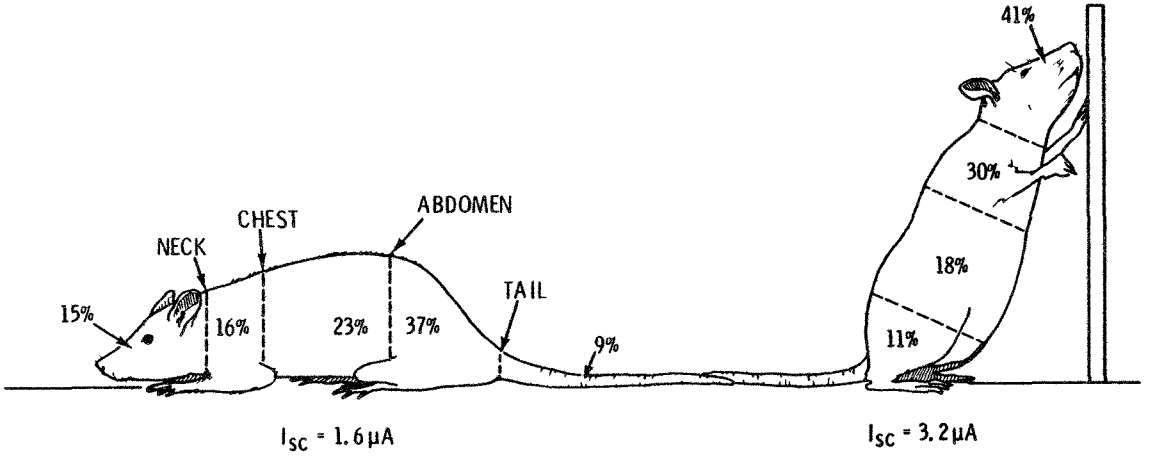



FIGURE XV-9. Grounded Rat Model Exposed to Vertical, 60-Hz, 10-kV/m Electric Field. Conducting body of model is divided into five segments by planes through neck, chest, abdomen, and tail. Fraction of total short-circuit current originating within each of five sections are shown as percentages.

4. Conclusions. These data can be used to compute total currents crossing sections through the necks, chests, abdomens and tails of prone and rearing rats. By using similar data for other species, it should be possible to compare the current densities and electric fields induced within the bodies, and derive approximate scaling relationships between species.

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# XVI. SUMMARY OF BIOLOGICAL INVESTIGATIONS

The purpose of this summary is to provide a list of biological experiments that have been completed on this project, and a quick reference to the location of detailed descriptions of the experiments in the 1978 and 1979 Annual Reports.

Table XVI-1 gives a summary of experiments completed within each major area of research, identifies each as an initial or replicate experiment, and cites the Annual Report in which the experiment is described in detail. Only experiments that have been completed are included in this list. Experiments that are in progress, and those completed since this report was prepared, will be discussed in monthly reports throughout FY 1980.

Table XVI-2 briefly summarizes those experiments in which confirmed effects have been observed in mice or rats exposed to 60-Hz electric fields. Experiments where we have failed to confirm effects in replicate experiments (i.e., those cases where effects were observed in one experiment but not replicated in a second experiment) are not included in this summary. To identify the latter studies, see Sections IV (Hematology and Serum Chemistry), IX (Endocrinology) and XI (Neurophysiology), in which effects were observed in a first experiment but not in the second (nor, in some cases, in the third) replicate experiments. In addition, some studies where effects were found in the first experiment are currently being replicated to confirm the earlier findings (e.g., Section IX, Endocrinology).

TABLE XVI-1. Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Behavior - D. L. Hjeresen and R. L. Sheldon

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Avoidance and traverse activity (rats, exposed 45 min) | I,R | 1978-1979 |
| Avoidance and traverse activity (rats, exposed 24 hr) | I,R | 1978-1979 |
| Serum corticosterone levels (rats, exposed 24 hr) | I | 1979 |
| Avoidance and traverse activity (rats, exposed 30 days before 45-min test) | I,R | 1979 |
| Audiogenic seizure sensitivity (rats, exposed 14 days) | I | 1979 |
| Audiogenic seizure sensitivity (rats, exposed 28 days) | I | 1979 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Bone Growth and Structure - B. J. McClanahan

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Bone growth and structure (rats, exposed 30 days) | I,R | 1978-1979 |
| Body weight (rats, exposed 30 days) | I,R | 1978-1979 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Cardiovascular Function - D. I. Hilton and R. D. Phillips

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Heart rate, ECG analysis (male rats, exposed 8 hr, 40 hr, 30 days) | I | 1978 |
| Heart rate, ECG (female rats, exposed 30 days) | I | 1979 |
| Heart rate, ECG (female rats, exposed 120 days) | I | 1979 |
| Heart rate, blood pressure, vascular reactivity (male rats, exposed 35 days) | I | 1979 |
| Heart rate, blood pressure, vascular reactivity (female rats, exposed 120 days) | I | 1979 |
| Response to cold stress (male rats, exposed 30 days) | | |
| Heart rate | I | 1979 |
| Thermoregulation | I | 1979 |
| Thyroid stimulating hormone | I | 1979 |
| Serum corticosterone level | I | 1979 |
| Heart rate and blood pressure during exposure (rats, exposed 1 hr) | I | 1979 |
| Serum corticosterone (rats, exposed 30 days) | R | 1979 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Central Nervous System - J. C. Hampton

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Histopathology of CNS (mice, exposed 30 days) | I | 1978 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Endocrinology - M. J. Free

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Endocrine organ weights (rats, exposed 30 days) | I,R | 1978 |
| Hormone levels in plasma: FSH,* LH,** testosterone, TSH,*** growth hormone, thyroxine (rats, exposed 30 days) | I,R | 1978 |
| Testis blood flow and androgen secretion rates (rats, exposed 30 days) | I | 1978 |
| Corticosterone levels (rats, exposed 30 days) | I,R | 1979 |
| Hormone levels in plasma (rats, exposed 120 days) | I | 1979 |
| Hormone levels in developing rats (exposed from weanling to adult, 21-56 days of age) | I | 1979 |
| Endocrine organ weights in developing rats (exposed from weanling to adult (21-56 days of age) | I | 1979 |
| Testosterone and corticosterone levels in plasma (rats, exposed 120 days) | I | 1979 |

*Follicle stimulating hormone
**Luteinizing hormone
***Thyroid stimulating hormone

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Hematology and Serum Chemistry - H. A. Ragan

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Hematology and serum chemistry (rats, exposed 15 days) | I,R | 1978 |
| Hematology and serum chemistry (rats, exposed 30 days) | I,R | 1978 |
| Hematology and serum chemistry (rats, exposed 60 days) | I,R | 1978 |
| Hematology and serum chemistry (rats, exposed 120 days) | I,R | 1979 |
| Hematology and serum chemistry (mice, exposed 30 days) | I,R | 1978 |
| Hematology and serum chemistry (mice, exposed 60 days) | I,R | 1978 |
| Hematology and serum chemistry (mice, exposed 120 days) | I,R | 1979 |
| Hematology and serum chemistry (mice, $F_1$, exposed 77 days) | I,R | 1979 |
| Hematology and serum chemistry (mice, $F_2$, exposed 77 days) | I,R | 1979 |
| Hematology and serum chemistry (mice, $F_3$, exposed 77 days) | I,R | 1979 |
| Iron kinetics (rats, exposed 60 days) | I,R | 1979 |
| Iron kinetics (mice, exposed 60 days) | I,R | 1979 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Immunology - J. E. Morris

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Levels of serum immunoglobulins (rats, exposed 15 and 30 days) | I | 1978 |
| Levels of serum immunoglobulins (mice, exposed 30 and 60 days) | I | 1978 |
| Serum levels of complement (mice, exposed 30 and 60 days) | I | 1978 |
| Distribution of T and B lymphocytes (mice, exposed 30 and 60 days) | I | 1978 |
| Cell-mediated immunity (mice, exposed 30 days) | I,R,R | 1979 |
| Antibody response to KLH*(mice, exposed 30 and 60 days) | I | 1979 |
| Lymphocyte mitogen stimulation (mice, exposed 90 and 140 days) | I | 1979 |

*Keyhole limpet hemocyanin

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Metabolic Status and Growth - R. D. Phillips

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Growth, food and water consumption (rats, exposed 30 days) | I,R | 1978 |
| Organ weights (rats, exposed 30 days) | I,R | 1978 |
| Liver composition (juvenile male and female rats, exposed 30 days) | I,R | 1978 |
| $O_2$ consumption and $CO_2$ production (rats, exposed 30 days) | I | 1978 |
| $O_2$ consumption and $CO_2$ production (rats, during exposure) | I | 1979 |
| Growth, food and water consumption (rats, exposed 120 days) | I,R | 1979 |
| Organ weights (rats, exposed 120 days) | I,R | 1979 |
| Growth and food consumption (mice, exposed 120 days) | I | 1979 |
| Organ weights (mice, exposed 120 days) | I | 1979 |
| Liver composition (rats, exposed 30 days) | I,R | 1979 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Mutagenesis* - F. P. Hungate

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Drosophila, recessive lethals (185 and 370 kV/m, dc) | I,R | 1979 |
| Salmonella, TA-100. Histidine reversion to phototrophy, base exchange (Ames Assay; 800 kV/m, dc) | I | 1979 |
| Salmonella, TA-98. Reversion to histidine phototrophy, frame shift (800 kV/m, dc) | I,R | 1979 |
| Photobacterium fisheri. Forward mutation to tetracycline resistance (800 kV/m, dc) | I,R | 1979 |
| E. coli, plasmid-borne drug resistance (500 kV/m, dc) | I,R | 1979 |
| E. coli, lysogen induction (500 kV/m, dc) | I,R | 1979 |
| E. coli, forward mutation: (500 and 750 kV/m, dc) | | |
| To nalidixic acid or rifampicin resistance | I,R | 1979 |
| To ampicillin, tetracycline, valine, or chloramphenol resistance | I | 1979 |
| Phage, reversion (nonreplicating) | I,R | 1979 |

*This research is funded by both the Environmental Protection Agency and the Office of Health and Environmental Research, Department of Energy. Results are included in Annual Reports to these sponsors. Research funded by the Electrical Energy System Division of the Department of Energy are included in the 1979 Annual Report on this project.

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Neurophysiology - R. A. Jaffe

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Synaptic transmission (rats, exposed 30 days) | I,R | 1978-1979 |
| Peripheral nerve function (rats, exposed 30 days) | I,R,R | 1979 |

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Pathology - R. A. Renne

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Histopathology (rats, exposed 30 days) | I,R | 1978 |
| Histopathology (mice, exposed 30 days) | I,R | 1978 |
| Body and organ weights (rats, exposed 30 days) | I,R | 1978 |
| Body and organ weights (mice, exposed 30 days) | I,R | 1978 |
| Histopathology (rats, exposed 120 days) | I,R | 1979 |
| Histopathology (mice, exposed 120 days) | I | 1979 |
| Body and organ weights (rats, exposed 120 days) | I,R | 1979 |
| Body and organ weights (mice, exposed 120 days) | I | 1979 |
| Virology (rats, exposed 30 days) | I | 1979 |

TABLE XVI-1 (Cont'd). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Reproduction, Growth and Development - M. R. Sikov

| Experiment | Initial (I) or Replicate (R) | Annual Report |
|---|---|---|
| Reproduction and fertility (rats, exposed 30 days) | I,R | 1978 |
| Teratology (rats, exposed in utero to 20 days of gestation) | I,R | 1978 |
| Postnatal growth and development (rats, exposed from conception to 8 days of age) | I,R | 1978-1979 |
| Growth and neuromuscular development (rats, exposed from 17 days of gestation to 25 days of age) | I,R | 1979 |
| Three-generation mouse study (exposed in utero and raised in the field through three generations) | I,R | 1979 |

TABLE XVI-2. Summary of Experiments Showing Confirmed Effects

| | |
|---|---|
| Behavior: | Rats spent significantly more time out of a 60-Hz electric field than in it at $\geq$ 90 kV/m during a 45-min test. Also, exposed rats made more traverses between the exposed and unexposed regions of a shuttlebox than sham-exposed controls. |
| Behavior: | Rats exposed to 75 or 100 kV/m spent more time out of the electric field than sham-exposed controls during the 12-hr light period of a 24-hr test (12 hr light:12 hr dark). Rats exposed to 25 and 50 kV/m spent more time in the field than sham-exposed controls during the 12-hr light period. During the 12-hr dark period the only significant effect was a preference by rats exposed to 100 kV/m to stay out of the field. |
| Cell-Mediated Immunity: | Mice exposed to 100 kV/m for 30 days showed a statistically significant decrease in immune response to an antigen protein (KLH*) applied to the skin when compared to the response of sham-exposed controls. |
| Growth and Development: | A smaller percentage of rats exposed to 100 kV/m from conception to 8 days after birth exhibited the righting reflex and a higher percentage showed motile behaviors than did sham-exposed rats at 14 days of age. |
| Synaptic Transmission: | Sympathetic ganglia of rats exposed to 100 kV/m for 30 days were more excitable than those of sham-exposed rats. |
| Hematology: | Male and female mice exposed to 100 kV/m from conception to 77 days after birth had higher leukocyte counts (all white-cell types) than sham-exposed controls.<br><br>Red-blood-cell parameters of the third generation of mice exposed in utero and for 77 days after birth had higher values than those of sham-exposed controls. |

* Keyhole limpet hemocyanin