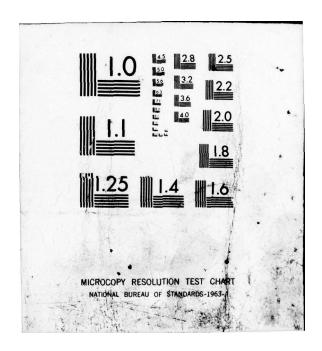
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Conditions were sacrificed; thyroid and adrenal tissues were weighed; intracardiac blood samples were fully analyzed. Most measures showed no statistically significant differences between experimentals and controls or between experimentals exposed to the various field strengths. Two activity measurements showed field effects: (1) size and duration of

Two activity measurements showed field effects: (1) size and duration of nighttime activity bout was reduced in the 1000 V/m condition, from the other conditions during initial exposure; (2) relative activity during the 0900-1000 period was consistently but not significantly greater in the 1000 V/m condition than the other conditions throughout exposure.

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FINAL REPORT

Project Title:

Reporting Period:

"Effects of 60 Hz Environmental Electric Fields on the Mammalian Central Nervous System"

October 1975 - September 1977

Attention:

Dr. Donald I. McRee Environmental Biophysics Branch National Institute of Environmental 2alth Sciences Post Office Box 12223 Research Triangle Park, NC 27709

Contract No. N00014-76-C-0421

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This study has examined effects of simulated high voltage AC power line fields on daily patterns of movement, renal functions, growth, organ weights and blood pictures in rats.

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## 1.0 SUMMARY

<u>1.0.1</u> The use of EHV and UHV lines in power transmission systems has grown dramatically in recent years; there is every reason to expect this growth will continue in the foreseeable future. This makes increasingly urgent the characterization of any biological effects of prolonged exposure to 60 Hz electric fields.

<u>1.0.2</u> The range of field strengths to be tested, species to be studied, and biological quantities to be measured is so great that no clear pattern of experimental results has yet emerged. For each combination of factors to be tested there is, however, a dual simulation problem that must be solved. The electrical field to be tested must be successfully generated and manipulated under laboratory conditions, meeting standards for uniformity, distortion and stability. The biological system being studied must be successfully maintained under laboratory conditions; the physiological and behavioral characteristics to be measured must be identified and means to accurately and systematically measure these characteristics must be devised and implemented. In each case, the simulation is selected to allow the evaluation of the adaptive significance of the animal's response, if any can be measured, to the 60 Hz electric field.

<u>1.0.3</u> The research pursued under the present contract was intended to simulate 60 Hz fields in the vicinity of power lines, with field gradients ranging from ambient levels in the laboratory (approximately 0.1 V/m) to 1000 V/m, with intermiediate values of 50 V/m and 500 V/m.

1.0.4 The test procedure that was utilized was intended to provide a first-order simulation of the continuous exposure of free-living rats to 60 Hz electric fields in this range. A wide range of measurements were made. To indicate effects of 60 Hz fields on behavior, general activity was continuously recorded and integrated over 15 min intervals. Activity behavior was measured with the intention of evaluating possible changes in circadian and infradian characteristics. To determine effects of the 60 Hz field on growth and development, water consumption was recorded on a daily basis, while food consumption and body weight were recorded on a weekly basis. To determine experimental influences on metabolic activity on a circadian basis, urine samples were taken by means of a fraction collector that allowed separate samples to be obtained from up to 60% of the members of a test cohort, 4 samples/d, while the remainder could be sampled twice daily. To determine the long term histopathological effects of the 60 Hz fields on three tissues representing vital organ systems, animals from the ambient, 500 V/m and 1000 V/m conditions were sacrificed at the conclusion of the experiment: blood was taken by cardiac puncture under ether anesthesia and comprehensively examined; adrenal and thyroid glands were removed and weighed.

<u>1.0.5</u> This wide variety of measurements was intended to provide a comprehensive picture of the effects of 60 Hz fields. Availability of this broad baseline allowed not only examination of individual data sets, as well as their interrelationships where these were deemed important.

1.0.6 A secondary but still important project goal was the development and refinement of the experimental techniques required by the simulations undertaken.

The results of the experiment reported here in general showed 1.0.7 no field-dependent effects; particularly in the measurements of growth and metabolic parameters. Most of the measurements of activity behavior also showed no field-dependent effects, but there were two possible exceptions. First, there appears to have been an increase in sensitivity to external sources of stimulation under the 1000 V/m condition. This increased sensitivity was not found to be significant, but it was also not an explicit goal of the present research to test for such an effect. Explicit testing would be required to evaluate this putative fielddependent effect. The second possible effect was reduction in the size and duration of the late night activity bout under the 1000 V/m condition. The amount of data analysis required to make detailed statistical tests of this putative effect was beyond the scope of the present project. Furthermore, a number of the features of the activity data acquisition procedures used in this experiment would reduce the power of any such expanded analysis, which would best be performed on a new set of data that eliminated these features.

<u>1.0.8</u> The results of this experiment make no clear case for or against the existence of field-dependent effects at 60 Hz over the range of field gradients at which determinations were made. The course for future, procedurally refined, and accurately targeted research has been more clearly charted.

## 1.1 INTRODUCTION AND EXPERIMENTAL RATIONALE

<u>1.1.1</u> The past 20 years have seen a vast increase in experimentation intended to determine the effects of environmental electromagnetic fields on living tissue, particularly in the central nervous system. Several extensive reviews of the literature have been published (Sheppard and Eisenbud, 1977; Phillips and Kaune, 1977; National Academy of Sciences, 1977). The following paragraphs are intended to briefly review those experiments that most directly relate to the biological simulation procedure selected here.

<u>1.1.2</u> Some of the most dramatic and clear-cut experimental evidence supporting biological sensitivity to environmental electromagnetic fields comes from study of naturally occurring behavior sequences that possess high adaptive significance for the species involved. For

example, sharks and rays have been shown (Kalmijn, 1971, 1972) to be sensitive to steady state and fluctuating electric gradients in the surrounding ocean at levels of  $10^{-8}$  V/cm. These gradients apparently play a role both in navigation and in prey detection. Similarly, Keeton (1972) showed that the earth's magnetic field could serve as an orientation guide in homing pigeons; the magnetic field was, however, not the primary navigational cue. Its influence could be measured only when the primary cue, the sun's position in the sky, was blocked by heavy cloud cover. More recently, Southern (1969, 1971, 1975) showed that fields produced at the Sanguine/Seafarer test facility in Wisconsin were capable of disrupting orientation during the migratory flight of a number of species of water fowl.

<u>1.1.3</u> Laboratory experiments have tested the effects of electric fields on conditioned behavior, behavior sequences developed in the laboratory that have no special significance and which are constructed by the experimenter to meet the requirements of a particular test procedure. In general, the results from these tests have proben less dramatic and convincing than those that employed highly adaptive behavior. This is no doubt due in large measure to difficulties in selection and development of sufficiently sensitive test procedures.

<u>1.1.4</u> Among those experiments that have shown positive results are those reported by König and Ankermüller (1960) and Hamer (1968) who reported reduced reaction times in humans during exposure to fields between 5 and 15 Hz in frequency and with gradients on the order of 10 V/m.

<u>1.1.5</u> A long series of experiments performed in this Laboratory (Gavalas, Walter, Hamer and Adey, 1970; Gavalas-Medici and Day-Magdaleno, 1976) offered substantial evidence that performance of monkeys on a subjective time estimation task (called a Differential Reinforcement for Low Rates of responding with a Limited Hold, DRL-1h) was modified by exposure to fields between 7 Hz and 75 Hz in frequency and between 1 V/m and 100 V/m field gradient. Specifically, the task required the monkeys to respond once every 5.0 sec to cnce every 7.5 sec to obtain a small quantity of apple juice. Since the effect of the fields was to cause the monkeys to underestimate the interval by from 0.3 sec to 0.5 sec (up to a 10% error), the fields tended to move the monkeys toward missing their rewards.

<u>1.1.6</u> These experiments suggested that the magnitude of the electric field effect was maximized with particular field frequency-field gradient combinations, indicating that the field tissue interaction was jointly determined by both variables.

1.1.7 A number of experiments from this Laboratory have provided evidence concerning the point of interaction between electromagnetic fields and nervous tissue as well as the biophysical basis of the interaction.

These experiments have been based on the concept of a "greater membrane" (Schmitt and Samson, 1969), which views the classic lipid bilayer as bearing on its outer surface a glycoprotein layer, loosely arranged, highly hydrated and carrying numerous fixed negative charges. The polyanionic character of this outer layer is seen as lending itself to a chemical and electrical transducer function based on its affinity for cations, particularly calcium and hydrogen.

<u>1.1.8</u> Testing of the possible role of this cell surface material in transductive coupling of weak EM fields has focused on measurement of the efflux of  $45Ca^{2+}$  from the isolated cerebral tissue of the chicken. It has been found that the calcium binding effect occurs across narrow domains of frequency and amplitude (Bawin, Kaczmarek and Adey, 1975; Bawin and Adey, 1976; Bawin, Sheppard and Adey, 1978; Bawin, Adey and Sabbot, 1978). These effects, which are qualitatively similar to those behavioral effects seen with monkeys, have recently been replicated in another laboratory (Blackman, et al., 1977).

<u>1.1.9</u> Each aspect of the test procedure employed in the present experiment was selected in recognition of the findings of the experiments just reviewed. While none of these experiments expressly demonstrated the biological potency of 60 Hz fields, it was considered important to develop procedures that would be most likely to show any effect of a 60 Hz electric field by virtue of that procedure's previously shown effect with some other weak environmental EM field.

<u>1.1.10</u> General activity and its circadian pattern would be expected to provide a good index of behavioral effects of fields since the circadian periodicity of activity is a robust phenomenon of high adaptive significance that is easily observed in all species under laboratory conditions.

<u>1.1.11</u> Measurement of growth and related metabolite parameters would be expected to provide a useful index of field effects on the health of the subjects.

<u>1.1.12</u> Measurement of urine electrolyte levels throughout the day would be expected to show circadian fluctuations in metabolic processes related to circadian fluctuations in general activity.

### 2.0 MATERIALS AND METHODS

2.1.1 The test facility was housed in the Animal Care Section of the UCLA Environmental Neurobiology Laboratory, a division of the UCLA Brain Research Institute. One room of the section was devoted to housing the test facility; access to the test facility was restricted and a log was kept of all persons entering the test area. The day-night cycle was regulated. The 12h day began at 0700.

2.1.2 The test facility comprised two units, illustrated in Figure 1. Each unit housed 5 rats in separate compartments 25cm x 25cm x 16cm; the compartments were arranged in a horizontal row with opaque plexiglass dividing individual compartments. Construction was entirely of plexiglass and other non-metallic materials with similar dielectric characteristics. Each compartment was provided with its own feeding and watering facility that allowed measurement of food and water consumption for each animal. Wastes were collected in a system that segregated fecal and other material from urine; the relatively uncontaminated urine from any of the rats could be transferred to an automated fraction collector (described below). A close-up on the test compartments appears in Figure 2.

2.1.3 Ventilation was provided by the building air conditioning system; no special controls for relative humidity, air ion or air pollution levels were imposed. Air flow through the individual compartments was facilitated by a number of holes machined in the compartment cover and the construction of the lcm nylon mesh floor.

2.1.4 The two units were placed end to end, separated by a distance of about 1.5m; the field generation apparatus and data acquisition equipment were located in this space. The nearest objects along the back of the units were about 2m distant while the nearest objects along the front were about 4m distant. The nearest objects along the ends were about 2m away.

2.2.1 Each of the 2 animal housing units was located on a trestle constructed of plywood which also supported the parallel-plate capacitor system which projected the vertically oriented electric field. The capacitor plates comprised 2 aluminum plates  $lm \times 2m$  protected by a .32cm plexiglass sheet. The plates were separated by a gap of 0.52m. Since the interior of the animal compartments had a clearance of 16cm, the animals could span no more than 31% of the inter-plate distance; this fell near the range required for adequate field uniformity, as discussed by Shih, et al. (1978).

2.2.2 The 60 Hz fields were generated by means of a power transformer connected to the field plates through RG-8 coaxial cable. The field plates were tied to ground through 300K  $\Omega$  resistors.

2.3.1 Each compartment in both of the animal housing units was equipped with a motion detection system. Two collimated beams of visible light were passed across the compartment 3.8cm above its floor level. The beams crossed the compartment 5.6cm from each end and separated by a distance of 13.8cm. Light was provided by a fibre optic illuminator equipped with a 50 Watt EKE-type quartz-halogen bulb. Light was transmitted to the collimating lens assemblies by a group of plastic fibre optic guides. Light was returned from the focusing lens assemblies, opposite the collimating lens assemblies, to the motion detection electronics packages through another set of plastic light guides.

2.3.2 The motion detection electronics packages were triggered whenever both light beams in a compartment were simultaneously interrupted. Motion counts were separately recorded for each compartment. Integral with the electronics packages were 4-digit impulse counters that recorded the total activity counts for each compartment. These counters were capable of counting rates of 20/sec; they recorded total activity during each 24h period.

2.4.1 The motion detection electronics packages also provided electrical outputs that were fed to a multi-channel event recorder that was used throughout the experiment. During the course of the experiment, a digital data-logging printer was added in parallel to the event recorder. The data logger recorded event counts and printed a cumulative count for each compartment every 15 min. Table 1 shows the method of recording activity in each phase on the experiment.

2.4.2 Patterns of circadian activity could be obtained from either of the two recording systems: the event recorder was capable of resolution into arbitrarily brief epochs while the data logger epochs as brief as 15 min. In practice, the event-recorder records proved so difficult to interpret (due to close packing of the data) that the data log numerical records have been relied upon entirely in the analysis of circadian patterns of activity.

2.5.1 The construction of each compartment in each animal housing unit allowed the urine produced by individual animals to flow into plastic tubes. Tubes from up to 3 animals per exposure unit could be connected to a fraction collector programmed for 6-hourly sample collections. Urine from the remaining rats was collected at the rate of 2 samples/day. This urine collection apparatus was located immediately below the trestle that supported the unit serviced. The timing and control electronics packages for the fraction collectors were located in an adjacent room. As far as possible, the fraction collectors were built of non-metallic components. Figure 3 shows one of the two fraction collectors employed in the experiment. Table 1 shows the method of urine collection in each phase of the experiment.

2.5.2 Urine was frozen and maintained at (0°C) when it was removed from the fraction collectors during the daily maintenance period between 0900 and 1000 Monday-Friday; urine collected between Friday morning and Monday morning was not frozen until Monday morning. The volume of urine produced by each subject each day was recorded. Selected urine samples were subsequently subjected to atomic absorption analysis to determine levels of Na, K and Ca.

2.6.1 At the conclusion of the ambient (control), 500 V/m and 1000 V/m conditions all subjects were sacrificed. Under ether anesthesia, blood samples were obtained by heart puncture and subjected to complete hema-tological analysis. Adrenal and thyroid tissues were surgically removed, weighed and examined for histological abnormalities. These procedures were performed in an autopsy room adjacent to the test facility.

## 2.7.0 Animal Care

2.7.1 Animal care and maintenance procedures on the data acquisition and urine analysis systems took place twice daily on a 5d/wk schedule. These operations began at 0900 and 1500; the morning maintenance period lasted for about 1h and the afternoon period for about 0.5h. During the morning period, the apparatus was cleaned, inside and out, and received any required maintenance.

2.7.2 Water consumed during the preceding 24h was measured and the nonmetallic waterers refilled. Measured amounts of food were placed in the feeders as required on a daily basis at this time. Food was placed in each compartment's feeder so as to be easily removed by the rats and at the same time minimally perturb the field. The contents of all feeders were removed once weekly, on Thursday, and food consumption for the week by each rat determined. The weight of each rat was measured at the same time weekly food consumption was determined.

2.7.3 During the morning period urine samples were removed from the fraction collector and new sample tubes put in place. Urine volume and pH levels were measured at this time, just prior to the freezing of the urine samples. The volume of urine from the fraction collector was measured for each fraction, but pH was determined only for the 0600-0900 fraction. Both measurements were made on the single sample from those animals not on the fraction collector.

2.7.4 During the morning maintenance period, 24-h activity counts for each subject were recorded and the 4-digit cumulating counters reset. Tapes from the event recorder and data logging system were removed as required.

2.7.5 During the afternoon maintenance period, the following measurements were made and maintenance procedures carried out. Water intake since morning was determined. Urine was collected both from the fraction collector and the pooled urine collectors; urine volume was measured and the urine frozen. Food was adjusted in the feeders to insure free access, but no food was added. Each of the animal housing units was again cleaned on the outside, but the interior of the compartments was not touched.

2.7.6 During maintenance periods, the field generating apparatus was shut off. The field was calibrated using a Tektronix high voltage probe (model #P6013) coupled to a Tektronix model 545 oscilloscope that was regularly calibrated (traceable to relevant national standards). Calibrations were performed prior to the beginning of each experimental condition and at random intervals during the 30d field exposure.

2.8.0 Experimental Design

2.8.1 The experimental procedure was carried out on 6 cohorts of 10 rats each. For the first 5 cohorts, all members received identical field-

exposure treatments. For the final cohort, 5 rats received field exposure at 1000 V/m and 5 received exposure at ambient levels (control condition).

2.8.2 Table 1 shows in the first column the order of presentation of the field conditions, the date of the beginning and end of the condition and the number of subjects employed. The remaining columns summarize the data collection procedures, previously described, employed under each experimental condition. Note that, in this pilot experiment, not all data collection procedures were employed under all experimental conditions.

2.8.3 The same testing procedure was followed under all experimental conditions. The test subjects were received from the supplier at 20d of age. They were housed in conventional metallic cages in the test facility for 14d; during this period, they received ad libitum food (Purina rat chow, used throughout the experiment) and water. During this period, rats were housed 5 per cage.

2.8.4 Following this period of adaptation to the test facility environment, the 10 test animals were placed singly into the 10 compartments of the test facility. The subjects were always placed in the test facility for the first time at the beginning of the morning maintenance and data collection period on Monday morning; in this manner all significant procedural landmarks occurred in a constant relation to the maintenance and data collection schedule already described. This schedule was imposed at the time the test subjects were placed in the test facility for the first time.

2.8.5 The subjects were allowed to adapt to the test facility and the experimental regimen for a 7d period. The field, if scheduled, was activated at the end of the morning maintenance period of the first field day. The field then continued for a 30d interval, interrupted only for the maintenance and data collection periods.

2.8.6 At the end of the 30d field exposure (or control) period, the subjects remained in the test facility for a 7d post-exposure study period, after which the subjects were removed from the test facility and sacrificed according to the protocol previously described. This procedure was performed beginning at 0900.

2.9.0 Statistical methods

2.9.1 This experiment yielded a number of measures made at regular intervals throughout the 44d course of each of the 6 field presentations. Among these are mean daily activity, weight, food consumed, water consumed, urine produced, urine electrolyte levels and pH. Even though some of the measures may not be entirely independent of each other, the most appropriate method of packaging and relating all these data, and for determining any field-dependent effects, would be through the performance of a multivariate analysis of variance (Winer, 1972).

8.4.

2.9.2 In addition to the measures just mentioned, a number related to blood and tissue characteristics were made just once. For this reason, these measurements would require a separate analysis of variance.

2.9.3 Finally, activity measurements were made 96 times per day. These data could be analyzed for changes in day-by-day circadian periodicity.

2.9.4 In each case, however, the pilot experiment characteristics of the present experiment caution against the full-dress analyses that have been mentioned. Specifically, the small number of subjects run, the unequal numbers of subjects run under each experimental condition as well as the failure to fully replicate all conditions caution against the performance of analyses of variance. With regard to the circadian activity data, the problem was compounded by the large amount of data that was collected, none in machine readable format.

2.9.5 The tactic adopted, therefore, was to begin (conservatively) by performing a number of graphical and numerical analyses that might reveal powerful field-dependent effects. The uncovering of any such effects could then lead to either further statistical analyses or the acquisition of additional data, or both. As a further step in this process, a number of comparable days throughout the experiment, from each field condition, were selected for intensive analysis. These days and analyses performed are summarized in Table 2. Only those field conditions under which data from 10 subjects were available from the automated data logging system were selected for intensive analysis.

## 3.0 RESULTS

<u>3.1.1</u> The analysis will first consider the activity data. Table 3 shows that average activity/24h period decreased from week to week throughout the experimental treatment under all field conditions. Table 3 also shows standard deviations of the mean that reflect inter-day variability within each week. Over the experimental treatment these standard deviations decrease, though not monotonically; this indicates that the day-to-day activity in each week became more and more similar during the course of the experiment.

<u>3.1.2</u> Figure 4 begins the intensive analysis of the activity data. It extends over 3 pages, corresponding to ambient, 500 V/m and 1000 V/m field conditions. We will first consider the data portrayed in the left-hand panel on each page, later turning to the right-hand panels.

3.1.3 The left-hand panels of Figure 4 show the average activity in all subjects during each of the days selected for intensive analysis. The error bars show the standard deviations, reflecting intra-subject variability. It is clear that under each field condition the average amount of daily activity decreased during the course of the experimental treatment. It is also clear that the standard deviations decrease during

the course of the experimental treatment, showing that the subjects became more similar in the amount of measurable general activity that they produced.

<u>3.1.4</u> The upper portion of Figure 5 shows the relation of the means and standard deviations just seen in Figure 4 by plotting the coefficient of variation for each of the data points graphed in Figure 4. Figure 5 shows that while the location of activity moved toward lower values and variability was reduced, the relation between the two was highly nonlinear. After the first week of field (or sham field) exposure, the difference between the subjects had reached a minimum; the increase in the coefficient of variability following this point reflects the continued decline in the mean and the stability of the measure of inter-subject variability.

<u>3.2.1</u> The variation in the mean and the complex relation seen between the mean and the measure of inter-subject variability argued that the analysis of circadian activity be made in terms of the relative amount of activity occurring during each hour of the 0900-0859 day. An analysis of relative amounts of activity would preserve the essential aspects of circadian pattern while masking out the obscuring variations in absolute amount of activity. This choice necessarily restricts the analysis of patterns of circadian activity to ordinal (greater than, less than, or equal) relations and statistics.

<u>3.2.2</u> Figure 6 shows the relative circadian activity (activity in epoch/ average activity per epoch for the day) from the 1000 V/m condition shown in Figure 4; each of the 9 panels in Figure 6 corresponds to one of the data points graphed in Figure 4. (Complete relative circadian activity from the analyzed sessions can be found in tabular form in Appendix A. The description provided here refers to all circadian activity reported in Appendix A.)

<u>3.2.3</u> Throughout the experiment, circadian activity is quite pronounced; activity falls to a minimum during the day and rises to a major peak during the hours after midnight. Note, however, that circadian variation may have been damped in part due to the use of visible light by the activity measurement system. With one exception to be noted in connection with Figure 8, there do not appear to be measurable shifts in the circadian pattern of activity either over the course of the experimental treatment or as a function of field condition. A striking feature of these data is the relative increase in amount of activity seen in the amount of activity observed during the 0900-1000 maintenance and data gathering period over the course of the experimental treatment.

<u>3.2.4</u> Figure 4, to which we must now temporarily return, shows that this increase was absolute as well as relative, and quite contrary to the pattern of activity during the session as a whole. The right-hand panels of Figure 4 show activity during the epoch of concern; in each case activity increases over the course of the experimental treatment. The error bars show the standard errors, reflecting inter-subject variability.

<u>3.2.5</u> Turning again to Figure 5, we may examine the relation between average amount of activity and inter-subject variability, now during the 0900-1000 epoch. In each case, a regular decline in the coefficient of variation is seen, indicating that the mean is growing faster than the index of variability. This shows that the increase in the mean is not due simply to the increased frequency of a few extreme values, rather it is due to an actual shift in location and a relatively smaller shift in variability.

<u>3.2.6</u> Figure 7 shows the relative amount of activity, as previously defined, as a function of session for the control, 500 V/m and 1000 V/m conditions. This figure shows that in the case of each condition, the increase over the course of the experimental treatment is by a factor of about 3. Note that of the 5 field sessions analyzed, the 1000 V/m condition is largest in 4 cases. This relation is not significant, however (binomial test p = .188) against H<sub>0</sub>: 1000 V/m condition relative scores are equal to or less than the other 2 conditions).

3.2.7 Figure 8 (which extends over 3 pages) shows the size and duration of the early morning activity bouts. Each bout is represented by a bar which is divided into filled and unfilled portions. The earliest experimental day is seen at the top and the latest at the bottom. Panel A of Figure 8 is for the control condition, Panel B for the 500 V/m condition, and Panel C for the 1000 V/m. The total area of each bar is proportional to the area between the relative activity curve and the mean. The broken line segments of the relative activity curves seen in Figure 6 indicate the areas used in the calculation of the activity bars seen in the 3rd page of Figure 8. The activity bars are divided into filled and unfilled portions to indicate the modal interval of early morning activity. Finally, the beginning of each activity bar is positioned to indicate the time at which the relative activity curve crossed the mean (or turned upward) after 0000 hours while the end of the bars is positioned to indicate the time at which the relative activity curve fell below the mean with the approach of day.

3.2.8 Figure 8 shows no apparent relation between field condition and time, amount or duration of late night activity with the exception of the first field session analyzed under the 1000 V/m condition. Here, activity both in amount and duration is very substantially reduced when compared to the 2 pre-field conditions that were analyzed. This is also the case for the 2nd field session analyzed under the 1000 V/m condition, and persists to some degree throughout the remainder of the experimental treatment. The control and 500 V/m conditions do not appear to show a comparable effect.

3.3.1 Attention is now turned to growth and metabolism measurements. Figure 9 shows the average weight of the subjects during each week of the experimental treatment for the experimental conditions where data from 10 subjects were available. Table 4 shows the weekly average weight for all experimental conditions as well as standard deviations. In this and succeeding tables, the standard deviations reflect inter-day variability.

This figure and table show a consistent increase in weight over the course of the experimental treatment. These data suggest no field-dependent effect.

<u>3.3.2</u> Tables 5 and 6 present data related to those just considered. Table 5 presents the change average weight from week to week over the course of the experimental treatment; i.e., the 1st derivative of the functions plotted in Figure 6. As would be expected on the basis of the foregoing, the data presented in Table 5 show no field-dependent effect. Table 6 shows the average weight of food consumed on a weekly basis thoughout the experimental treatment. The data presented in Table 6 show no field-dependent effects.

<u>3.3.3</u> Figure 10 shows the average urine volume per animal per day as a function of weeks over the course of the experimental treatment. The conditions where data from 10 subjects are available and/or the automated data logger operated has been plotted. Table 7 shows the urine volume for all experimental conditions. Table 8 shows a related measure, average daily water consumption. Taken together, these data do not suggest any field-dependent effect. The control conditions suggest a somewhat lower level of production, but this lower level appeared in the data from the outset. The relatively small change in urine output during the 50 V/m condition, the first condition run, was due principally to the need to perfect urine collection techniques early in the experiment.

<u>3.3.4</u> Figure 11 shows the amount of electrolytes in the collected urine in  $\mu E/vol$  urine excreted/mean body weight, as determined by atomic absorption spectroscopy over the course of the experimental treatment for all conditions. These highly regular data suggest no field-dependent effects, either on the electrolytes themselves or on steroid levels in the urine.

3.3.5 Table 9 shows the pH of the urine samples contributing to Figure 11. These pH data do not suggest any field-dependent effects.

<u>3.4.1</u> The final two Tables 10 and 11 deal with the morbid anatomy results obtained from the control, 500 V/m and 1000 V/m animals. The gross characteristics of the glandular tissue showed no apparent field-related effects; neither did the detailed clinical picture of the blood that was taken at the completion of the experimental treatment.

## 4.0 DISCUSSION

4.1.1 The overall pattern of results in this experiment showed no substantial field-dependent processes, though 2 potential field-related effects were noted, both behavioral measures: the relatively larger amount of activity during the 0900-1000 period during the 1000 V/m condition, when compared with the other field conditions, and the reduced amount and duration of late night activity under the 1000 V/m condition when compared with the other conditions.

4.1.2 There was a strong field-independent effect: the reduction in absolute activity over the course of the experiment. It is of substantial

interest because the first of the putative field-related effects, the increase of activity between 0900 and 1000, was found in spite of the overall reduction in number of responses/hour. The reduction in number of responses/ hour is most likely due to an unfortunate combination of test compartment size and motion detection logic design. As the experimental treatment proceeded, the rats approached maturity, which of course meant they became larger. As this occurred, there was a reduction in the probability that the rats' movements would not interrupt both light beams, triggering a counter. The animal's weight, which should be highly related to length and girth during development given ad <u>libitum</u> access to food, was measured. The correlation coefficient between activity counts/h and weight was quite high, r = -.84, thus accounting for about 71% of the variability in the activity data.

4.1.3 This correlation was dependent on 3 factors: the size of the housing compartment, the size of the rat and the design of the activity detection circuits. Any procedural modification that would eliminate the dependency (such as using mature rats or redesigning the circuit) would break down the correlation seen here.

<u>4.1.4</u> While the activity-weight relation is itself not too important from the point of view of this experiment, it is important because it stands in strong contrast to the increases in 0900-1000 activity: the two measures of different aspects of activity, go in opposite directions, suggesting the operation of different processes. This interpretation is further buttressed by the very different time courses of the coefficients of variability associated with the two measures of activity. The plot of relative activity in the 0900-1000 epoch for control, 500 V/m and 1000 V/m conditions suggests a field-dependent relationship though statistical significance was not achieved.

<u>4.1.5</u> A possible explanation for the putative field-dependent increase may be made in terms of an increased sensitivity to external stimuli on the part of the 1000 V/m rats as compared to the other rats whose data were plotted for comparison. Other possible sources of the increase, such as adaptation to handling by the technical staff, do not seem credible since such sources of variation are either produced by the procedure or are fieldindependent, or both.

4.1.6 Statistical evidence supporting a field-dependent effect here might be obtained by subjecting more of the data that were collected to the analysis employed on the data presented here. Any important increase in the amount of data analyzed in this way would, however, involve a very substantial commitment of resources and time. It would be our recommendation that, rather than follow this course, experiments be designed and conducted that would specifically test the hypothesis of increased irritability in laboratory rats exposed to 1000 V/m fields compared with other rats exposed to fields of smaller gradient.

4.2.1 The other putative field-dependent effect involved the apparent reduction of the late night activity bout in the 1000 V/m rats as opposed

to the control and 500 V/m rats. Considerable additional data will be needed for analysis before it would be appropriate to perform statistical analyses, if one wished to test for a difference on a particular field day. Testing for a difference over several days would be possible from the present experimental data set. The "on"-effect for the 1000 V/m condition suggested by the present data would be of considerable interest if its existence was borne out by further analysis and data collection. Data should be collected at a number of field gradients to determine the range characteristics of this putative effect.

4.2.2 The mechanism by which a 1000 V/m electric field might change the size and duration of the late night activity bout is unclear. The data from the present experiment do not appear to bear upon this question. More extensive and detailed data collection would reveal the mechanism of such an interaction.

<u>4.3.1</u> The remainder of the other experimental results failed to manifest any obvious field effects. In general, these data showed that adequate experimental control had been established and that the subjects had adequately adapted to the test facility and procedures.

<u>4.3.2</u> The results of the present experiment seem to fall into the same category as most related experiments performed in other laboratories: small or no field-dependent effects have been seen. Two factors relating to the failure to detect field-dependent effects (in addition to their genuine absence) apply, singly or together, to most field research, including the present experiment. First is the choice of experimental procedure: the experimental procedures that are chosen, even when substantial care is employed, as here, may miss field effects entirely or poorly map onto what are in fact robust field-dependent phenomena, in which case low magnitude effects would be seen. Second, if the field-dependent effects are small, much data will be required before field-dependent variability can be distinguished from other sources of experimental variability.

<u>4.3.3</u> Another feature of the physiological and behavioral measures employed here may have been of significance in the nil or small fielddependent effects measured here: the inherent stability of the processes measured. If one thinks of the experimental problem in this class of research to be the design and construction of a sensitive 60 Hz radiation detector, using biological components, attention is focused on the need for measuring processes that are stably balanced in the absence of the field, but easily driven from that balance by the field but by no other cause, in accordance with well established engineering practise.

4.3.4 Good basic biological engineering, on the other hand, has dictated that many of the parameters measured in the present experiment be extremely stable under a wide range of environmental influences. Consequently, it is reasonable to assume that at least some of the measures employed here may have involved processes not easily perturbed under most circumstances.

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4.3.5 The present experiment makes clear the need for the implementation of procedures that are most likely to measure any field effects that may exist. The results of the present experiment do not afford any specific conclusions concerning significant biological hazard associated with 60 Hz fields; they do strongly suggest an appropriate course for continued investigation.

## REFERENCES

Bawin, S. M. and Adey, W. R. Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency, <u>Proc. Nat. Acad. Sci., U.S.A.</u>, 1976, 73:1999-2003.

Bawin, S. M., Adey, W. R. and Sabbot, I. M. Ionic factors in the release of  $^{45}Ca^{2+}$  from chicken cerebral tissue by electromagnetic fields, <u>Proc. Nat. Acad. Sci., U.S.A.</u>, 1978, <u>75</u>:6314-6318.

Bawin, S. M., Kaczmarek, L. and Adey, W. R. Effects of modulated VHF fields on the central nervous system, Ann. New York Acad. Sci., 1975, <u>247</u>: 74-81.

Bawin, S. M., Sheppard, A. and Adey, W. R. Possible mechanisms of weak electromagnetic coupling in brain tissue, <u>Bioelectrochemistry</u> and Bioenergetics, 1978, 5: 67-76.

Blackman, C. F., Elder, J. A., Weil, C. M., Benane, S. G. and Eichinger, D. C. Two parameters affecting radiation-induced calcium efflux from brain tissue, URSI International Symposium on the Biological Effects of Electromagnetic Waves, 1977, Proceedings, p. 101.

Gavalas, R. J., Walter, D. O., Hamer, J. and Adey, W. R. Effect of low-level, low-frequency electric fields on EEG and behavior in Macaca nemestrina, Brain Research, 1970, <u>18</u>: 491-501.

Gavalas-Medici, R. J. and Day-Magdaleno, S. R. Weak, low frequency electric fields affect schedule-controlled behavior of monkeys, <u>Nature</u>, 1976, <u>261</u>: 256-259.

Hamer, J. R. Effects of low-level, low-frequency electric fields on human reaction time, <u>Communications in Behav. Biol</u>., 1968, <u>2(A)</u>: 217-222.

Kalmijn, A. J. The electric senses of sharks and rays, <u>J. Exper</u>. <u>Biology</u>, 1971, <u>55</u>: 371-383.

Kalmijn, A. J. The role of electroreceptors in the animal's life. I. The detection of electric fields from inanimate sources other than electric organs. In: <u>Handbook of Sensory Physiology</u>, Vol. III, A. Fessard, ed., 1972, Springer-Verlag, Berlin.

Keeton, W. T. In: <u>Animal Orientations and Navigation</u>, S. R. Galler, <u>et al</u>., eds., 1972, Government Printing Office, Washington, D. C., p. 579.

König, H. and Ankermüller, F. Uber den Einfluss besonners niederfrequenter elektrischer Vorgange inder Atmosphere auf den Menschen, Naturweiss, 1960, <u>47</u>: 486-490.

National Academy of Sciences. Biologic Effects of Electric and Magnetic Fields Associated with Project Seafarer, 1977, Washington, D. C.

Phillips, R. D. and Kaune, W. T. Biological effects of static and low frequency electromagnetic fields: an overview of United States literature, Battelle Northwest Laboratories, 1977, Publication 2262, Richland, Washington.

Schmitt, F. O. and Sampson, F. E. Brain cell microenvironment, <u>MIT</u> Neurosci. Res. Prog. Bull., 1969, <u>7</u>: 277-417. Sheppard, A. R. and Eisenbud, M. <u>Biologic Effects of Electric and</u> <u>Magnetic Fields of Extremely Low Frequency</u>, 1977, New York Univ. Press, New York.

Shih, C. H., dePlacidio, J., and Ware, B. J. Analysis of parallel plate simulation of transmission line electric field as related to biological effects laboratory studies, <u>IEEE Transactions on</u> <u>Power Apparatus and Systems</u>, Vol. PAS-96(3), 1977, pp. 962-968.

Southern, W. E. Orientation behavior of ring-billed gull chicks and fledglings, <u>Condor</u>, 1969, <u>71</u>: 418-425.

Southern, W. E. Gull orientation by magnetic cues: a hypothesis revisited, <u>Ann. New York Acad. Sci.</u>, 1971, <u>188</u>: 295-311.

Southern, W. E. Influence of disturbances in the earth's magnetic field on ring billed gull orientation, Condor, 1975, 74: 102-105.

Winer, B. J. <u>Statistical Principles in Experimental Design</u>, 2nd ed., 1971, McGraw-Hill, New York.

		Blood and Tissue	Not analyzed	Blood and tissue analyzed	Blood and tissue analyzed	Blood and tissue analyzed	Blood and tissue analyzed	Blood and tissue analyzed	Blood and tissue analyzed
	ta Acquired	Urine Collections	2 samples/day/rat (for all 10 rats)	<pre>Fraction collector on 3 rats, 4 samples/day/rat 0ther 7 rats, 2 samples/day</pre>	As in 2.	<pre>Fraction collector on 6 rats, 4 samples/day/rat 0ther 4 rats, 2 samples/day</pre>	As in 4.	<pre>3 rats on fraction collector, 4 samples/day 2 rats, 2 samples/day</pre>	As in 6a.
TABLE 1	Field Conditions and Data Acquired	Body Activity	Event recorder (chart)	Event recorder	Event recorder	Event recorder <u>and</u> digital data logger	Event recorder <u>and</u> digital data logger	Event recorder <u>and</u> digital data logger	Event recorder <u>and</u> digital data logger
		Field Condition	1. 50 V/m Oct-Nov 76 (10 rats)	2. 500 V/m Jan-Feb 77 (10 rats)	<ol> <li>No field control Mar-Apr 77 (10 rats)</li> </ol>	4. 1000 V/m Apr-Jun 77 (10 rats)	5. 500 V/m Jul-Sep 77 (10 rats)	<pre>6a. No field control     Sep-Oct 77     (5 rats)</pre>	6b. 1000 V/m Sep-Oct 77 (5 rats)

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# DAYS SELECTED FOR INTENSIVE ANALYSIS OF CIRCADIAN ACTIVITY AND METABOLIC ACTIVITY UNDER CONTROL, 500 V/m AND 1000 V/m CONDITIONS

DAY OF TREATMENT 2 7 9 16 23 30 37 38

\*

MEAN NUMBER OF ACTIVITY COUNTS AND STANDARD DEVIATIONS PER 24h PERIOD

Condition and Date	and	Pre-Field Days 1-7	Field Days 1-7	Field Days 8-14	Field Days 15-21	Field Days 22-30	Post-Field Days 1-7
No-Field 3/77	3/77	1872±215ª	1687±263	1401±112	1209±64	995±154	815±66b
	11/6	1960±118 <sup>c</sup>	1858±107	1734±82	1485±75	1307±105	1216±1
50 V/m	10/76	1756±187	1565±71	1069±368	1069±106	992±156	923±85b
500 V/m	1/11	1789±173	1843±141	1538±193	1305±110	1153±76	1003±172b
	1717	1910±106	1802±75	1623±162	1382±98	1212±75	1170±74
1000 V/m	4/77	1973±209	1518±76	1290±92	1171±42	931±64	844±21b
	1779	2179±205 <sup>c</sup>	2071±159	1857±102	<b>1549±185</b>	1287±123	11122±111

<sup>a</sup>Unless noted otherwise, n = 10 bn = 9 CFor all cases in this row, n = 5

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AVERAGE WEIGHT OF ALL SUBJECTS IN 9

Condition and Date	and	Pre-Field Days 1-7	Field Days 1-7	Field Days 8-14	Field Days 15-21	Field Days 22-30	Post-Field Days 1-7
No-Field 3/77	3/77	267.6ª	320.5	356.1	394.1	428.0	447.8
	1776	280.8 <sup>b</sup>	323.0	360.0	392.6	425.0	435.8
50 V/m	10/76	261.9	322.5	371.5	392.7	425.0	445.5
500 V/m	11/1	263.9	309.7	350.8	378.0	402.6	422.3
	ררור	285.6	331.2	376.0	412.4	452.2	474.2
1000 V/m	4/77	287.0	335.9	383.4	414.7	452.3	470.4
	1776	287.8 <sup>b</sup>	341.5	380.4	415.0	447.6	461.0

aUnless otherwise noted, n = 10bFor all cases in this row, n = 5

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AVERAGE WEIGHT CHANGE IN 9 PER DAY

Condition and Date	and	Pre-Field Days 1-7	Field Days 1-7	Field Days 8-14	Field Days 15-21	Field Days 22-30	Post-Field Days 1-7
No-Field 3/77	3/77	4.4ª	7.6	5,1	5,4	3.8	3.3
	1776	6.3b	6.0	5.3	4.7	3.6	3.4
50 V/m	10/76	3.0	8.7	7.8	5.2	4,3	3.4
500 V/m	11/1	3.6	13.7	5.7	4.0	2.7	2.8
	1111	6.4	6.5	6.4	5.2	4.5	3.7
1000 V/m	4/77	8.8	7.0	6.8	4.5	4.2	4.3
	17/6	7.3b	7.7	- 5.5	4.9	3.6	3.4

aunless otherwise noted, n = 10bFor all cases in this row, n = 5

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AVERAGE FOOD INTAKE IN 9 PER 24 HOURS

Condition and Date	and	Pre-Field Days 1-7	Field Days 1-7	Field Days 8-14	Field Days 15-21	Field Days 22-30	Post-Field Days 1-7
No-Field 3/77	3/77	22.3ª	27.9	25.0	30.6	28.1	26.4
the second	11/6	21.4 <sup>b</sup>	25.5	26.3	26.5	28.7	26.0
50 V/m	10/76	21.0	29.2	28.4	26.6	21.0	27.3
500 V/m	1/11	24.5	24.5	24.2	22.8	25.6	15.6
	ננונ	22.5	26.5	28.1	26.6	21.0	27.3
1000 V/m	4/77	23.1	28.2	29.2	27.4	28.2	27.9
	1779	22.9b	27.2	29.8	28.5	28.7	28.1

aUnless otherwise noted, n = 10bFor all cases in this row, n = 5

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## AVERAGE MILLILITERS AND STANDARD DEVIATION OF URINE EXCRETED PER 24h PERIOD

Condition and Date	and	Pre-Field Days 1-7	Field Days 1-7	Field Days 8-14	Field Days 15-21	Field Days 22-30	Post-Field Days 1-7
No-Field 3/77	3/77	3.8±0.9ª	5.6±1.7	7.8±1.0	8.1±1.3	8.8±1.1	10.6±0.9
	11/6	8.3±1.3 <sup>b</sup>	8.6±0.7	<b>10.5±0.8</b>	13.5±1.3	13.6±0.5	13.4±0.6
50 V/m	10/76	5.8±0.6	5.7±0.8	4.2±0.4	5.3±0.8	<b>5.7±0.8</b>	7.1±0.6
500 V/m	1/11	4.8±0.6	5.9±1.2	10.6±2.7	<b>10.8±1.2</b>	12.5±1.5	13.0±1.0
	111	<b>9.6</b> ±1.0	9.5±1.1	11.4±1.2	<b>13.7±1.0</b>	13.2±0.8	13.6±0.5
1000 V/m	4/77	7.8±1.3	11.7±0.9	12.4±0.9	<b>13.0±1.4</b>	13.8±0.9	15.1±1.0
	11/6	9.2±1.3 <sup>b</sup>	9.8±1.6	10.7±1.1	11.7±1.1	13.8±1.5	10.6±3.0

aUnless otherwise noted, n = 10bFor all cases in this row, n = 5

AVERAGE WATER INTAKE AND STANDARD DEVIATION IN m1 PER 24h

Condition and Date	and	Pre-Field Days 1-7	Field Days 1-7	Field Days 8-14	Field Days 15-21	Field Days 22-30	Post-Field Days 1-7
No-Field 3/77	3/77	29.6±6.8ª	37.3±2.4	37.9±2.8	39.2±1.5	37.7±4.4	39.7±1.5
	11/6	33.1±6.9 <sup>b</sup>	36.1±1.2	42.0±3.2	<b>43.3±6.7</b>	45.2±2.8	<b>45.5</b> ±3.0
50 V/m	10/76	31.5±7.1	39.2±5.8	36.0±4.0	35.7±3.0	36.0±2.5	36.2±5.0
500 V/m	1/11	30.2±8.3	35.6±3.0	<b>41.7±4.5</b>	40.4±4.9	42.5±5.6	37.5±5.3
	ננונ	<b>33.8±6.8</b>	36.2±4.1	41.2±3.8	40.8±5.1	39.9±2.3	<b>39.3±1.4</b>
1000 V/m	4/77	39.4±8.9	41.4±2.8	46.0±3.8	44.9±1.9	45.5±2.6	45.5±3.0
	11/6	31.8±7.6 <sup>b</sup>	37.1±4.5	40.3±2.3	37.3±4.2	43.1±3.4	41.2±0.3

<sup>a</sup>Unless otherwise noted, n = 10 <sup>b</sup>For all cases in this row, n = 5 TABLE 9 ph level of urine samples from days subjects to intensive analysis

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DAY	CONTROL	500 V/m	1000 V/m
PR 1	8.3	8.2	7.52
F 1	8.6	8.67	8.95
F 2	8.7	8.74	8.75
F 3	8.8	8.75	8.82
F 4	8.7	8.65	8.65
F 5	8.8	8.62	8.62
P0 2	8.8	8.65	8.7

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## QUANTATIVE RESULTS OF EXAMINATION OF GLANDULAR TISSUES

			6/77 <sup>D</sup>		51.1		20.3	
		1000 V/m:	4/77a		50.8		20.3	
	FIELD	7/77 <sup>a</sup>			48.5		18.1	
		500 V/m:	1/77a		57.8		18.5	
	_1		d/17b		48.6		18.2	
	CONTROL		3/77 <sup>a</sup>		43.1		19.0	
			<	Adrenal Glands	mg wet tissue	Thyroid Glands	mg wet tissue	

an = 10 bn = 5

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QUANTITATIVE RESULTS OF COMPLETE BLOOD TESTING

	TATIVITINNON		AUNITIALITY ALOOLID OF CONFELLE DECOD ILDITIA			
	CONTROL	ROL		FIELD		
	3/77 <sup>a</sup>	9/776	500 V/m: 1/77 <sup>a</sup>	7/77a	<u>1000 V/m</u> : 4/77 <sup>a</sup>	q/1/6
BLOOD						
Hematocrit (PVC)	44.4	41.1	49.0 <sup>c</sup>	43.8	44.7	43.6
Hemoglobin	14.8	15.3	16.7 <sup>c</sup>	15.0	15.3	14.8
WBC	11,340	9.060	11,100 <sup>c</sup>	7,300	11,650	10,480
DIFFERENTIAL						
Band Cells	0	0.2	0	0	0	0
Segmented Cells	4.6	8.8	9.0	10.1	8.7	0.6
Lymphocytes	92.3	89.4	87.0	86.6	86.9	87.0
Monocytes	1.5	3.2	2.7	2.2	3.0	3.0
Eosinophils	0.5	0.2	1.4	1:1	1.4	0.6
Basophi1s	0	0	0	0	0	0
Platelets	Adequate	Adequate	Decreased <sup>c</sup>	Adequate	Adequate	Adequate

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an = 10 bn = 5 <sup>c</sup>Incomplete data

## APPENDIX A

## PART I

				L CONDITION
AND	MEAN ACT	TIVITY C	DUNTS PER	HOUR <sup>a</sup>

TIME	PR 1	PR 2	F 1	F 2	F 3	F 4	F 5	PO 1	PO 2
0900	0.64	1.05	1.26	1.39	2.16	2.64	2.25	2.81	3.28
1000	0.61	1.73	1.39	1.06	1.01	0.89	0.24	1.49	0.72
1100	0.30	0.45	0.91	0.81	0.26	0.99	0.66	0.76	0.44
1200	0.21	0.79	0.66	0.78	0.42	0.64	1.38	0.53	0.34
1300	0.49	0.98	1.00	1.12	0.79	0.72	0.79	0.42	1.02
1400	0.79	0.85	0.89	0.80	0.74	0.71	0.33	1.37	1.12
1500	0.93	0.89	0.85	0.83	1.26	1.18	0.65	0.77	1.29
1600	0.70	0.23	0.49	0.38	0.35	0.38	0.79	0.45	0.79
1700	0.38	0.94	0.63	1.01	0.49	0.33	0.84	0.45	0.39
1800	1.00	1.18	1.37	1.26	0.62	0.82	0.68	0.82	0.53
1900	1.30	0.26	0.96	0.63	1.13	0.74	1.05	0.63	0.75
2000	1.10	0.35	0.63	1.06	0.74	0.93	1.08	0.68	1.19
2100	1.33	1.24	0.66	0.97	1.07	1.10	1.04	0.82	1.08
2200	1.19	1.22	0.91	1.26	1.20	0.82	0.78	1.34	1.33
2300	1.23	0.71	1.27	1.20	1.71	0.96	1.19	1.14	1.16
2400	1.32	0.84	1.20	1.12	1.28	1.28	1.07	1.06	1.23
0100	1.13	0.97	0.63	0.71	1.08	1.50	1.19	1.33	0.72
0200	0.93	1.24	1.17	1.56	1.01	1.15	0.63	1.26	1.16
0300	1.59	1.66	0.98	1.33	1.59	0.99	1.85	1.17	1.25
0400	1.40	1.66	1.33	1.14	1.25	1.13	1.03	0.95	1.17
0500	2.06	1.23	1.21	0.90	1.17	1.49	1.54	0.91	1.03
0600	2.01	1.17	0.98	0.96	1.11	1.16	1.31	0.74	0.98
0700	0.60	1.12	0.78	1.12	0.69	0.89	0.58	0.97	0.39
0800	0.75	1.20	0.59	0.56	0.80	0.54	1.05	1.11	0.62
X	97.16	85.88	84.55	75.29	58.16	51.06	45.8	42.8	38.88

a n = 10

## APPENDIX A

## PART II

## RELATIVE ACTIVITY BY HOUR AT 500 V/m AND MEAN ACTIVITY COUNTS PER HOUR<sup>a</sup>

TIME	PR 1	PR 2	F 1	F 2	F 3	F 4	F 5	PO 1	P0 2b
0900	0.92	1.83	1.59	2.07	1.99	2.27	2.97	3.39	3.08
1000	0.69	1.37	1.22	1.33	0.93	1.05	1.21	1.07	1.71
1100	0.45	0.64	0.89	0.69	0.67	0.77	0.57	0.68	0.50
1200	0.55	0.56	1.05	0.52	1.05	0.62	0.47	0.57	0.35
1300	0.56	0.62	1.00	0.86	0.83	0.75	0.33	0.32	0.40
1400	0.45	0.75	0.83	0.73	0.40	0.91	0.82	0.77	0.91
1500	0.45	1.18	1.24	0.69	1.16	1.44	1.86	1.26	1.05
1600	0.64	0.51	0.54	0.71	0.62	0.66	0.80	0.51	0.41
1700	0.88	0.57	0.57	0.72	0.93	0.52	0.50	0.37	0.41
1800	0.73	0.46	0.75	0.96	0.81	0.61	0.57	0.78	0.52
1900	1.14	0.43	0.75	0.95	0.87	0.80	0.80	0.93	1.01
2000	1.24	0.48	0.73	0.94	0.97	0.77	1.00	1.04	1.17
2100	1.10	1.23	0.72	1.12	0.87	1.00	1.13	0.86	0.80
2200	1.20	0.91	0.89	1.06	0.82	1.17	0.76	0.94	1.00
2300	1.12	0.97	0.88	1.32	1.45	0.76	1.09	1.28	0.93
2400	1.14	1.01	1.10	1.20	1.44	1.00	0.93	1.43	0.88
0100	1.68	1.22	1.19	0.90	0.94	1.59	1.24	1.23	1.22
0200	1.58	1.67	1.19	1.00	0.79	1.09	1.00	0.94	1.16
0300	1.22	1.57	1.24	0.93	1.16	1.25	1.20	.17	0.90
0400	1.34	1.45	0.94	1.06	1.03	1.19	1.42	1.32	0.88
0500	1.35	1.24	1.30	1.31	1.02	1.32	1.56	0.73	1.04
0600	1.71	1.17	1.23	1.00	1.66	1.13	1.34	0.83	1.32
0700	1.00	0.80	1.01	0.98	0.82	0.54	0.64	0.53	1.67
0800	0.86	1.46	1.14	0.90	0.76	0.76	0.90	1.01	0.62
X	75.93	76.33	76.13	63.41	61.08	52.29	44.08	46.67	41.57

<sup>a</sup>Unless otherwise indicated, n = 9<sup>b</sup>n = 8

## APPENDIX A

## PART III

## RELATIVE ACTIVITY BY HOUR AT 1000 V/m AND MEAN ACTIVITY COUNTS PER HOUR<sup>a</sup>

TIME	PR 1b	PR 2	F 1	F 2	F 3	F 4	F 5	PO 1	PO 2
0900	0.55	1.28	1.07	2.18	2.39	2.76	3.41	3.53	2.76
1000	0.60	1.56	1.80	0.58	0.22	0.72	0.76	1.22	0.65
1100	0.88	1.27	1.09	0.40	0.89	0.95	0.47	0.57	0.24
1200	0.33	0.86	0.84	0.94	0.49	0.52	0.38	0.37	0.14
1300	0.41	1.22	1.07	0.61	0.97	1.29	0.71	0.60	0.51
1400	0.45	0.64	1.22	0.58	0.62	0.47	0.89	1.08	0.61
1500	0.39	0.91	1.35	0.67	1.18	0.81	0.86	1.10	0.84
1600	0.37	0.65	1.05	0.58	0.60	0.53	0.50	0.28	0.85
1700	0.38	0.48	0.59	0.79	0.41	0.38	0.78	0.57	0.57
1800	1.20	0.66	0.78	0.72	0.61	0.84	0.93	0.93	1.09
1900	0.46	0.51	0.75	1.00	1.12	0.89	0.62	0.89	0.55
2000	1.15	0.42	0.70	0.66	1.27	0.61	0.93	0.71	1.04
2100	1.79	1.32	0.61	1.29	0.80	0.92	1.06	0.72	1.04
2200	1.36	0.77	0.94	1.40	0.86	0.82	1.27	0.65	1.06
2300	1.22	1.32	0.95	0.98	1.19	1.44	1.01	0.89	1.18
2400	0.96	1.12	0.95	1.57	1.05	1.07	0.77	1.37	1.13
0100	1.57	1.40	0.93	1.61	1.42	0.78	0.86	0.98	1.63
0200	2.04	1.15	1.32	1.25	1.09	1.31	1.63	0.77	1.53
0300	1.52	1.58	0.82	1.04	0.61	1.13	1.38	2.06	1.45
0400	1.36	1.67	1.19	1.79	1.18	1.22	1.11	1.11	0.84
0500	1.93	1.72	1.62	1.54	1.36	1.51	1.66	1.09	1.20
0600	0.81	0.96	0.97	0.80	0.79	0.89	1.20	0.91	0.57
0700	0.95	1.21	1.23	0.61	0.76	1.01	0.42	0.63	1.16
0800	1.31	0.67	1.11	0.39	0.49	1.12	0.37	0.94	1.35
X	81.56	74.32	68.36	59.31	55.55	42.49	33.87	34.52	33.09

<sup>a</sup>Unless otherwise indicated, n = 6<sup>b</sup>n = 5

## FIGURE CAPTIONS

Figure 1. This figure shows both test units in use in the Animal Care Facility. A rat is visible in the nearest of the test compartments. Below each compartment the plumbing for the urine collection system and fraction collector for the unit in the foreground can be seen on the floor. The fibre optic guides of the motion detection system can be seen connecting to the electronics packages. The light sources are hidden by the test units. Visible between the units are the data logger and event recorder.

Figure 2. A closeup view of the individual compartments is shown. Note the complete non-metallic construction of the apparatus, including the optical components. Also note the size of the rats, when fully extended, in relation to the size of the compartments.

Figure 3. A detailed view of a fraction collector.

Figure 4. Mean number of activity counts per hour and mean number of activity counts during the 0900-1000 epoch are plotted as a function of sessions (days) subjected to intensive analysis. The error bars show standard errors, reflecting intersubject variability. The 3 parts show 0 V/m, 500 V/m and 1000 V/m conditions. Table 2 shows the sessions analyzed and their mnemonics. In this and the remaining figures, the following key applies:  $\triangle$  control;  $\blacksquare$  control partial replication;  $\square$ 50 V/m;  $\triangle$  500 V/m;  $\bigcirc$  500 V/m replication;  $\bigcirc$  1000 V/m;  $\Diamond$  1000 V/m partial replication. <u>Figure 5</u>. The coefficients of variation for daily activity and for activity in the 0900-1000 epoch are plotted as a function of sessions subjected to intensive analysis.

<u>Figure 6</u>. Covering 3 pages, this figure shows the relative hourly activity throughout the day under the 1000 V/m test. Each panel shows the circadian pattern during one of the days subjected to intensive analysis; the day, number of subjects contributing and mean activity counts/hour for the day are indicated for each panel. The broken lines indicate the late-night activity bouts graphed in Figure 8.

Figure 7. The relative activity in the 0900-1000 epoch during the control, 500 and 1000 V/m conditions are plotted against sessions subjected to intensive analysis.

Figure 8. The size, duration and time of the late-night activity bouts are shown for each of the sessions subjected to intensive analysis. The figure covers three pages, the first showing the control condition, the second the 500 V/m condition and the third the 1000 V/m condition. Each bar begins at the time the bout started and ends at the time of the conclusion of the bout. The area of each bar is proportional to the area between the relative activity curve and the mean. The bars are divided to indicate the mode of the bout.

Figure 9. The average weight of the test subjects from those conditions where data from 10 subjects were available is plotted for each week of the experiment.

Figure 10. Urine output of the test subjects from those conditions where data from 10 subjects were available is plotted for each week of the experiment.

<u>Figure 11</u>. The calcium, sodium and potassium levels in collected urine, expressed in Equivalents per volume of urine excreted per day, normalized for body weight is shown for sessions subjected to intensive analysis. Data from all experimental conditions are shown.



