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Publication Date
1984-10-01
Presented at the Twenty-third Annual Hanford Life Sciences Symposium, Richland, WA, October 2-4, 1984

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MONITORING OF CIRCADIAN WAVEFORMS IN RODENTS
EXPOSED TO HIGH-INTENSITY STATIC MAGNETIC FIELDS

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October, 1984


This work was supported by the U.S. Department of Energy under Contract DE-AC03-76SF00098.
ABSTRACT

A system has been developed for the noninvasive monitoring of circadian variables in mice exposed to a 1.50 T (1 Tesla = 10⁴ Gauss) static magnetic field. The ambient light level, temperature and relative humidity within the exposure chamber are closely regulated, and physiological monitoring systems provide simultaneous measurements of seven circadian variables: (1) climbing activity on a triangular bar, (2) migratory activity, (3) body mass, (4) respired carbon dioxide, (5) nutrient consumption, (6) urine excretion, and (7) fecal excretion. Data from the various transducers and environmental monitoring devices within the exposure system are recorded on magnetic tape at 5-min intervals throughout experiments of 50-60 days duration, and the circadian waveforms of behavioral and physiological parameters are analyzed by a modification of the cosiner method using a high-speed computer. Exposure of adult female LAF-1 mice to a 1.50-T homogeneous field for 5 continuous days, or for 10 consecutive days with intermittent daily exposures on an 8-hr-on/16-hr-off cycle, has been found to produce no significant alterations in the circadian waveforms of behavioral or physiological parameters.
The maintenance of normal circadian regulation is an important factor in assessing the response of living organisms to static and ELF magnetic fields. Although there is relatively little information on this subject, several reports in the literature suggest that weak magnetic fields may alter circadian variables. Bennett and Huguenin (1969) reported that diurnal variations in the geomagnetic field intensity may influence the light withdrawal response time of earthworms. A cancellation of the geomagnetic field by Helmholtz coils was found by Bliss and Hepner (1976) to alter the circadian activity pattern of birds when the normal light/dark cycle had been removed. Brown and Skow (1978) observed a modulation of the normal 24-hr circadian activity cycle in hamsters when a static magnetic field with a maximum intensity of 26 µT was applied in 26-hr cycles. Semm and his associates have reported that the electrical activity of rodent and avian pineal cells can be altered by artificial changes in the strength and direction of the local geomagnetic field (Semm, Schneider, and Vollrath, 1980; Semm et al., 1982; Semm, 1983), and Welker et al. (1983) have observed that the circadian variation in pineal melatonin content and serotonin-N-acetyltransferase activity is also modified by changes in the ambient magnetic field. Electrophysiological measurements by Raybourn (1983) indicate that circadian variations may exist in the sensitivity of turtle retinal cells to static magnetic fields at levels greater than 2-3 mT. A recent report by Kavaliers, Ossenkopp, and Hirst (1984) indicates that the nocturnal sensitivity of mice to morphine may be diminished when the subjects are exposed to a rotating magnetic field with an intensity ranging from 0.15 - 9.0 mT.

As part of our program to assess the response of living organisms to high-intensity, static magnetic fields, a study of the influence of these fields on circadian variations in rodent behavioral and physiological parameters has been
undertaken. In this report a description is given of an exposure chamber that was developed for parallel measurements of seven circadian variables in mice exposed to static fields up to 1.50 T. The environmental control systems within the exposure chamber, the noninvasive physiological and behavioral monitoring systems that are employed, and the data analysis procedures are described in detail. Data from an 8-week experiment during which rodents were exposed either continuously for 5 days, or intermittently for 10 days, to a 1.50-T static field are also presented.
EXPOSURE SYSTEM AND EXPERIMENTAL METHODS

General Description

A block diagram and schematic drawing of the system developed for monitoring circadian variables in mice exposed to high-intensity static magnetic fields are presented in Figs. 1 and 2. A population of 40 mice resides within a square aluminum exposure cage with a floor area of 0.37 m². The floor is constructed from cross-hatched aluminum bars with a 1-cm spacing, thereby allowing excreta to drop into a waste collecting tray below the cage. The exposure chamber lies entirely within the uniform field region of a large-volume electromagnet. The air temperature, relative humidity and magnetic field intensity within the exposure chamber are continuously regulated and recorded.

The exposure chamber contains monitoring devices for the simultaneous measurement of seven circadian variables: (a) total body mass and migratory activity of the rodent population determined from the loads registered on four strain gages that support the corners of the exposure cage; (b) climbing activity based on the loads registered on two strain gages that support the ends of a triangular exercise bar; (c) respired carbon dioxide content of the effluent air measured by an infrared analyzer; (d) rate of nutrient consumption based on the volume of liquid diet intake; (e) rate of urine and feces excretion based on a gravimetric analysis of the contents of a feces collecting screen and urine reservoir located below the cross-hatched aluminum floor of the exposure cage.

Environmental Controls

Regulation of ambient temperature at 22 ± 1 °C and relative humidity at 50 ± 10 percent is achieved by passing conditioned air from a plenum (Wedco, Silver Spring, MD) through the exposure chamber at a rate of approximately 10
air exchanges per hr. Regulation of the air temperature is achieved by a feedback control circuit which responds to a thermistor probe inserted in the air outlet port of the exposure chamber. Ambient temperature is continuously recorded by copper-constantin thermocouples inserted in the air inlet and outlet ports on the exposure chamber, and the relative humidity is recorded in the outlet port by a Hygrocon probe (Phys-Chem Research Corp., New York).

Uniform lighting throughout the exposure chamber is provided by a light pipe that serves as the roof of the lucite support frame. The light pipe consists of a 1.27-cm-thick sheet of lucite that is 1.07 m long and 0.61 m wide. The 0.37 m² area over the mouse exposure chamber was sand-blasted in order to diffuse the light. The source of white light is an array of 15 incandescent GE bulbs, each with 18 W output, that are mounted in an aluminum housing at the end of the light pipe protruding beyond the magnet gap. In order to focus the light over the 0.37 m² sand-blasted surface, the remainder of the light pipe was coated with a layer of silver foil. Complete uniformity of the light intensity over the rodent exposure chamber has been achieved by covering the sand-blasted surface of the light pipe with a countergradient filter composed of a cellulose acetate sheet sprayed with India ink. By appropriate variation of the opacity across the surface of the filter, a uniform light intensity of 155 ± 6 candles/m² was obtained over the entire exposure chamber. The incandescent bulbs at the end of the light pipe are cooled by a continuous flow of air through the lamp housing, and protected by an overtemperature regulator. Light/dark cycles are regulated automatically by a timer circuit.

**Physiological and Behavioral Monitoring Systems**

(1) **Strain Gage Measurements.** The floor of the exposure cage is supported by 4 corner strain-gage transducers that continuously monitor the variation in
body mass and the center-of-mass coordinates of the rodent population. A triangular exercise bar constructed from aluminum is located along a diagonal line of the exposure cage, and is supported on each end by a strain-gage transducer. Photographs of the cage floor, exercise bar and strain-gage transducers are shown in Fig. 3. The 6 strain gages were custom-made by NDT Consultants (Los Altos, CA) using nonmagnetic materials, and their voltage outputs are a linear function of the applied load (1 μV/g from the cage-corner strain gages and 10 μV/g from the exercise-bar strain gages). The outputs of the strain gages are amplified by $10^3$ in a bank of signal conditioners (2100 System, Vishay Inst., Malvern, PA) located external to the exposure chamber.

The center-of-mass coordinates of 40 mice within the exposure chamber are illustrated in Fig. 4. The data points represent the center-of-mass coordinates calculated at 5-min intervals from the load applied to each of the four strain gages under the cage corners. These data demonstrate a preference of the rodents for one quadrant of the exposure cage, away from the air inlet port and the 6 liquid diet feeder ports. Based on a day-to-day analysis of the average center-of-mass coordinates, this preferential location of the rodent population has been shown to be unaffected by the application of a 1.50 T static magnetic field, either continuously for 5 days or intermittently for 10 days in an 8-hr-on/16-hr-off daily cycle. Fig. 4 also demonstrates that in a rodent population entrained on a diurnal light/dark cycle, the scatter of the center-of-mass coordinates as a function of time is much greater during the dark phase. This circadian migratory activity is closely correlated with other behavioral and physiological indices, as illustrated in a later section of this paper. Quantitation of the rate of animal migration is achieved by computing the lineal distance over which the center-of-mass coordinates move during consecutive 5-min intervals.
(2) **Carbon Dioxide Analysis.** Effluent air from the chamber is pulled by a small pump through a dehumidifier and into a LIRA infrared monitor (Mine Safety Appliances Co., Pittsburgh, PA) for the measurement of carbon dioxide content. The voltage output of the infrared monitor is calibrated with standard CO$_2$/N$_2$ mixtures, and has completely linear response characteristics for carbon dioxide concentrations up to 2%.

(3) **Quantitation of Excreta.** Urine and feces are collected in a tray that is inserted along lucite guide rails beneath the cross-hatched floor of the rodent exposure cage. The waste collecting tray consists of a lucite trough overlaid by a screen made from nonmagnetic stainless steel with a 1.27-mm mesh diameter (Fig. 3). The trough is V-shaped and the sides have a 7° downward slope that facilitates drainage of urine into a reservoir along the center line of the trough. The mesh of the stainless steel screen is sufficiently small to allow collection of fecal pellets with 100% efficiency. The excreta collecting tray and waste separating screen can be rapidly removed from beneath the exposure cage and replaced with an identical unit. After removal of the waste collector, the fecal pellets on the waste separating screen are placed in a preweighed plastic envelope for the subsequent gravimetric measurement of excreted solids. The urine is collected by syringe and placed in preweighed glass vials for gravimetric analysis. The weight of collected urine is converted to units of volume using a value for the specific gravity of 1.01 g/cc, which was determined by pycnometry of mouse urine samples. The volume of collected urine is also corrected for evaporative loss, which varies as a function of relative humidity within the exposure cage (50 ± 10 percent). Based on studies of the rate of water evaporation from the waste collecting tray as a function of relative humidity over the range 40 - 60 percent, empirical formulae have been derived by regression analysis to correct urine...
volumes for evaporative loss over 3-hr and 24-hr collection intervals. For 3-hr intervals used in circadian waveform measurements, the percent evaporation is related to the percent relative humidity (R.H.) by the formula \( r^2 = 0.98 \):

\[
\log_e(\% \text{ evaporation}) = 9.803 - 1.667 \cdot \log_e(\% \text{ R.H.})
\]  

(1)

For 24-hr daily collection intervals, evaporative loss is described by the formula \( r^2 = 0.96 \):

\[
\log_e(\% \text{ evaporation}) = 10.996 - 1.799 \cdot \log_e(\% \text{ R.H.})
\]  

(2)

The temperature within the exposure chamber is regulated at 22 ± 1 °C, and no correction is required for evaporative urine loss as a function of temperature.

Following gravimetric analysis, 50 units/ml penicillin and 50 μg/ml streptomycin (Gibco, Grand Island, NY) are added to the collected urine samples, which are then stored at -20 °C. Chemical analysis of selected urine samples has been performed by The Pathology Institute (Berkeley, CA) using standard clinical procedures.

(4) Quantitation of Nutrient Consumption. From preliminary studies with a conventional metabolic cage, it became apparent that the collected excreta could become significantly contaminated with fragments of pellet food supplied to the rodents from a hopper. In order to circumvent this problem, the nutritional and water requirements of the rodent population have been met by providing them with ad libitum access to a complete liquid diet (Gibco, Grand Island, NY). The liquid diet is supplied to the exposure chamber from a 250 ml bottle and 6 lines of PVC tubing. Each tube is terminated by a springloaded nozzle to prevent dripping of the liquid (Fig. 3). Six brass guides position the nozzles along one wall of the exposure chamber. All of the components of the liquid diet feeder assembly are autoclaved at the initiation of an
experiment to ensure antiseptic conditions. The bottle that supplies the liquid diet is connected to the 6 PVC tubes via a manifold and a quick-disconnect valve. During the course of an experiment, the bottle is removed once a day for addition of new liquid diet, and is replaced by a freshly sterilized bottle at 2-3 day intervals. The consumption of liquid diet by the rodent population is recorded using volumetric marks on the side of the supply bottle.

(5) Data Acquisition and Recording. Analog outputs from the various physiological and environmental monitoring systems are monitored continuously and recorded at 5-min intervals on an Autodata Nine 100-channel data acquisition unit (Acurex, Mountain View, CA). The Autodata Nine unit is serially linked to a Silent 700 ASR terminal (Texas Instruments, Dallas, TX), which transfers data onto magnetic tape for subsequent analysis on a CDC 7600/6600 computer system (Control Data Corp., Minneapolis, MN).

Performance of Environmental Monitors and Transducer Systems

Prior to the initiation of each experiment, the performance of the physiological and environmental monitoring systems are evaluated for a period of several days as a test of accuracy and stability. The results of tests during a 24-hr monitoring interval are shown in Fig. 5. Both the temperature and relative humidity within the exposure chamber exhibit low-amplitude fluctuations that reflect the feedback regulation of the temperature of the incoming air supplied from the plenum. The average carbon dioxide content of the air varies from 0.03 - 0.04 percent, with a mean value of 0.035 percent. The strain-gage transducers that support the cage floor and the exercise bar exhibit excellent long-term stability, both in an unloaded state as shown in Fig. 5 and under loads applied by calibrated brass weights. Data recorded from the various environmental monitors are routinely fit to a cosine curve (see
Fig. 5) as a test for diurnal variations that might influence rodent behavior and other physiological indices. In general, the low-amplitude daily oscillations in temperature and relative humidity have been found to exhibit an irregular pattern that is not correlated with the circadian oscillations in rodent behavioral and physiological parameters.

Magnetic Field Exposures

The exposure cage is placed within the gap of an iron-core DC electromagnet that achieves a maximum vertical field strength of 1.6 T. The flat 73.7 cm x 82.6 cm magnet pole faces are aligned horizontally, with a vertical separation of 19.4 cm. The operating characteristics of this electromagnet have been described in previous publications (Tenforde et al., 1983; Davis et al., 1984). Based on measurements with a search coil and a transverse Hall-effect probe (F. W. Bell Co., Orlando, FL), the magnetic flux density is uniform to within 0.1 percent throughout the volume of the rodent exposure chamber. Continuous recording of the magnetic field strength during an experiment is made with a Hall probe located at the geometric center of the exposure cage. The probe is covered by the triangular exercise bar for protection against rodent molestation (Fig. 3).

Circadian Waveforms and Computer Analysis Techniques

Circadian waveforms are characterized by a modification of the time-series analysis technique described by Halberg et al. (1972). Each of the several circadian variables under study is fit by a least-squares technique to a function of the form:

$$ Y(t) = Y_0 + \tilde{Y} \cdot \cos(\omega t + \phi) $$

where $Y(t)$ is the value of the circadian variable at time $t$. $Y_0$, $\tilde{Y}$, $\omega$ and $\phi$ are, respectively, the level, amplitude, angular frequency, and acrophase of
the cosine function. The angular frequency is equal to 360° divided by the period of the oscillation. The acrophase represents the phase angle at which the cosine function reaches a maximum value. The acrophase can be expressed in clock hours by multiplying the phase angle by (24 hr/360°).

In the time-series analysis technique described by Halberg et al. (1972), the period is assigned a value of 24 hr and a least-squares analysis technique is used to obtain the best-fit values of the remaining three variables. This technique cannot be used for the analysis of nonstationary circadian states in which the period varies during the course of an experiment. A computer code has therefore been developed in which the assigned period is incremented in 15-min steps from 10 to 40 hr, and the best-fit values for $Y_0$, $\dot{Y}$ and $\phi$ are determined by regression analysis at each value of the assigned period. The value of the assigned period that produces a minimum sum of residuals between the raw data and the fitted cosine curve is considered to be the true period of the circadian oscillation. The 95% confidence intervals of the best-fit amplitude and acrophase are calculated following the method described by Halberg et al. (1972).

Examples of circadian waveforms in respired carbon dioxide, body mass, climbing activity registered on the triangular exercise bar, and locomotor activity determined from changes in the center-of-mass coordinates as a function of time are shown for a 5-day interval in Fig. 6. The population of 40 mice from which these data were obtained had been placed in a free-running circadian state by maintaining constant illumination within the exposure chamber, under which condition the best-fit circadian periods are typically 25 - 26 hr. When entrained on a daily light/dark cycle, the best-fit periods of these circadian variables decrease to 24 ± 0.5 hr, as illustrated by data presented in a later section of this paper. Fig. 7 shows circadian waveforms
in liquid diet consumption and excreta measured over a two-day interval for a group of 40 mice entrained on a 12/12 light/dark cycle. The circadian waveforms of these parameters were fit to data recorded at consecutive 3-hr intervals throughout a 39-hr monitoring session, and exhibit large-amplitude oscillations with periods close to 24 hr.

EXPERIMENTAL RESULTS

Following completion of the exposure chamber, several pilot tests with durations up to two weeks were carried out to analyze the long-term transducer performance and animal adaptation to the chamber environment. A series of three experiments, each of two months duration, was then undertaken to assess behavioral and physiological variables in rodent populations exposed to a 1.50 T static magnetic field. The first two of these experiments were conducted with mice entrained on a 12/12 light/dark cycle, and the third was carried out with rodents maintained in a free-running circadian state by constant dim illumination. The first of these three experiments will be described in detail as an illustration of the physiological and behavioral data obtained with the exposure system.

Experimental Design

Experimental subjects were female LAF-1 mice from the Jackson Laboratory (Bar Harbor, ME). The mice were 16 weeks old at the initiation of the experiment, and were entrained on a 12/12 light/dark cycle with the light phase from 06:00 - 18:00 daily. Three separate groups of mice were randomly selected from the lot: (A) 40 mice were placed in the exposure chamber; (B) 10 mice were placed in a conventional plastic cage with a wire top, and fed liquid diet nutrient ad libitum; (C) 10 mice were placed in a conventional plastic cage with a wire top, and fed pellet food and water ad libitum. The primary purpose
of control groups (B) and (C) was to assess the influence, if any, of sustaining the experimental subjects with liquid diet for a two-month period. The cages for groups (B) and (C) were placed in the magnet facility at a distance of approximately 0.6 m from one corner of the electromagnet. The stray magnetic field at the location of the control cages was 0.2 mT when the magnet was energized to a 1.50 T level.

Following an initial 3-day period in which the rodents acclimated to the exposure chamber environment, the baseline circadian parameters of the 40 experimental subjects were monitored for 8 days (designated as Control Period No. 1). The mice were then subjected continuously for 5 days to a 1.50 T static field, followed by an 18-day control monitoring period (Control Period No. 2) to assess the occurrence of any delayed effects subsequent to the magnetic field exposure. The mice were next subjected for 10 consecutive days to an intermittently applied 1.50 T field in an 8-hr-on/16-hr-off daily cycle, followed by a 13-day control monitoring period (Control Period No. 3).

Measurements at 5-min intervals of respired carbon dioxide, body mass, climbing activity, and migratory activity were made continuously throughout the experiment by the automated techniques described above. Measurements of circadian variations in nutrient consumption, urine excretion and fecal excretion were made at consecutive 3-hr intervals over a 39-hr monitoring period in the latter half of each of the 5 segments of the experiment. In parallel with these measurements on the experimental rodent population, recordings were also made during each of the five 39-hr monitoring sessions of the rates of liquid diet, pellet food and water consumption by the two control rodent populations [groups (B) and (C)].

**Circadian Variables in Exposed and Nonexposed Rodent Populations**

The results of time-series analyses across the five segments of the
experiment are shown in Fig. 8 for the four continuously recorded circadian variables. Based on a nonparametric Wilcoxon U-test (van der Waerden, 1969), none of the four circadian parameters showed significant shifts at the $p < 0.05$ level when analyzed across consecutive segments of the experiment. One apparent exception was the change in body mass circadian parameters between Control Period No. 1 and the 5-day magnetic field exposure that followed ($p = 0.015$). From a day-to-day analysis of the best-fit circadian parameters across these two segments of the experiment, it was evident that the change in body mass data distribution was primarily associated with a rapid weight gain of 3.4 percent during the first 4 days of Control Period No. 1. When the second half (4 days) of Control Period No. 1 was compared with the subsequent 5-day magnetic field exposure period, the difference in body mass data was not significant ($p > 0.25$). This result indicates that the change in body mass circadian data was not associated with the magnetic field exposure per se, but most likely reflected an adaptation to the liquid diet nutrient. The conclusion is further supported by the finding discussed below of a significant increase in body mass over the same period of time by the mice in control group (B), which were also maintained on liquid diet nutrient.

Circadian variables for nutrient consumption and excreta are shown in Fig. 9. Based on a Wilcoxon nonparametric analysis of the data distributions between consecutive segments of the experiment, none of these three circadian parameters showed significant changes as a function of "field on" versus "field off" conditions. Measurements were also made of circadian variation in liquid diet consumption by control group (B), and in pellet food and water consumption by control group (C). None of these circadian variables showed significant changes at the level $p < 0.05$ during the course of the experiment.

In addition to measurements of nutrient consumption and excreta in the
experimental rodent population during the five 39-hr monitoring sessions, daily measurements were made of these parameters throughout the course of the experiment (Fig. 10). Based on a Student t-test of differences between consecutive segments of the experiment, there were no significant changes in these variables associated with exposure to a 1.50 T static field. However, the excreted mass of fecal solids showed a progressive increase during the course of the experiment, and the average daily fecal excreta during Control Period No. 3 was 18.9 percent greater than the average value during Control Period No. 1. This difference was statistically significant based on a Student t-test (p < 0.001), and may be an effect associated with prolonged maintenance on a liquid diet nutrient.

Daily measurements were also made of liquid diet, pellet food, and water consumption by the mice in control groups (B) and (C). In addition, the body masses of these rodents were measured on a pan balance at 3-4 day intervals throughout the experiment. These data are presented in Fig. 11. Based on a Student t-test of data obtained during consecutive weeks of the experiment, none of these 5 parameters exhibited a change with the exception of the average body mass of rodents in control group (B), which were fed liquid diet. Similar to the experimental rodents maintained on liquid diet in the exposure chamber, the rodents in control group (B) exhibited a significant 2.4 percent increase in average body mass during the first week of the experiment (p = 0.03). These control mice also showed a qualitative trend towards increasing weight throughout the 8 weeks of the experiment, although the consecutive week-to-week changes were not statistically significant after the first week.

**Analysis of Organ and Tissue Parameters**

The pooled urine samples from each of the five 39-hr circadian monitoring sessions were subjected to routine clinical chemistry analysis. The
concentration ratios of 5 major urine components during "field on" versus "field off" conditions were: electrolytes (Na⁺, K⁺, Cl⁻): 0.95; protein: 1.02; creatinine: 0.94; urea: 1.04; 17-OH ketosteroid: 0.91. As compared to unity, all of these ratios are within the variability of the values determined for the 3 urine samples collected during Control Periods No. 1, 2, and 3, and indicate the absence of any significant magnetic field effect.

Upon termination of the experiment, blood samples were drawn by cardiac puncture from 10 mice in the experimental group and 5 mice each in control groups (B) and (C). The hematologic parameters for these 3 groups of mice are summarized in Table 1. Based on a Student t-test, none of the blood parameters differed at the p < 0.05 level between the experimental mice and the group (B) control mice that were fed liquid diet. The average hematocrit of the experimental mice was significantly higher than that of the group (C) control mice that were fed pellet food and water (p = 0.02). However, the larger average hematocrit of the experimental mice was not paralleled by a statistically significant difference in RBC concentration, hemoglobin, mean corpuscular volume, or mean corpuscular hemoglobin content. This finding suggests that the small, but significant, difference in hematocrit may have been a consequence of variability in the measurement technique or the small sample sizes used in this analysis. The percentage of lymphocytes also differed significantly (p = 0.04) between the experimental group and the control group (C) mice. It should be noted, however, that the average percentage of lymphocytes was lower in the experimental group, whereas the total WBC concentration was higher for this group than the average value determined for the group (C) control mice. Because lymphocytes are the major component of the total WBC population, this observation again suggests that the significantly lower concentration of lymphocytes in the experimental group
relative to the control group (C) mice may have resulted from measurement variability or the small sample sizes used in this study.

Three groups of mice were also analyzed for organ weights upon termination of the experiment. These data are presented in Table 2, and demonstrate that with one exception there were no significant differences in the average organ weights between the experimental mice and the mice in control groups (B) and (C). The exception was a significantly lower average weight of the adrenals in the experimental mice relative to the mice in control group (C) that were fed pellet food and water throughout the experiment. Because the measurement accuracy of the Sartorius balance (Brinkmann, Westbury, NY) used for the determination of organ weights was estimated to be ± 0.5 mg, the 0.7 mg difference in average adrenal weights between these two groups may be a consequence of measurement variability rather than a true biological difference. After excision and weighing, all of the organs and sections of femoral bone marrow and jejunum from each animal were fixed in Bouins, dehydrated in a graded series of alcohols, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin for histological examination. No microscopic lesions were observed in the organs from the three groups of rodents that were suggestive of infection or degenerative changes.
DISCUSSION

The development of an exposure chamber with completely noninvasive monitoring techniques has made possible the simultaneous analysis of seven circadian variables in rodent populations exposed to a high-intensity, static magnetic field over prolonged time intervals. Because of the long-term stability of the environmental controls and the various physiological monitoring systems, the rodent population within the exposure chamber can serve as its own control for the analysis of changes in circadian variables over prolonged periods of time and as a function of "field on" versus "field off" conditions. Data from the eight-week experiment described in this paper indicate that no consistent alterations occur in behavioral and physiological circadian variables in response to the application of a uniform 1.50 T field. This lack of responsiveness to the field was observed both with a continuous exposure for 5 days, and with intermittent exposures in an 8/16 daily cycle during 10 consecutive days. Various clinical measures of organ and tissue parameters also revealed no significant alterations in the exposed group of rodents as compared with nonexposed control animals.
ACKNOWLEDGMENTS

Research support was received from the Office of Energy Research, Health and Environment Research Division, of the Department of Energy under Contract No. DE-AC03-76SF00098 with the Lawrence Berkeley Laboratory. The authors gratefully acknowledge the skillful engineering and shop support provided by P. Dowling, F. Upham, C. Dols, P. Banchero, R. Hall, and R. Armer. We also thank Dr. S. Ebbe and the members of the Donner Clinic staff, P. Garbutt, D. Carpenter and C. Allan, for carrying out the hematology measurements. V. Havens provided skillful assistance with histology sections, and histopathology assessments were kindly performed by Dr. M. R. Culbertson of the Laboratory for Energy-Related Health Research, University of California at Davis.
REFERENCES


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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exposed Group (N = 10)</th>
<th>Liquid Diet Control Group (N = 5)</th>
<th>Pellet Food + Water Control Group (N = 5)</th>
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<td>RBC, x10^6/mm³</td>
<td>8.44 ± 0.12</td>
<td>8.38 ± 0.14</td>
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<td>Hemoglobin, g/dl</td>
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<td>Hematocrit, %</td>
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<td>Segmented, %</td>
<td>18.1 ± 2.9</td>
<td>17.8 ± 3.2</td>
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* All values are given as the mean ± S.E.

# Compared to the pellet food + water control group, p = 0.02.

+ Compared to the pellet food + water control group, p = 0.04.
<table>
<thead>
<tr>
<th>Organ</th>
<th>Liquid Diet (N = 5)</th>
<th>Pellet Food + Water (N = 5)</th>
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<td>5.2 ± 0.2</td>
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<td>460 ± 11</td>
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<td>Liver</td>
<td>1141 ± 91</td>
<td>1180 ± 47</td>
</tr>
<tr>
<td>Lung</td>
<td>160 ± 4</td>
<td>149 ± 6</td>
</tr>
<tr>
<td>Spleen</td>
<td>63 ± 2</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>Stomach (empty)</td>
<td>212 ± 14</td>
<td>240 ± 24</td>
</tr>
</tbody>
</table>

* Organ weights (mg) are given as the mean ± S.E. The average body masses (g) of the three groups at the time of autopsy were: (1) exposed mice: 25.66 ± 0.8; (2) liquid diet control mice: 26.25 ± 1.03; (3) pellet food + water control mice: 25.25 ± 0.68.

# Compared to the pellet food + water control group, p = 0.03.
FIGURE LEGENDS

Fig. 1. Block diagram of magnetic field exposure system and circadian variables measured by noninvasive techniques.

Fig. 2. Schematic drawing of magnetic field exposure system.

Fig. 3. Photographs of exposure chamber (with light pipe roof removed) and various accessories used for physiological monitoring.

Fig. 4. Center-of-mass coordinates recorded at 5-min intervals during an entire day and during the 12-hr dark and light cycles. The center of the ellipse represents the average value of the center-of-mass coordinates \((X_c, Y_c)\), and the semi-minor and semi-major axes represent one standard deviation of the mean values for the \(X_c\) and \(Y_c\) coordinates. The coordinates given at the four corners represent the actual dimensions of the exposure cage expressed in units of feet.

Fig. 5. One day of data recorded at 5-min intervals is shown for various environmental monitors and transducer system with no experimental subjects in the exposure chamber. The solid curves represent best-fit cosine curves and 95% confidence intervals (see text for explanation).

Fig. 6. Circadian waveforms of respired carbon dioxide, body mass, climbing activity and migratory rate are shown over 5 serial days for a population of 40 LAF-1 mice maintained in a free-running circadian state by exposure to constant dim illumination within the exposure chamber.

Fig. 7. Circadian waveforms in liquid diet consumption, urine excretion, and fecal excretion are shown for a population of 40 LAF-1 mice maintained on a 12-hr-light/12-hr-dark cycle within the exposure chamber.

Fig. 8. The results of a time-series analysis of four circadian variables are shown during the five segments of an experiment in which mice were
subjected to a 1.50 T static field, continuously for 5 days and intermittently in daily 8-hr-on/16-hr-off cycles for 10 days. The average values of the respired carbon dioxide in the effluent air have been corrected for the ambient level determined from pre-experiment monitoring (0.04%).

Fig. 9. Results of a time-series analysis of circadian variations in nutrient consumption and excreta in a rodent population subjected to a 1.50 T static field, continuously for 5 days and intermittently in daily 8-hr-on/16-hr-off cycles for 10 days. The best-fit circadian parameters are plotted at the midpoints of the five 39-hr monitoring sessions that were conducted during the course of the experiment.

Fig. 10. Average daily values of nutrient consumption and excreta are shown for the five segments of an experiment in which 40 LAF-1 mice were subjected to a 1.50 T static field, continuously for 5 days and intermittently in daily 8-hr-on/16-hr-off cycles for 10 days.

Fig. 11. Average values of body mass and daily nutrient and water consumption are shown for two control populations of LAF-1 mice monitored over an 8-week experimental period.
CIRCADIAN RHYTHM MEASUREMENTS

ENVIRONMENTAL CONTROLS AND MONITORS
- Continuous flow of conditioned air
- Feedback regulation of inlet air temperature
- Thermocouple measurements of inlet and outlet air temperatures
- Relative humidity probe
- Magnetic field Hall-effect probe

PHYSIOLOGICAL AND BEHAVIORAL MEASUREMENTS
- 4 strain gages under cage floor corners
- 2 strain gages under exercise bar
- Infrared carbon dioxide monitor
- Volumetric analysis of liquid diet intake
- Gravimetric analysis of urine and feces

DATA RECORDING AND ANALYSIS
- Data acquisition unit with interval timer
- Terminal with printer and magnetic tape drive
- High-speed computer for analysis of circadian waveforms

CIRCADIAN VARIABLES
- Climbing activity (exercise bar)
- Migratory activity (center-of-mass variation per unit time)
- Body mass
- Respired carbon dioxide
- Nutrient consumption rate
- Urine output
- Feces output

FIGURE 1
Area of Uniform Field Intensity
[61 cm x 61 cm x 19.4 cm (ht.)]

1.5 Tesla Electromagnet

Exposure Cage

Humidity Probe and Thermocouple

Air Conditioner (Temperature and Humidity Control, Forced Draft Fan)

FIGURE 2
A) Exposure Cage and Support Frame

B) Triangular Exercise Bar Support Frame With Strain Gages and Hall Probe

C) Cage Corner Strain Gage

D) Exercise Bar Strain Gage

E) Urine Collecting Tray and Feces Separating Screen

F) Spring-loaded Nozzle Tip of Liquid Diet Feeder Line

FIGURE 3
Center-of-Mass Coordinates of Rodent Population
Entrained on a 12-Hour-Light/12-Hour Dark Cycle

FIGURE 4
Performance Tests on Transducers and Environmental Monitors

Air Temperature
Average = 22.16 °C

Relative Humidity
Average = 57.16%

Carbon Dioxide Content of Effluent Air
Average = 0.035%

Cage Floor Strain Gages
Average = 1.03 g

Triangular Exercise Bar Strain Gages
Average = 0.08 g

FIGURE 5
Circadian Waveforms (Free-Running State)

A. Carbon Dioxide Content of Effluent Air
- Average = 0.0714%
- Amplitude = 0.006%
- Period = 25.6 h

B. Total Body Mass of Rodent Population
- Average = 1071.8 g
- Amplitude = 15.1 g
- Period = 24.75 h

C. Climbing Activity Based on Load Applied to Triangular Exercise Bar
- Average = 0.26 g
- Amplitude = 0.24 g
- Period = 26.76 h

D. Migration Rate Based on Center-of-Mass Displacement Per Unit Time Interval
- Average = 2.56 cm/5 min
- Amplitude = 0.78 cm/5 min
- Period = 25.0 h

FIGURE 6
Circadian Waveforms of Nutrient Consumption and Excreta
(40 Female LAF-1 Mice Per Group)

(A) Liquid Diet Consumption

Output (ml per 3-h interval)

Average Per 3-h Interval: 30.47 ml
Amplitude: 17.42 ml
95% C.I. = 12.35–22.48 ml
Acrophase: 20.43 h
95% C.I. = 19.13–21.74 h
Period: 23.5 h

(B) Urine Excretion

Output (ml per 3-h interval)

Average Per 3-h Interval: 30.47 ml
Amplitude: 17.42 ml
95% C.I. = 12.35–22.48 ml
Acrophase: 20.43 h
95% C.I. = 19.13–21.74 h
Period: 23.5 h

(C) Fecal Excretion

Output (grams per 3-h interval)

Average Per 3-h Interval: 0.94 g
Amplitude: 0.78 g
95% C.I. = 0.37–1.15
Acrophase: 20.10 h
95% C.I. = 18.06–22.17 h
Period = 24.0 h

FIGURE 7
Circadian Variables in Exposed Rodent Population
(40 Female LAF-1 Mice)

- Control Period No.1 (8 Days)
- Continuous B = 1.5 Tesla (5 Days)
- Control Period No.2 (18 Days)
- B = 1.5 Tesla, 00:00-08:00 Daily (10 Days)
- Control Period No.3 (13 Days)

(A) Respired Carbon Dioxide

- Period (hours): 00:00-08:00
- Average (percent) (± 1 S.D.): 0.08 ± 0.05, 0.04 ± 0.03, 0.02 ± 0.02
- Amplitude (percent) (± 95% C.L.): 0.015 ± 0.010, 0.005 ± 0.005
- Acrophase (Clock Hours): 18:00

(B) Total Body Mass

- Period (hours): 00:00-08:00
- Average (grams) (± 1 S.D.): 1015 ± 1055, 1000 ± 975, 975 ± 950
- Amplitude (grams) (± 95% C.L.): 40 ± 30, 20 ± 10, 10 ± 0

(C) Climbing Activity

- Period (hours): 00:00-08:00
- Average Load (grams) (± 1 S.D.): 1.5 ± 1.0, 1.0 ± 0.5, 0.5 ± 0.0
- Amplitude (grams) (± 95% C.L.): 7.5 ± 5.0, 5.0 ± 2.5, 2.5 ± 0.0
- Acrophase (Clock Hours): 18:00

(D) Migration Rate

- Period (hours): 00:00-08:00
- Average (cm/min) (± 1 S.D.): 4 ± 3, 3 ± 2, 2 ± 1
- Amplitude (cm/min) (± 95% C.L.): 1.5 ± 1.0, 1.0 ± 0.5, 0.5 ± 0.0
- Acrophase (Clock Hours): 18:00

FIGURE 8
Circadian Variables In Exposed Rodent Population
(40 Female LAF-1 Mice)

(A) Liquid Diet Consumption

<table>
<thead>
<tr>
<th>Calendar Days</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (hours)</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- ○ Control Period No.1 (8 Days)
- ● Continuous B = 1.5 Tesla (5 Days)
- □ Control Period No.2 (18 Days)
- □ B = 1.5 Tesla, 00:00-08:00 Daily (10 Days)
- △ Control Period No.3 (13 Days)

Average (ml) Per 3-h Interval (±1 S.D.)

Amplitude (ml) (±95% C.I.)

06:00 - 24:00

Dark Phase

18:00

Light Phase

06:00

24:00

ACrophase

[Clock Hours] 18:00

(±95% C.I.)

12:00

06:00

(B) Urine Excretion

<table>
<thead>
<tr>
<th>Calendar Days</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (hours)</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average (ml) Per 3-h Interval (±1 S.D.)

Amplitude (ml) (±95% C.I.)

06:00 - 24:00

Dark Phase

18:00

Light Phase

06:00

24:00

ACrophase

[Clock Hours] 18:00

(±95% C.I.)

12:00

06:00

(C) Fecal Excretion

<table>
<thead>
<tr>
<th>Calendar Days</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (hours)</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average (grams) Per 3-h Interval (±1 S.D.)

Amplitude (grams) (±95% C.I.)

06:00 - 24:00

Dark Phase

18:00

Light Phase

06:00

24:00

Acrophase

[Clock Hours] 18:00

(±95% C.I.)

12:00

06:00

FIGURE 9
Daily Nutrient Consumption and Excreta in Exposed Rodent Population (40 Female LAF-1 Mice)

Calendar Days

0 10 20 30 40 50 60

Daily Liquid Diet Consumption (ml) (± 1 S.D.)

Daily Urine Excretion (ml) (± 1 S.D.)

Daily Fecal Excretion (grams) (± 1 S.D.)

- Control Period No.1 (8 Days)
- Continuous B = 1.5 Tesla (5 Days)
- Control Period No.2 (18 Days)
- B = 1.5 Tesla, 00:00-08:00 Daily (10 Days)
- Control Period No.3 (13 Days)

FIGURE 10
Body Mass and Nutrient Consumption in Control Rodent Populations (10 Female LAF-1 Mice Per Cage)

Calendar Days

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
</table>

Daily Liquid Diet Consumption (ml) (± 1 S.D.)
- 120
- 110
- 100
- 90
- 80

Body Mass (grams) (± 1 S.D.)
- 27
- 26
- 25
- 24

Daily Water Consumption (ml) (± 1 S.D.)
- 70
- 60
- 50
- 40
- 30

Daily Pellet Food Consumption (grams) (± 1 S.D.)
- 40
- 35
- 30
- 25
- 20

Body Mass (grams) (± 1 S.D.)
- 26
- 25
- 24
- 23

FIGURE 11
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