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A Digital Ultrasound Recorder for Rat Stress Analysis

by

© R. B. Paranjape

A THESIS
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Abstract

An experimental technique is proposed which may quantify behavioral effects resulting from chronic, low-level, microwave radiation of rodents. Rats, for example, make spontaneous calls indicative of their behavioral patterns. From these calls it may be possible to recognize either the thermal or athermal (stress) effects of exposure to electromagnetic radiation. A microprocessor-based, data collection system is developed which will monitor these ultrasonic calls in the frequency range 10 kHz - 90 kHz.

Data from vocalizations are analysed using recognized statistical methods. The technique is applicable to any behavioral experimentation involving rodents. In this work it is used specifically for the analysis of thermal stress in rat pups. It is a positive step towards determining whether or not the neurasthenic syndrome results from low-level, electromagnetic radiation. The data collection system is used to monitor the vocalizations of rat pups when exposed first to a cold environment and then warmed in a microwave field (6.17 mW/cm^2 at 2450 MHz). Vocalizations are compared to those of pups which are subjected just to a thermal (cold) stress. Statistically significant differences are observed ($p \leq 0.005$).

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I. Introduction

The biological effects of exposure to electromagnetic (EM energy are a source of both public and scientific concern. Justesen (1979) when speaking for the IEEE's Committee on Man and Radiation stated:

> " Whatever the loss or gain, it is the net advantage or disadvantage that must be weighed. The potential trade-offs associated with man-made emitters of electromagnetic energy are therefore legitimately associated with concern for the safety of those who make and those who use the products of radiofrequency technology."

In practice, exposure to EM radiation can be divided into two classes: short-term exposure to high-intensity fields and long-term exposure to low-intensity fields. High-intensity radiation can be defined in terms of power density, dosimetry, and specific absorption rate. Low-intensity, chronic radiation is, on the other hand, somewhat more difficult to quantify. The conditions of exposure often change with the time and position of the experimental subjects.

The responses of living organisms to these different irradiation regimes are divergent. Whereas effects of high-intensity radiation are normally defined in terms of physiological function, the effects of low-intensity, chronic radiation are more subjective and indeterminate (Baranski and Czerski, 1976; Livshits, 1958). The actual biological effects have been classified into two major categories: thermal and nonthermal. When EM energy is absorbed by a living organism, the effects are defined as being thermal if the temperature rise is significant (≤ 0.1 °C). The whole organism (or a major portion of it) participates in the heat-transfer process. The organism reacts to heat as a direct stimulus. The capacity of microwave radiation to produce a measurable temperature increase in tissue is well-known. The thermal effects of radiation are well defined (Baille et al., 1969; D'Andrea et al., 1975, 1977; Frey and Feld, 1975; Michaelson, 1969).

Nonthermal effects, on the other hand, are not explained as reactions to direct heating. They can occur when the electric field or the magnetic field of the incident EM radiation interacts directly on the molecular or macroscopic level with the irradiated tissue (Schwan, 1969). Nonthermal effects are difficult to identify. They often occur only in certain frequency regions and usually exhibit saturation at low intensities. It has been suggested that thermal effects may drown-out any evidence of nonthermal effects (Fröhlich, 1980).

Nonthermal effects have been reported in the areas of neural, cardiovascular, blood, and endocrine function. The effects of chronic, low-intensity radiation may be very subtle, affecting general mental and physical health and longevity (Moe et al., 1975). It is also speculated that long-term exposure may be mutagenic, affecting future generations (Healer, 1969). Justesen (1979), in a review of the behavioral and psychological effects of electromagnetic radiation, states:

> "Microwaves and other radiofrequency radiations of the electromagnetic spectrum can have highly predictable effects on behavior at modest and even low-levels of irradiation. Introduction of weak fields into sensitive tissue promotes bona fide physiological reactions that give rise to changes in behavior."

One established nonthermal effect is the ability of irradiated subjects to detect particular kinds of incident radiation fields. Specifically, subjects are able to hear "clicking" and "popping" sounds when radiated by pulsed EM fields. This effect was first studied by Frey (1961). It is thought to result from a sudden expansion of tissues in the head. Yet, the power density of this pulsed field can be of an order such that it produces only 10^{-5} °C temperature elevation in the subject. It is speculated that the effect may be due to a change in density which results from the sudden expansion of tissue. The shock wave which follows is detected by the inner ear (Chou and Guy, 1975). This reaction is defined as the result of microthermal stimulation. It would not be possible to apply any direct heating technique to synthesize this effect.

Neurasthenia, a reversible syndrome akin to mild depression, has been attributed to weak microwave fields but an etiological connection has yet to be demonstrated (Justesen, 1979). Some of the symptoms of neurasthenia are: irritability, headache, lethargy, insomnia, impotence, and loss of libido. These and similar complaints have been made by individuals exposed to weak radiation fields.

Nonthermal effects in the behavioral and psychological realm are difficult to define (Justesen and King, 1969). Behavioral experimentation in particular can be easily biased because of experimental technique. There are many variables that must be considered before good experimental technique can be ascertained.

Data describing human response to EM radiation come almost exclusively from clinical studies of accidentally or inadvertently exposed individuals. The conditions of their exposure cannot be precisely assessed and their numbers are not sufficiently large to carry out proper epidemiological studies.

Since any procedure involving irradiation of human subjects at any level is unethical, experimental exposures of small animals must be used. Behavioral and psychological reactions of rodents are often manifested in terms of ultrasonic vocalization. It may therefore be possible to model the effects of low-level, microwave radiation on man using rodents as experimental subjects (Voss, 1979; Voss _et al._, 1980).

Chronic microwave irradiation can be considered as a parallel to chronic exposure to stress. Emotional and psychosocial stress can, through chemical imbalance, result in injury to elements of the immunological apparatus of the body. This leaves the irradiated subject vulnerable to viral and other incipient pathological processes normally held in check by the body (Riley, _et al._, 1976). Behavioral and psychological reactions to radiation provide a means to monitor this stress. Thus, vocalizations and the changes in them can be the basis of modelling stress.

Vocalizations can be quantified and then analysed using statistical methods. This technique allows systematic replication of experiments and, in essence, the conformation or rebuttal of the neurasthenic effect of microwaves in rodents. Monitoring ultrasonic vocalizations is attractive because it is a remote sensing technique and it perturbs neither the rodents nor the microwave field. Further, a wide variety of situations (maternal (Noirot, 1965); aggression and submission (Sales ,1972), sexual (Barfield and Geyer, 1972)) are accompanied by vocalizations. Table 1 summarizes many of the calls of Wistar and Long Evans rats and the circumstances in which they are detected.

The technique of monitoring vocalizations requires the use of datalogging equipment able to pick up the characteristic features of rodent ultrasound. These features include number of calls, frequency, amplitude and duration of calls. This information, correlated with exposure time and power level, should provide sufficient data to quantify behavioral and physiological response. For prolonged experimentation, a steady stream of this information would soon exceed the capacity of any conventional ultrasound datalogging equipment.

The analysis of murine vocalization has been difficult because satisfactory broad-band ultrasound detecting devices

TABLE 1 - Ultrasonic Vocalizations

STRAIN	FREQ. RANGE in kHz	TYPICAL DURATION in ms	RATE OF CALLING	CAUSE	NOTES
WISTAR	0-90	5-65	Not given.	Isolation from nest.	Pups 4-16 days. Lower frequency if pups>16 days. Sewell (1968)
WISTAR	35-55	Not given.	Calls per minute = 48,45,25.	Lower temp. 3, 12, 22 °C	Pups 0-22 days. Homiothermy is developing. Okon (1971)
WISTAR	22-30	700-1000	Many calls per minute.	Submission to strong male.	Male rats. Inhibits aggression. Sales (1972)
WISTAR	40-70	3-65	Many calls per minute.	Domination of weak male.	Male rats. Indicate dominance. Sales (1972)
WISTAR	30-56 34-120	100-800 2-40	Many calls per minute. Very sudden.	Heterosexual activity. Genital sniffing.	Male or female. Sales (1972)
LONG EVANS	22-25	1-3000	Not given.	Post ejaculation.	Depression call deters female. Male exhaustion. Anisko *et al.* (1978)

Table 1. Ultrasonic vocalizations. The calls of Wistar and Long Evans rats and the conditions of their occurrence.

are not available. There are five different ultrasound detecting devices currently used. Two of these devices depend on the conversion of high-frequency, acoustic signals into signals that can be directly perceived by the researcher. The electric response of an ultrasonic microphone can be amplified and viewed directly on an oscilloscope screen. The number of calls can then be manually recorded. Photographic techniques can be employed and traces measured with a ruler to assess the frequency and duration of sound (Griffin, 1958).

The heterodyne conversion of high-frequency sound into audible signals can also be used to detect ultrasound signals. This detector (known as an ultrasound detector) produces sound signals which are partially representative of the input signals. If the instrument is tunable, the original frequency can be approximately determined. This assumes that the original signal is unchanging in frequency, and that the vocalization is of sufficient duration to allow tuning of the ultrasound detector. The amplitude of the vocalization is not measured (Sales and Pye, 1974).

The technique of recording ultrasound directly by high-speed tape-recorders can also be used. The low-speed replay of these tapes effectively converts high-frequency ultrasound into low-frequency, audio-sound. The tape-recording technique provides an ideal mechanism for the collection and storage of ultrasound. A per[illegible] record of the vocalization is made. The analysis of the recordings is, however, slow and cumbersome. Tapes can be studied by a sonographic method. By replaying short sections of the tape hundreds of times through narrow, tunable filters the recorded frequencies can be precisely measured. Short recordings require a long analysis time and silent intervals take up much of the tape (Sales and Pye, 1974).

Recently, techniques have been developed which categorize ultrasonic calls and use computer scanning techniques to collect data. Harrison and Holman (1978) developed the first computerized device which monitors ultrasound. The ultrasonic recorder tests the frequency spectrum over four bands. If the relevant frequency is present in an ultrasonic call, a digital state of "one" is registered at one of four parallel twin-T filter circuits. These four outputs are periodically scanned by a computer and their levels are stored for future analysis (White, 1971). The scope of this technique is limited by the small set of frequencies tested.

Other devices which count the number of calls in a specific frequency band have also been developed. They do not provide a complete analysis of the spectrum of rodent vocalization. These devices are basically ultrasonic event recorders (Brain et al., 1980; Morget, 1972).

The analysis of behavior and psychology based on these types of instruments could be misleading. Many of these devices do not provide complete information about the vocalizations of rodents. Most have no amplitudinal parameters and the frequency is often only approximated. The duration of vocalizaton cannot be considered at all, except when using a tape-recording technique. Tape-recordings of ultrasound, although initially attractive, are so difficult to analyse that often recording sessions are extremely short. If vocalizations are to be used as the bases of study into behavior, then it is necessary that all factors be considered.

Due to advances in digital electronics, equipment capable of properly monitoring signals across the ultrasonic frequency range can now be constructed. A device has been designed and constructed which can monitor ultrasound across the frequency range of 10 kHz - 90 kHz. It stores data directly on a computer for subsequent analysis. The development of this device is now detailed. Its ability to identify specific signals is tested.

Before large scale studies can be carried out, it must be shown that microwaves can affect the vocal activity of rodents. Therefore, the device is used to monitor the vocalizations of infant rats that are exposed to hypothermal stress and then placed in a low power microwave field. It is well known that rat pups vocalize in response to changes in body temperature. This experiment tests the ultrasound recorder's ability to distinguish between vocalizations of rat pups exposed to different irradiation conditions.

II. The Digital Ultrasound Recorder

A technique to detect the ultrasonic vocalizations of rats was developed in the course of this study. The microprocessor-based, detection system was constructed to log vocalizations and to store information on the Amdhal 5860 computer. This system records vocal frequency, sound pressure, time, and duration of calls. It detects ultrasonic vocalizations in the range 10 kHz - 90 kHz. These data are analysed using the Michigan Interactive Data Analysis System (MIDAS). A determination of the difference between the vocal activity of experimental and control animals can then be made.

Rats vocalize over the ultrasonic frequency range of 20 kHz - 150 kHz. The most heavily used range is 20 kHz - 70 kHz. Their calls are sporadic and usually are between 5 ms and 800 ms in duration. Thus, an ultrasonic detection system must be capable of recognizing a call quickly. It must be able to monitor animals for long periods of time, and, when there are no calls, must not produce null data as a tape recorder running continuously would. The rate of vocalization is dependent on the individual animal, and on the experimental protocol during recording. Up to 300 calls per minute have been detected from rat pups exposed to thermal stress. The frequency of vocalization is generally constant through out a call. The amplitude of vocalization can be difficult to assess because of its dependence on the microphone position, animal position and orientation, and finally on the acoustic properties of the environment.

A. Theory of Operation

A broad-band ultrasound recorder that automatically monitors signals in the frequency range of 10 kHz - 90 kHz has been developed. The high speed digital components of this system trigger data collection on detection of an ultrasonic call. The storage of null data is eliminated. The output of the system is automatically stored in a random-access, memory (RAM) buffer. For extended periods of data collection, information can be transfered to any digital mass storage device, such as a parallel printer, a disc drive, or a mainframe computer. Subsequently, more detailed analysis of ultrasonic calls can be made.

This ultrasonic detection system is based on a set of digital circuits.[1] The microprocessor which forms the central processing unit of the recorder is a Motorola MCM 6802. The microprocessor can operate in either of the two operating systems. One is Motorola's D5 Bug (MEK6802D5 Operator's manual), and the other is described in detail later. These two operating systems form the basis of the recorder's functions.

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[1] A technical manual for the Digital Ultrasound Recorder is provided. The manual contains a complete set of circuit diagrams, an IC layout for all circuit boards, a memory map, and a listing of the programs currently available for the recorder. This manual is intended to be kept with the recorder and to be updated as changes are made to the equipment.

Programs were written for the recorder in assembly language on the Amdhal 5860 computer. They were loaded into the recorder by programming a set of erasable, programmable, read-only memories.

The recorder system was designed with two major goals in mind, cost effectiveness and speed. The recorder uses a combination of standard TTL, low power Schottky TTL, CMOS and NMOS integrated circuits (ICs). The circuits were wire wrapped onto three perforated (Vector) boards. The wire wrapping allows dense packing of ICs and is also convenient for making changes in the wiring. The circuits were designed and tested in small sections before being interconnected. Grounding problems in the recorder were avoided by adopting a tree structure on each board, thereby eliminating the possibility of grounding loops. A view of the recorder's three major boards is shown in Figure 1.

All digital circuits produce, and are vulnerable to, interference from external electromagnetic fields. The recorder was therefore housed inside a grounded aluminum box with a small opening to allow access to the key pad. The recorder requires three different power levels +5 V, and ±15 V. The +5 V source is the primary supply for the recorder and must provide 20 W to the system. The ±15 V sources are required for the frequency-to-voltage, analog-to-digital converters and for the sample-and-hold circuits only, and must supply 5 W. A view of the recorder inside this box is shown in Figure 2.

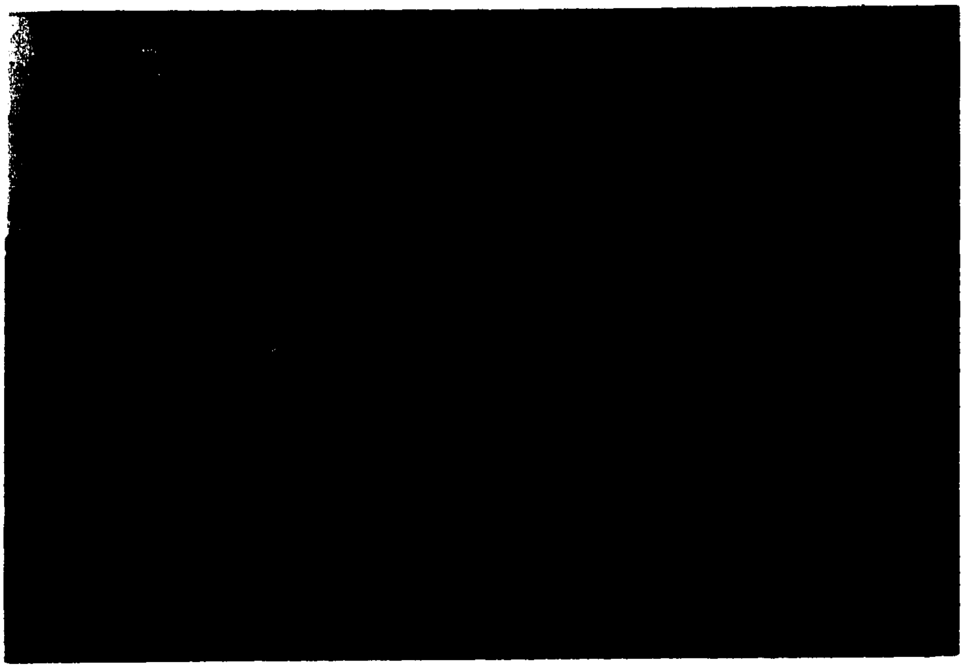

Figure 1. Ultrasound recorder - circuit boards. A photograph of the three main IC boards in the ultrasound recorder.

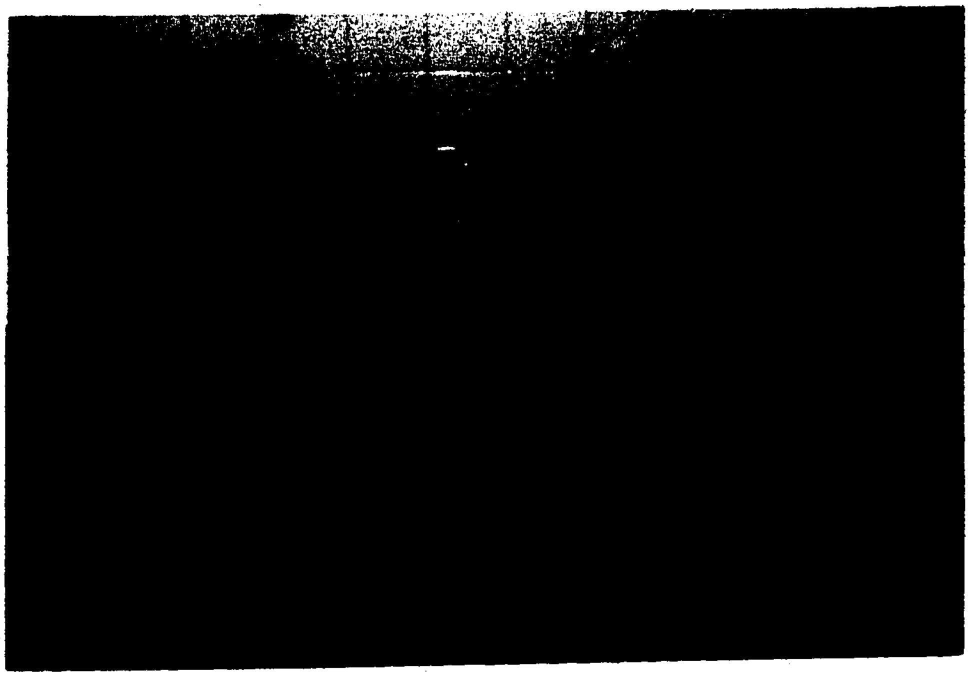

Figure 2. The digital ultrasound recorder. A photograph of the front panel of the recorder

The ultrasound recorder converts any 10 kHz - 90 kHz call into a digital signal. Figure 3(a), a block diagram, divides the recorder into five discrete units. When an ultrasonic call is received by the condenser microphone, it is directed to the analog (input-conditioning) unit, No. 1. This unit prepares the call for the digital conversion unit, No. 2. The control unit, No. 3, directs all the system's functions: it triggers events in all sections and controls the flow of data among them. The memory unit, No. 4, is a temporary storage depot; data are passed from it, through the output unit, No. 5, for final transfer to a device such as a computer or parallel printer. In this way, the components work together to detect, to digitize, to store and to finally transmit data on the ultrasonic calls.

The control unit contains the system's central processing unit (CPU), an 8-bit microprocessor (MCM 6802). This processor executes programs stored in a set of erasable, programmable, read-only memories (EPROMs). The operating system is the largest program in this memory bank. When the operating system starts execution, it interrogates the user for the system-timer speed, the rate of data collection, and the method of data transfer. Once these basic parameters are defined, the system begins to monitor ultrasonic signals.

A flow chart of the system is presented in Figure 3(b). During operation, the recorder constantly tests for ultrasonic signals. On detection of an ultrasonic call, the

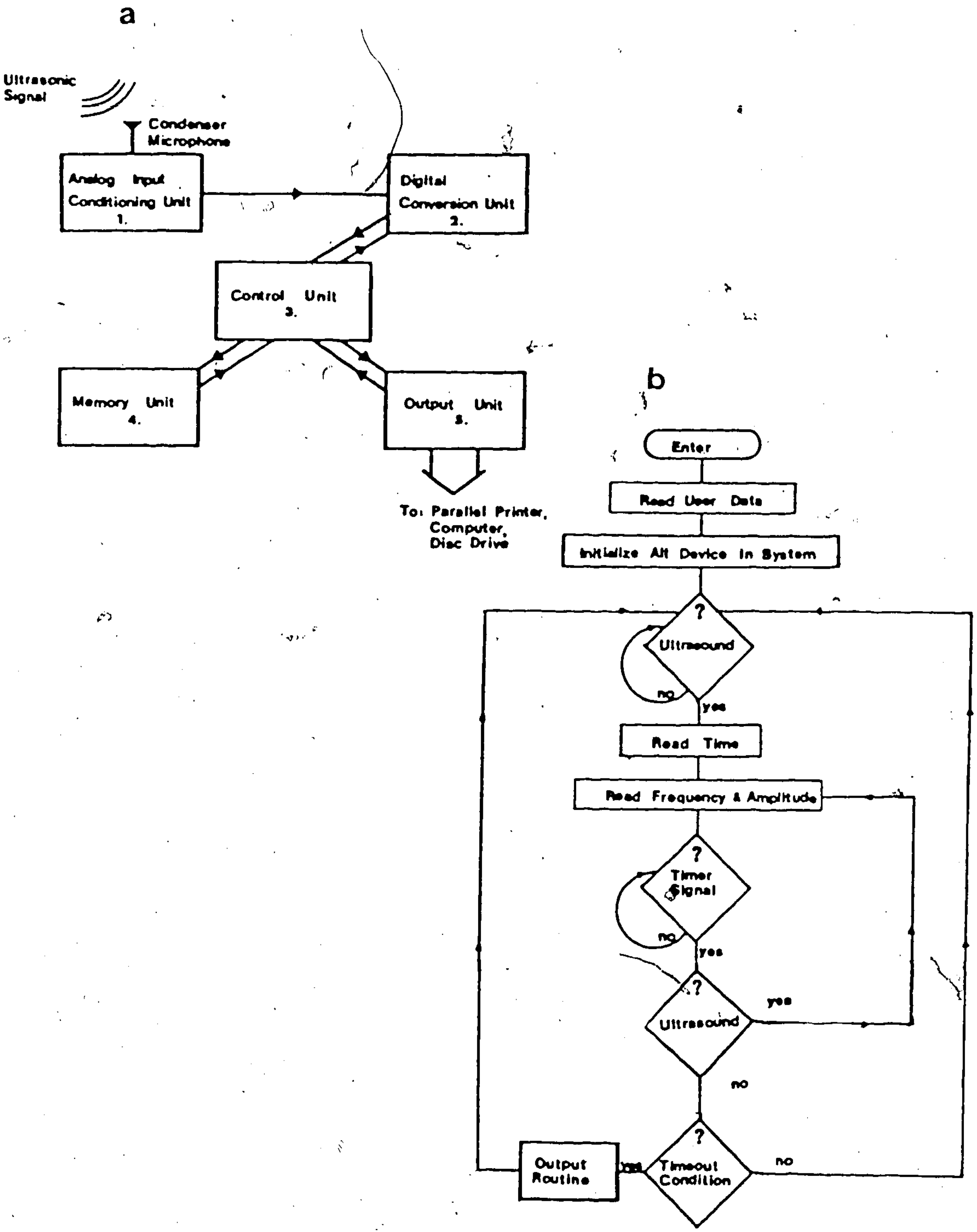

Figure 3. Block diagram of the recorder. (a) Divides the recorder into its five major units. (b) A flowchart of the operating system of the recorder.

system begins data collection. It first reads the timer to find out when the call began and then measures the amplitude and frequency of the call. This information is stored in the local memory buffer. The recorder then waits for the timer to trigger it to start making further measurements of the amplitude and frequency. This process continues for the duration of the ultrasonic call. When the call ends, the recorder returns to its earlier mode and begins testing for the occurrence of additional ultrasound.

The cycle of testing for calls and making readings continues until a time-out condition is reached. The time-out condition can be brought on by: (1) local memory overflow; (2) system timer termination of the experiment; or (3) user selection of a continuous transfer mode of operation. The presence of a time-out condition results in part of the data being transmitted out of the system by the output unit. After the transfer is completed, the time-out condition is automatically removed and the operating system continues its normal flow.

The analog input-conditioning unit of the recorder essentially guarantees that all signals directed through it to the digital conversion unit are within prescribed tolerances. The block diagram, in Figure 4(a), shows the output of the condenser microphone first amplified by a factor of 2000. This improves the signal-to-noise ratio for the rest of the system, and also isolates the microphone output from the digital network. (The microphone and

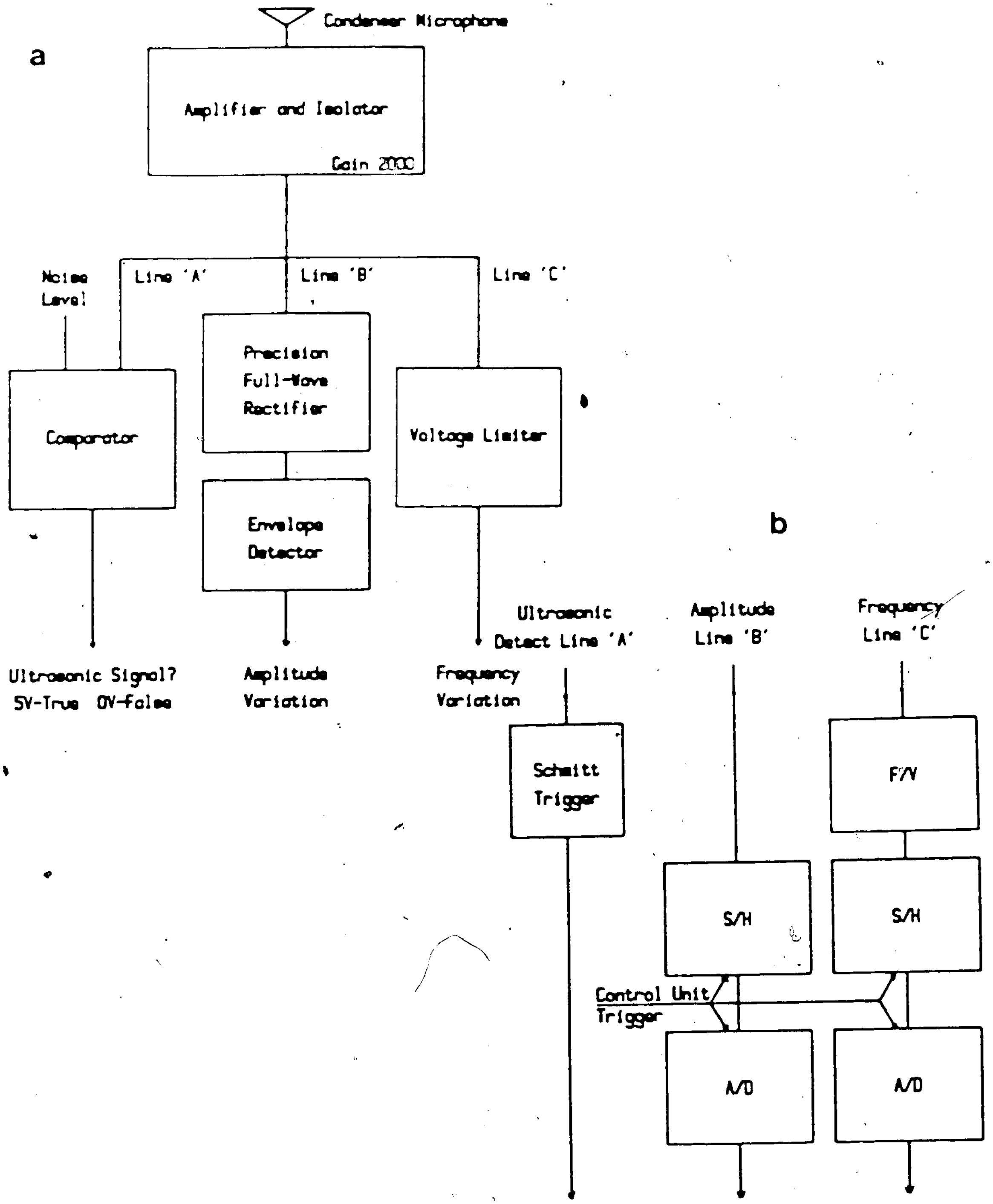

Figure 4. Block diagram of the input conditioning unit and the digital conversion unit. (a) The input unit splits the call onto three test lines. (b) The conversion unit digitizes these lines.

amplifier are part of an S100 Bat Detector - QMC Instruments Limited, Queen Mary College, University of London, London, England). The signal is then split into three parallel signals: A, B, and C. Signal A is compared to a user-specified noise level. Setting the appropriate value for this level is critical, because the sound pressure of the ultrasonic call must be greater than this noise level, for the system to collect data. The position of the noise level is determined by the following factors: (1) the level of ambient interfering noise; (2) the proximity of the microphone to the experimental subject(s); and (3) the electronic noise. It is centered at a frequency of 1.0 MHz, a frequency which cannot trigger the system. The amplitude of the noise could, however, mean that some low intensity signals are not recorded: a variable resistor within the unit controls the threshold of the noise level which, is set at 0.1 V. At this level, an ultrasonic signal must have an amplitude of more than 5 mV in order to be registered. If the incoming signal is greater than the noise level, the output of a comparator is set to 5 V, which indicates to the control unit that a valid call is present; this output is labeled "the ultrasonic detect line". The second parallel signal, B, is fed into a precision rectifier and envelope detector. Only the positive envelope of the original call passes through these components. It is a slow moving signal and represents the amplitudinal variation and frequency modulation of the ultrasonic call. The third parallel signal, C, is tested to ensure that its voltage levels will not damage subsequent circuits. These three signals A, B, and C are directed to the digital-conversion unit.

The primary task of the digital conversion unit is to couple incoming analog signals to the CPU (in the control unit). The three analog signals of the previous unit are converted into digital signals by the processes shown in Figure 4(b). The ultrasound detect line, A, is technically a digital signal when it leaves the analog unit. It has two states 0 and 5 V. To add a small amount of hysteresis and to ensure that the transition between states is rapid, the signal is fed into a Schmitt Trigger. This signal can now be read by the CPU.

The amplitudinal variation of the ultrasound, line B, is fed into a sample-and-hold circuit (S/H) which is directly connected to an analog-to-digital (A/D) converter. The CPU triggers the S/H and the A/D to convert the input analog signal, B, to its equivalent digital signal at user defined time intervals (this digital signal can be accessed by the CPU).

The frequency variation of the ultrasonic call, line C, is converted to a digital number by a frequency-to-voltage (F/V) converter. The module counts the number of zero crossings of the ultrasonic call. It is, therefore, a measure only of the fundamental frequency of the call. The number of zero crossings is translated into an analog voltage, which is fed into an S/H and an A/D, in a fashion similar to that of the previous signal. The CPU is able to trigger simultaneously a measurement of frequency and of amplitude. These three signals, which are directly related to the incoming ultrasonic call, are available to the CPU within 100 µseconds of detection.

The CPU stores these digital numbers, which represent the amplitude and the frequency, in the local memory buffer as part of its normal execution of the operating system. The memory buffer can hold up to 10 kilobytes of information (5000 ultrasound measurements). Depending on the rate of animal vocalization and data collection, the memory buffer will be filled in anywhere from 2 minutes to the duration of the experiment. The buffer is made up of static, random-access, memory chips (MCM2114), and the data stored in them can be maintained for any length of time, if the line current is not interrupted.

Transfer of data through the output unit must occur before useful information can be extracted from the recorder. A transfer will occur if the buffer is filled. It will also result if the user selects the active transfer mode of data transfer. In this mode, the operating system is always trying to empty the local buffer and transmit data to a mass storage device. If there are data in the buffer, the system will continue to transmit data unless the rat vocalizes, or until the buffer is emptied. Rat vocalizations generate "interrupts" for the system. On detection of an "interrupt", the output routine stops transmission and the recorder starts to collect data. The system collects data as long as the rat continues its vocalization. The system then returns to the output routine and continues the transmission of data.

Data to be transmitted are then taken from the bottom of a data stack. As the data are collected they are put on the top of the data stack. Thus, the transfer process is in a race with the collection process. When the transfer process reaches the top of the data stack the entire stack is reinitialized to the first location memory in the local RAM buffer. If the transfer rate is not sufficiently high there will be an overflow. Data will be lost and the recording session will be wasted. The use of this mode is contingent on a slower rate of data collection than the rate of data transfer.

The format of the recorder output is:

255 255 000 000 T1 T2 A1 F1 A2 F2 ---- An Fn

The numbers, 255 255 000 000, are a marker that indicate the start of a call. T1 and T2 are the measurement of the time that the calls begin, relative to the start of the recording session. A1 F1, A2 F2, ---- An Fn are measurements of the amplitude and the frequency of a single call. These measurements are made in parallel and thus are always in pairs. The time interval between A1 and A2 can be programmed before the recording session. The number of pairs of measurements is therefore an indicator of the length of the call. There can be thousands of calls in a single recording session.

B. Experimental Verification

Artificially produced high frequency signals were used to determine the effectiveness of the detection system. This tests the system's ability to reconstruct ultrasonic calls accurately.

Three test frequencies, 22 kHz, 50 kHz, and 70 kHz were chosen to cover the broad range of calls commonly made by rodents. Amplitude modulation was introduced so that amplitudinal variations could also be determined. A 4 Hz modulation frequency was used. The signals were fed directly into the recorder, by-passing the microphone, thereby eliminating the problem of ensuring the integrity of the modulation of the signal through a loud speaker. The results are shown in Figure 5. There is a significant similarity between the output of the recorder and the input high frequency signals. The uncertainty of the frequency measurement is less than 5%, and that of the amplitude measurement, less than 10% [2] (Any nonlinearities in the system can be determined by testing its output across the entire frequency spectrum. If data are to be transferred to

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[2] The uncertainty is defined as the average deviation of the measured signal from the input signal, divided by the magnitude of the input signal.

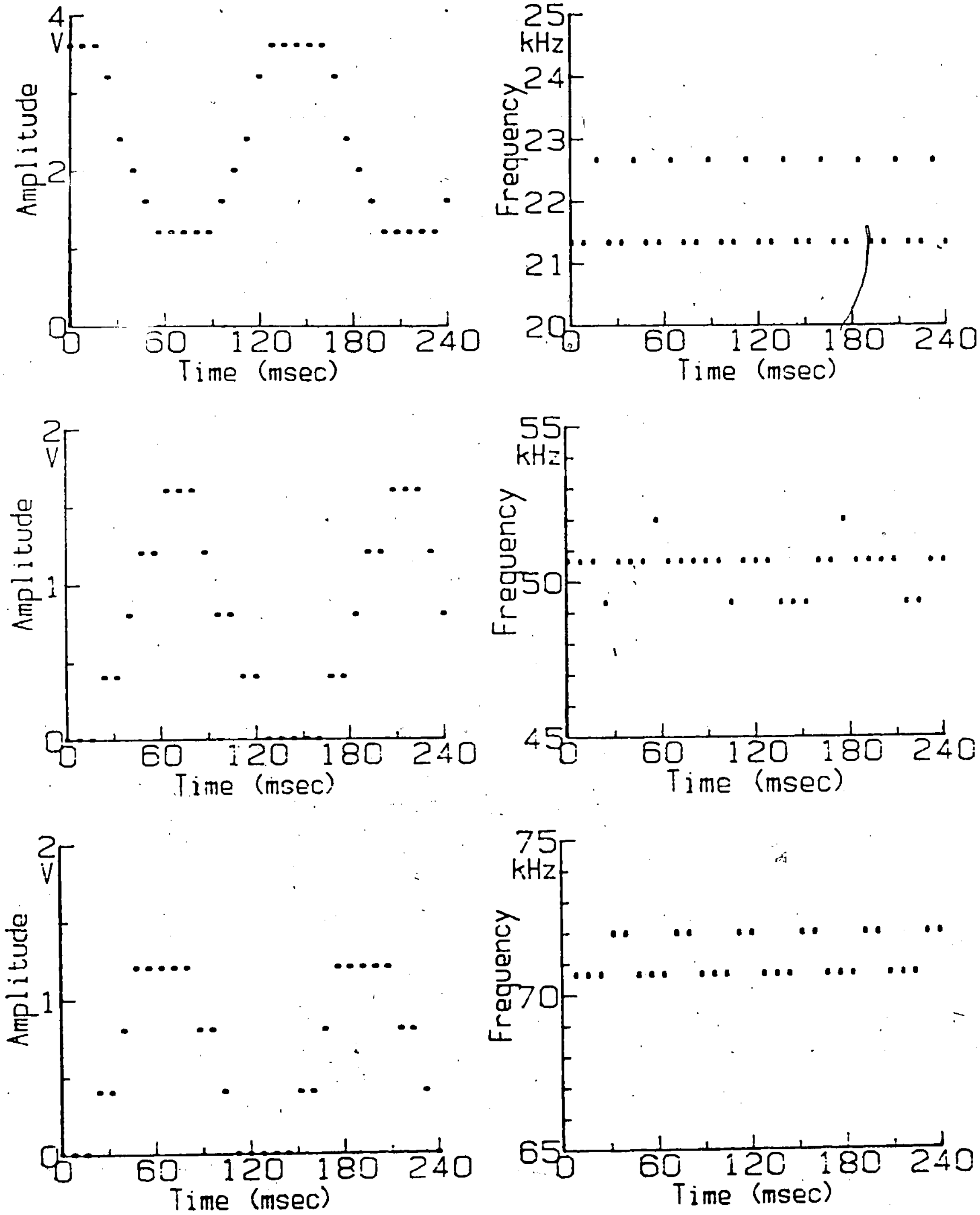


Figure 5. Recorder output for high-frequency signals. 22 kHz, 50 kHz, and 70 kHz carrier with 4 Hz modulation were used as input signals.

a computer, for statistical analysis, this kind of error can be easily suppressed by multiplying a datum by the appropriate scaling function.).

To use any of the statistical packages available on the Amdhal 5860, it is necessary to reformat the data. The amount of reformating required is dependent on the statistical package used. For example to use SPSS (Statistical Package for Social Sciences) it is necessary to place a single call on a record in a file. The reorganization used for invoking MIDAS is more extensive. Individual measurements must be placed on a single line. Thus, a single call may extend to several lines. Additional parameters such as Run number, Exposure number, and Call number must be added. Appendix III has the Pascal program used to reformat data for MIDAS. The analysis techniques used after the data is reformatted are dependent on the experimental protocol. Some parameters that can be considered are: amplitude, frequency, number of calls, length of the call, total vocal time, ...

III. Rodent Experiments

A. The Microwave Irradiation System

The microwave irradiation system that was used in these experiments was originally developed by Guy and Chou (1975) and later refined by Guy, Wallace and McDougall (1979). It is designed for exposure of rodents, on a long-term basis, to microwave radiation without disturbing their normal, living patterns. Other techniques were considered, such as exposure to a plane wave field in an anechoic chamber, and to a standing wave field in a metallic cavity. These techniques result in power being coupled to experimental subjects as a function of animal size and orientation, and the position of the food and water dispensers. The amount of power coupled to the subjects can vary over a range of three orders of magnitude, under these conditions (Guy and Chou 1975). Thus, the anechoic chamber and the resonant cavity irradation systems were rejected.

The exposure system that was used, consisted of a section of cylindrical waveguide, constructed of wire mesh screen, in which circularly-polarized, propogating TE(11) and TM(11) mode, field configurations are excited. A 50-Ohm coaxial feedline is matched to a small diameter cylindrical waveguide with two orthogonal excitation probes. A TE(11) mode is excited in the small waveguide. A short distance down the guide from the excitation probes, there is a circular polarizer consisting of four stubs that convert the linearly-polarized TE(11) mode into a circularly-polarized TE(11) mode. The circularly-polarized mode is matched to the large diameter wire mesh waveguide by means of four additional tuning stubs. The two radiating modes (TE11 and TM(11)) are excited and travel down the wire mesh waveguide, irradiating experimental subjects in a plastic animal holder in the center of the guide. A photograph of the system is seen in Figure 6(a).

The plastic animal holder is of an adequate size to house rodents as large as guinea pigs. The floor is constructed of plastic rods so that waste falls through and is collected outside the irradiation system. The food dispenser is designed to allow only a fixed amount of the dry food pellets (which absorb very little power) into the microwave field. Water is dispensed from a standard water bottle with a glass nozzle. The water bottle is decoupled from the animals by two concentric quarter wavelength coaxial choke sections. The animals can move freely in the holder. The power coupling characteristics of the incident circularly-polarized wave ensures that the animals are uniformly illuminated by the propogating field. An explanation of this phenomenon is found in Appendix II.

Power that is transmitted past the animal is coupled to four matching stubs, similar to those used to launch the waves. From these stubs the power is coupled to a 50-Ohm line which can be terminated by a matched load. A directional coupler and meter may be placed before the load

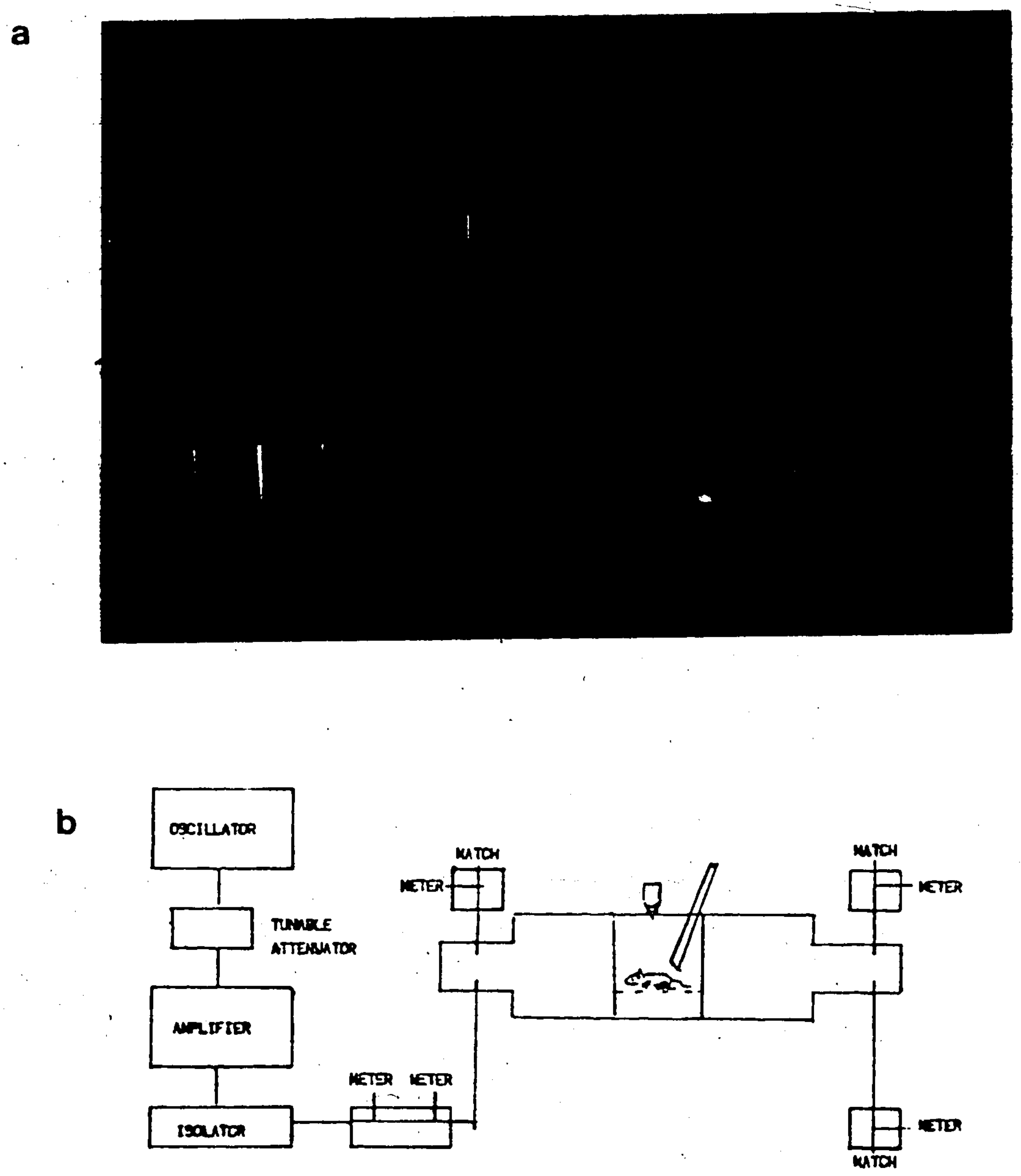


Figure 6. Arrangement of the irradiation system. (a) A photograph of the system. (b) A block diagram indicating the components of the irradiation system.

to measure the power absorbed by the load.

The power that is reflected from the experimental subject is polarized in the opposite direction to the incident microwave field. Therefore, reflected power is coupled to a fourth port on the irradiation system. This port is also terminated by a matched load. There is good isolation between the microwave radiation source and the reflections from within the waveguide. Figure 6(b) shows the layout of the irradiation system.

The absorption of the system can be measured by summing the reading of transmitted and reflected power and subtracting from the incident power. By using the straight substitution technique, and comparing the power absorbed by the system, with and without the animal holder loaded, it is easy to measure the power absorbed by an irradiated animal.

The detailed descriptions of the microwave techniques used in this exposure system, and an analysis of the dosimetery of the irradiated subjects, can be found in the original reports (Guy and Chou, 1975; Guy, _et al._, 1979).

B. Methodology

An experimental protocol was developed to monitor rat pup ultrasound under conditions of microwave heating. The irradiation system previously described was used. The digital ultrasound recorder was used to monitor the vocal activity of the rat pups (10-11 days old) for 20 minute test intervals. The recorder (operating in an active mode) directly stored vocalization data in a computer file on the Amdal 5860 computer (the major computing facility on the University of Alberta campus). Data collection occured on two nights during which 14 recording sessions were made. The subjects were eight rat pups of randomly bred Sprauge-Dawely strain (Rattus Norvegicus from the Biosciences Animal services, The University of Alberta, Edmonton, Canada). They were housed in a standard 21 by 21 by 45 cm cage in the Animal Unit in the Surgical-Medical Research Institute,the University of Alberta. Food and water were available *ad libitum*. The room temperature was maintained at 22(±1.5) °C and a light-dark schedule of 12:12 was employed. The infant rats were maintained under maternal care, except for the duration of the experimental periods.

Ten minutes before the recording began, a pup was removed from its nest and was placed in a closed ice-bath. The bath temperature was 1-2 °C. The pup was kept in the bath for 10 minutes to allow its temperature to decrease by approximately 12 °C (See Appendix I for measurement of rate of cooling.). The pup was then removed from the bath and placed in a standard 250 ml glass beaker (at room temperature). The beaker was placed on its side in the animal holder of the irradiation system. An irradiation field of 2 W was switched on for experimental runs, while control runs were sham-irradiated. A microphone was positioned outside the irradiation system opposite to the mouth of the beaker. The microphone was connected to the ultrasound recorder and the recording system was turned on. A chart recorder with inputs from the transmitted and reflected power signals was started, to measure indirectly the power absorbed by the pup. The ultrasound recorder monitored vocal activity for a 20 minute period. After the recording session the radiation field was switched off and the pup was removed from the animal holder. The mass of the pup was measured and then the pup was returned to its nest. Nesting material was sprinkled over the pup so as to mask any odor the pup may have picked up during experimentation (Belacostra _et_ _al._, 1980).

This procedure was used 14 times to monitor the vocalizations of rat pups. Six of the recording sessions involved microwave radiation and are referred to as experimental, and six did not, and are referred to as control. Two additional sessions were used to determine the vocal activity of rat pups isolated from the nest for 30 minutes. The same procedure, as previously described, was used with these pups, except that the ice-bath was not loaded and the radiation field was not switched on. The vocalizations of this group can be considered as a base level for the control and the experimental data.

In all sessions, the water bottle and the food dispenser were left empty. All experiments were carried out between 7:00 pm and 12:00 pm to minimize the effects of noise in the building.

The data collected during recordings was reformatted to allow analysis by the MIDAS statistical package, which is available on the Amdhal 5860 computer.

To analyse the level of energy absorbed by the pups in the 2 W field a glass vial holding 20 ml of distilled water, was placed in the radiation field for 20 minutes. The temperature of the water was noted before and after irradiation. From the temperature increase of the water the total energy deposited into the water by the microwave field can be calculated. This value can be compared with the number calculated when using the straight substitution technique of measuring energy deposition.

IV. Experimental Results

Before evaluating the effects of microwave radiation on the vocal activity of rat pups, an exact description of the conditions of irradiation must be made. Control and experimental animals were handled in exactly the same way, with the exception that the RF section of the microwave oscillator and amplifer was not switched on for the control animals.

For experimental runs, the frequency of the microwave radiation was set to 2450 MHz. The field pattern is nominally defined as TE(11) and TM(11), circularly-polarized and propagating in the forward direction. The field is marginally perturbed by the non-ideal materials used in the construction of the waveguide, and in the plastic animal holder in the center of the guide. When a rat pup is placed in the animal holder, the incident microwave field is further perturbed. The conditions of exposure for the rat pups are to some extent directly related to the pups. Their mass, volume, and movements will govern the amount of energy that they will absorb. To measure the amount of power they will absorb, a straight substitution technique is employed. The power transmitted through the cage is measured with and without the rat pup in the animal holder. Table 2 summarizes the results of the tests made during each experimental run.

Table 2 - Power flow through the irradiation system

	PI (W)	PD (mW/cm²)	PT (W)	PAC (W)	PAR (W)	MASS (g)	ENERGY (J)	SAR (W/kg)
empty	2	6.17	1.07	0.93	0.00	00.0	000	00.0
run3	2	6.17	0.57	0.93	0.50	18.4	600	27.2
run4	2	6.17	0.64	0.93	0.43	21.6	516	19.9
run5	2	6.17	0.66	0.93	0.41	19.6	492	20.7
run6	2	6.17	0.60	0.93	0.47	20.6	564	18.1
run9	2	6.17	0.72	0.93	0.35	23.3	420	15.1
run10	2	6.17	0.50	0.93	0.57	20.8	684	27.4

Table 2 - Power flow through the irradiation system.

1. PI (power incident) - is set to 2 W for all runs.
2. PD (power density) - is the average power of the radiation field.
3. PT (power transmitted) - is the power that flows out of the irradiation system. This includes all reflected power.
4. PAC (power absorbed by the cage) - is the power absorbed by the system.
5. PAR (power absorbed by the rat) - is the power absorbed by the rat pup when it is placed in the animal holder, and the 2 W field is on. PAR is equal to PI-(PT+PAC).
6. MASS - is the mass of each rat pup.
7. ENERGY - is the energy gained by the rat pup, assuming that all energy absorbed by the pup remains in the pup.
8. SAR (specific absorption rate) - is a measure of the power absorbed by a kg of matter, if it were exposed to the same microwave field.

The measurements described in Table 2 are based on a technique of straight substitution. These results can be compared to the energy absorbed by a vial of distilled water, placed in the same 2 W microwave field for 20 minutes. The vial contained 20 ml of water which is approximately the same mass as the average rat used in these experiments. The temperature of the water in the vial before radiation was 24.9 °C. After irradiation for 20 minutes its temperature was 29.0 °C. The water gained 351 Joules of energy. There are some differences between these values and the numbers calculated using the substitution technique. This is due to the different chemical composition of the rats. Further, the movements of the rat pups will affect their absorption patterns.

An analysis of the vocal activity in the 20 minute recording periods was made using the Michigan Interactive Data Analysis System. The parameters used in the analysis were derived from the output of the ultrasound recorder. Each line of the data file used was organized in the following format:

Run No., Exposure, Interval, Call No., Amplitude, Frequency

* Run No. - Identifies the run that was being analysed.

* Exposure - Identifies the experimental condition; exposure to microwave radiation, to sham-irradiation or to simple isolation from the nest and maternal care.

* Interval - Identifies the time interval in which the call was made. In the 20 minute recording period there were nine full intervals. In most cases, however, the recording period started in the middle of the first interval and therefore ended in the middle of the tenth interval. The analysis was done assuming there were ten full intervals. If a session had only nine intervals, a tenth was added with zero readings.

* Call No. - Identifies calls in the same time interval. If a call is of sufficient duration, to cause the recorder to make more than one reading of its amplitude and frequency, the call number is repeated. The total number of calls, as well as the length of calls, is coded into this datum.

* Amplitude - Identifies the measurement of the amplitude of the condenser microphone signal. This is a measure of the sound pressure of the ultrasonic call. The reading is in mV.

* Frequency - Identifies the frequency of the ultrasonic signal. A scale of 0-255 is used to define signals from 0 kHz - 90 kHz.

Number of calls is the first parameter considered in the analysis of the data. Figure 7 and 8 are a set of graphs indicating the number of calls recorded in each interval for the control animals. Figures 9 and 10 are a set of graphs indicating the number of calls recorded in each interval for experimental animals.

These graphs clearly show a difference in the vocal activity of the rats. The control animals begin vocalization at a low rate and increase their vocalization rate as the recording period continues. The experimental animals begin vocalization at a high rate and decrease their vocalization rate as the recording period continues. A graph of the average of number of calls for each group is presented in Figure 11. To investigate the significance of these results, statistical tests are used to determine if the number of calls in each interval are significantly different. The variation between each recording period within the same type of exposure is too large to apply standard techniques satisfactorily. The number of calls is, therefore, normalized by dividing by the total number of calls in the recording session. In most cases, this ratio has too many variations. To further reduce the variation between recordings in a single type of exposure, the average number of calls in the first five intervals is calculated, and the number of calls in the second five intervals is also calculated. The slope of the line joining these points is used as the basis of a Student T test ($n=6$; $x_1=57.67$,

x_2=-31.09; var_1=26.66, var_2=9.94; normal distribution is assumed). Figures 12 and 13 show the average slope for the control and experimental animals. The standard deviation of the slope is also shown. The results of the T test clearly show the trend that is apparent in the original data. The slopes of the control recording sessions have a probability of 99.26% of being less than the slopes of the experimental recording sessions. A p-value of $\leq$0.005 is calculated for the slopes of these two groups which indicates highly significant differences.

A second parameter considered for the analysis of data is the total vocal time. The total vocal time is calculated as the total time that a pup vocalizes in a given interval. The unit of measure is 60 ms, because the recorder measures a call every 60 ms. Thus, each measurement is equated to a 60 ms vocal time. Figures 14 and 15 show how the total vocal time varies over the 10 time intervals for the control animals. Figures 16 and 17 show the total vocal time for the experimental animals. The same trend of increasing vocalizations for the control animals and decreasing vocalizations for the experimental animals is seen. Again, due to the wide variations in the standard deviations of this variable, standard statistical techniques are not applied. Using the same techniques applied to the number of calls, the total vocal time is normalized. Then the slope of the two large intervals (1-5, 6-10) is calculated. The results are shown in Figures 18 and 19. A p-value of $\leq$0.007

is calculated from the Student T test of the slopes ($n=6$; $x_1=57.77$, $x_2=-30.23$; $var_1=27.10$, $var_2=12.97$; normal distribution is assumed). This shows that the slopes of the control group are significantly different from the slopes of the experimental group.

Figures 20 and 21 are graphs of number of calls and total vocal time for the rat pups that were isolated. These pups experienced all the physical handling and the isolation from their nest which the control and experimental animals faced. The isolated animals did not experience any thermal stress. The number of calls and the total vocal time for this group is extremely low. Isolation is not a significant factor in the vocalizations of the control or experimental animals.

The frequency measured by the ultrasound recorder was used as a basis of further analysis. Histograms of frequency for each type of exposure are presented in Figure 22. A Two-sample test of the median of all vocalization data shows that: experimental versus control p-value ≤ 0.061 ($n=4850$, median$=40.00$), experimental versus isolation p-value ≤ 0.001 ($n=2040$, median$=40.00$), control versus isolation p-value ≤ 0.001 ($n=2864$, median$=40.00$). The experimental and control data are not significantly different. The experimental and control groups do have highly significant differences in frequency from the isolation group. It should be noted that, because the number of calls made by the isolated animals are low, the percentage of noise in their sample will be high.

The amplitude of the vocalizations shows some variation also. Histograms of the amplitude of each type are presented in Figure 23. A Two-sample test of the median of these data show that: experimental versus control p-value ≤0.001 (n=4850, median=16.50), experimental versus isolation p-value ≤0.378 (n=2040, median=16.50), control versus isolation p-value ≤0.575 (n=2864, median=40.00). The differences between control and experimental data are explained by the differing distributions of the two populations about the median.

The length of each call was also considered as a source of variation between groups. Histograms of this parameter are shown in Figure 24. These data show no significant variation between groups.

Finally a graph of the total number of vocalizations versus mass is presented in Figure 25. There are no trends to indicate that larger pups vocalize more often than smaller pups or vice versa.

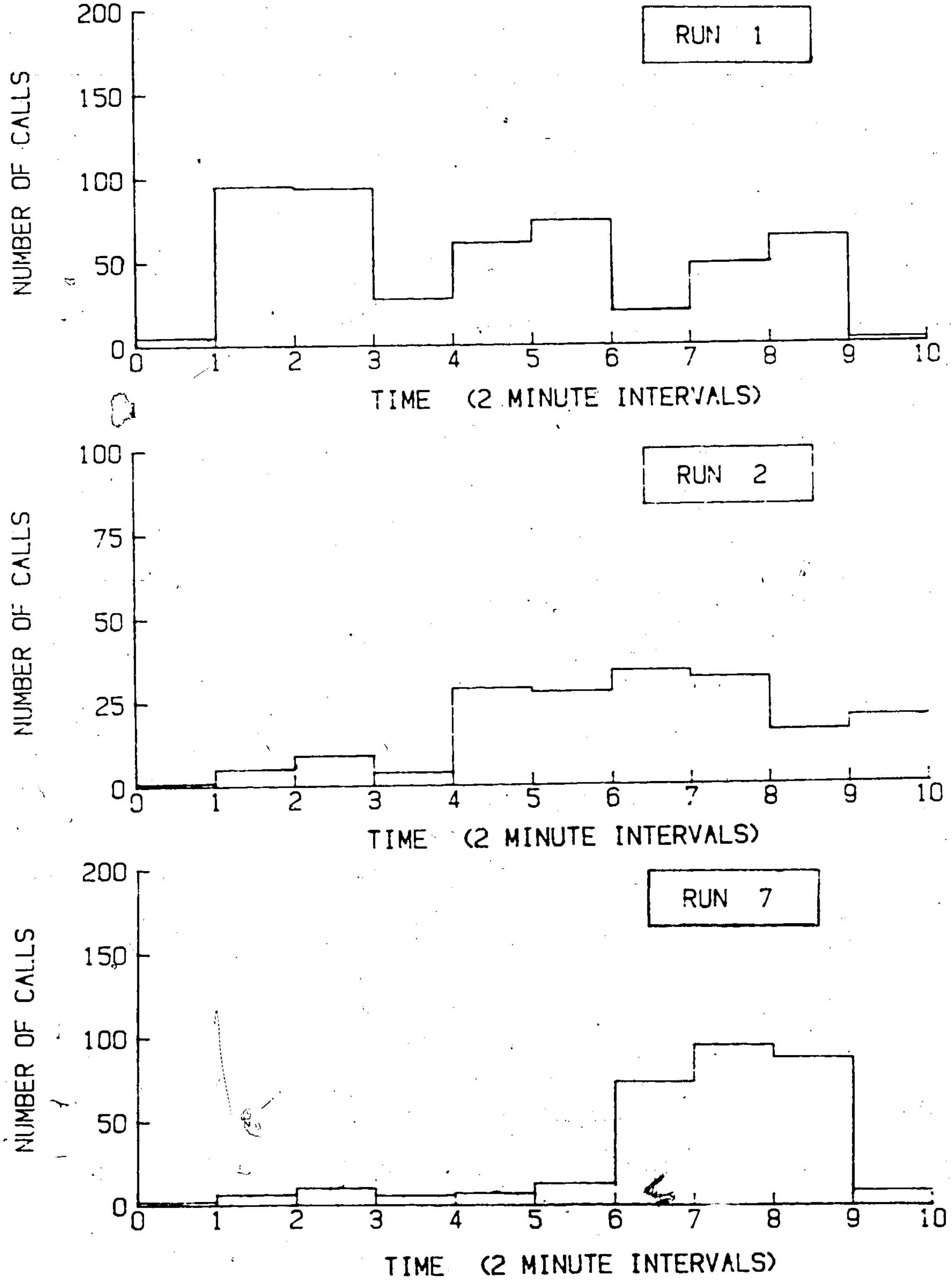


Figure 7. Number of calls for control animals. The number of calls registered in each 2 minute interval, by the control animals, increases during most recording sessions (Runs 1,2,7).

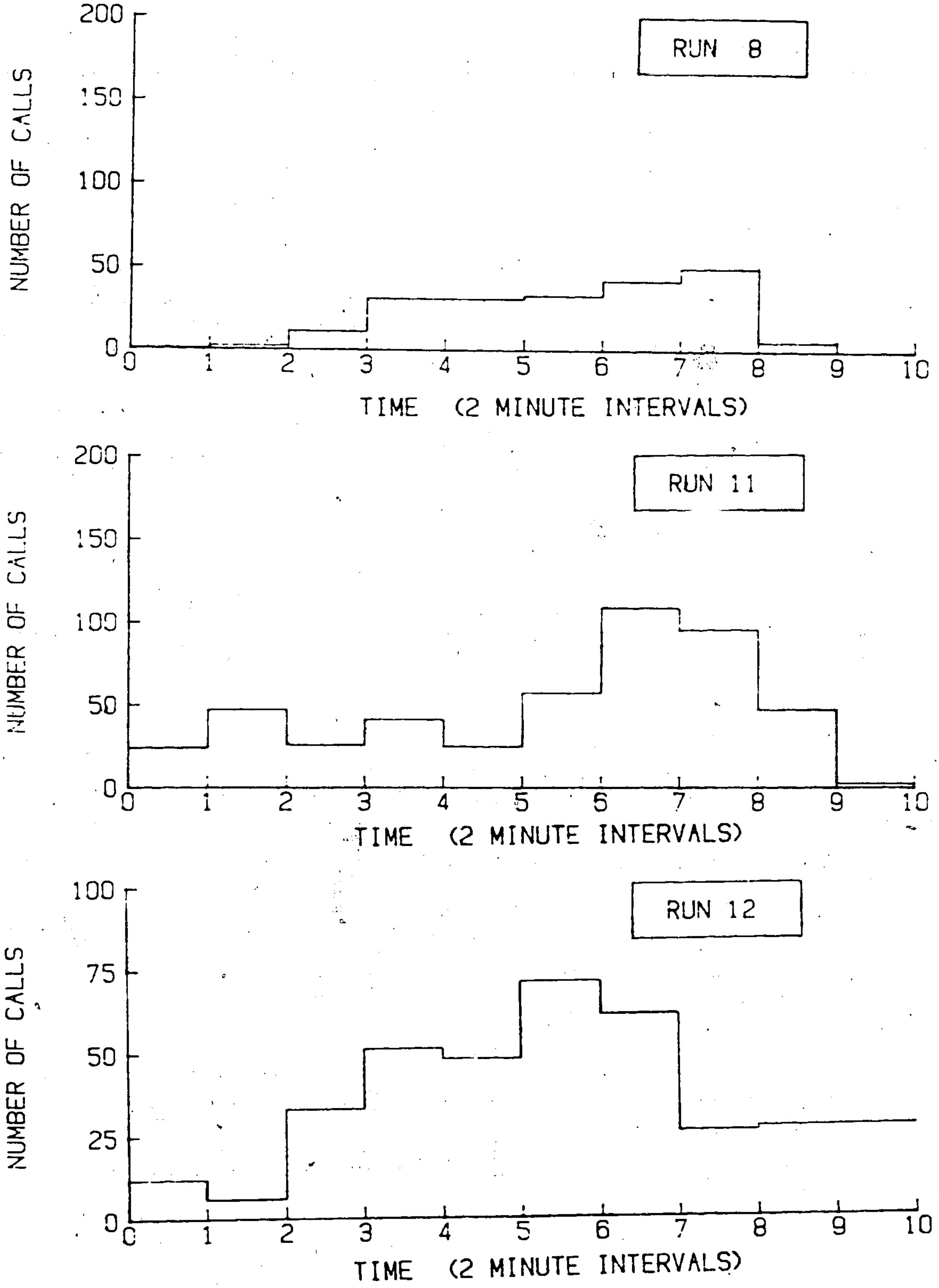


Figure 8. Number of calls for control animals. The number of calls registered in each 2 minute interval, by the control animals, increases during most recording sessions (Runs 8,11,12).

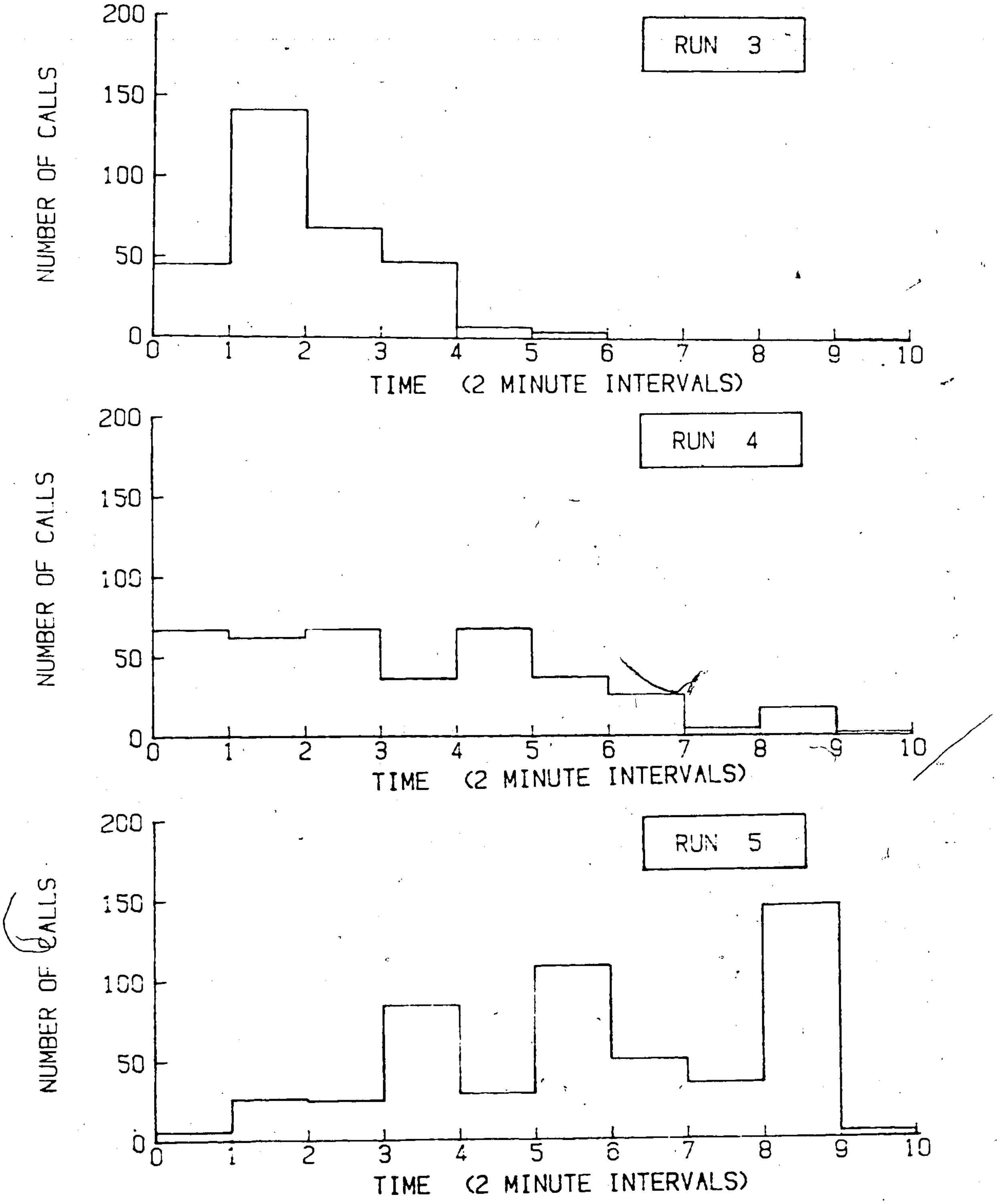


Figure 9. Number of calls for experimental animals. The number of calls registered in each 2 minute interval, by the experimental animals, decreases during most recording sessions (Runs 3,4,5).

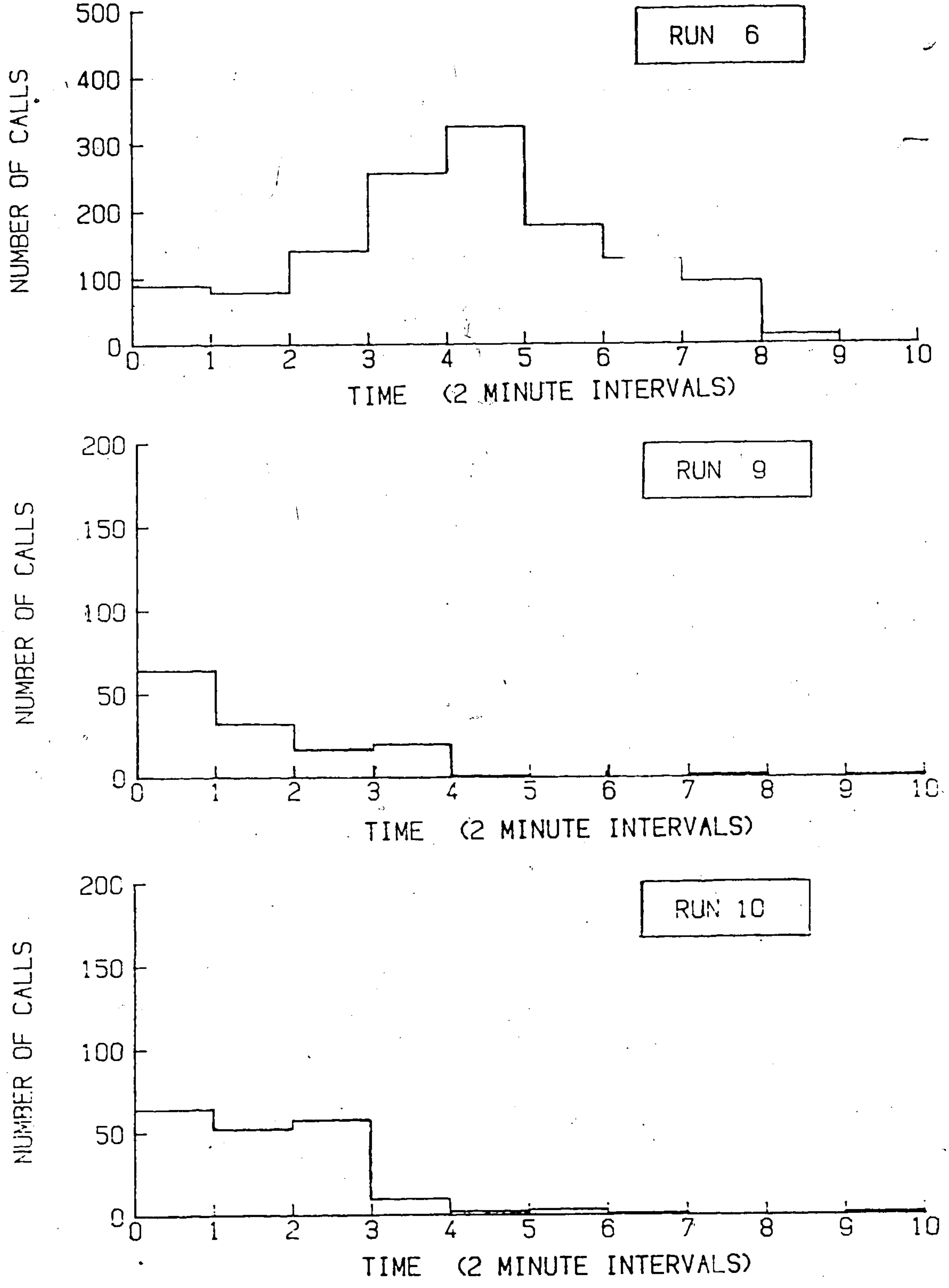


Figure 10. Number of calls for experimental animals. The number of calls registered in each 2 minute interval, by the experimental animals, decreases during most recording sessions (Runs 6,9,10).

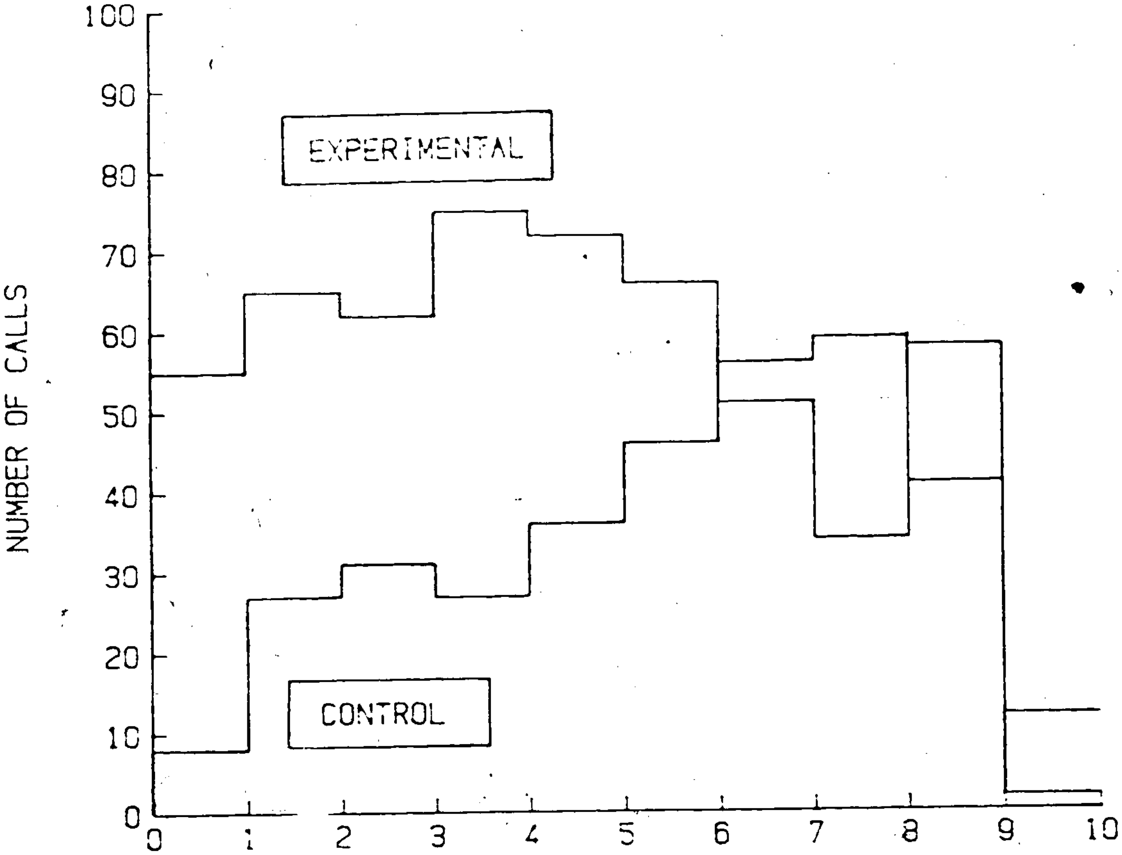


Figure 11. Average number of calls for both control and experimental animals. The average is calculated, for each time interval of the control and the experimental recording sessions.

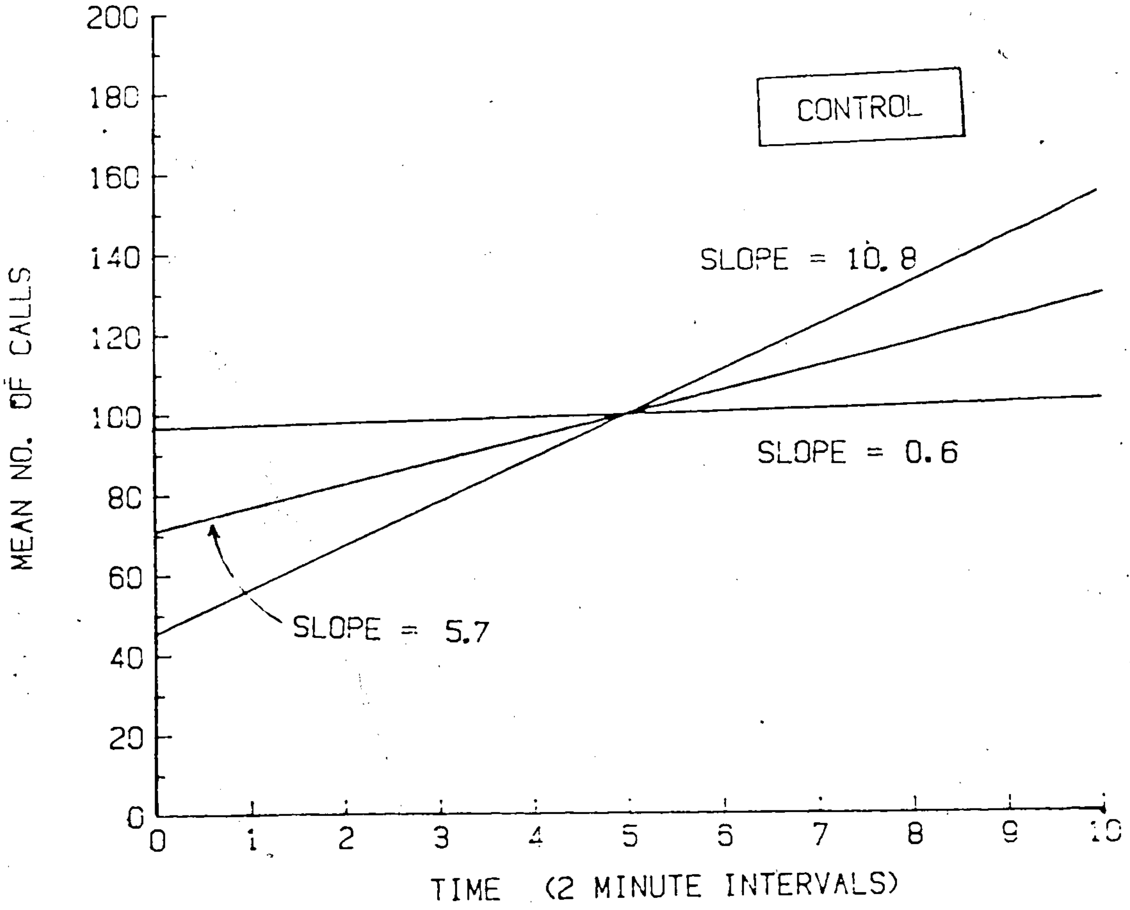


Figure 12. Normalized slopes of the number of calls for the control animals. The center line is the mean slope and the peripheral lines are one standard deviation away. All lines are standardized to intersect the point (5,100).

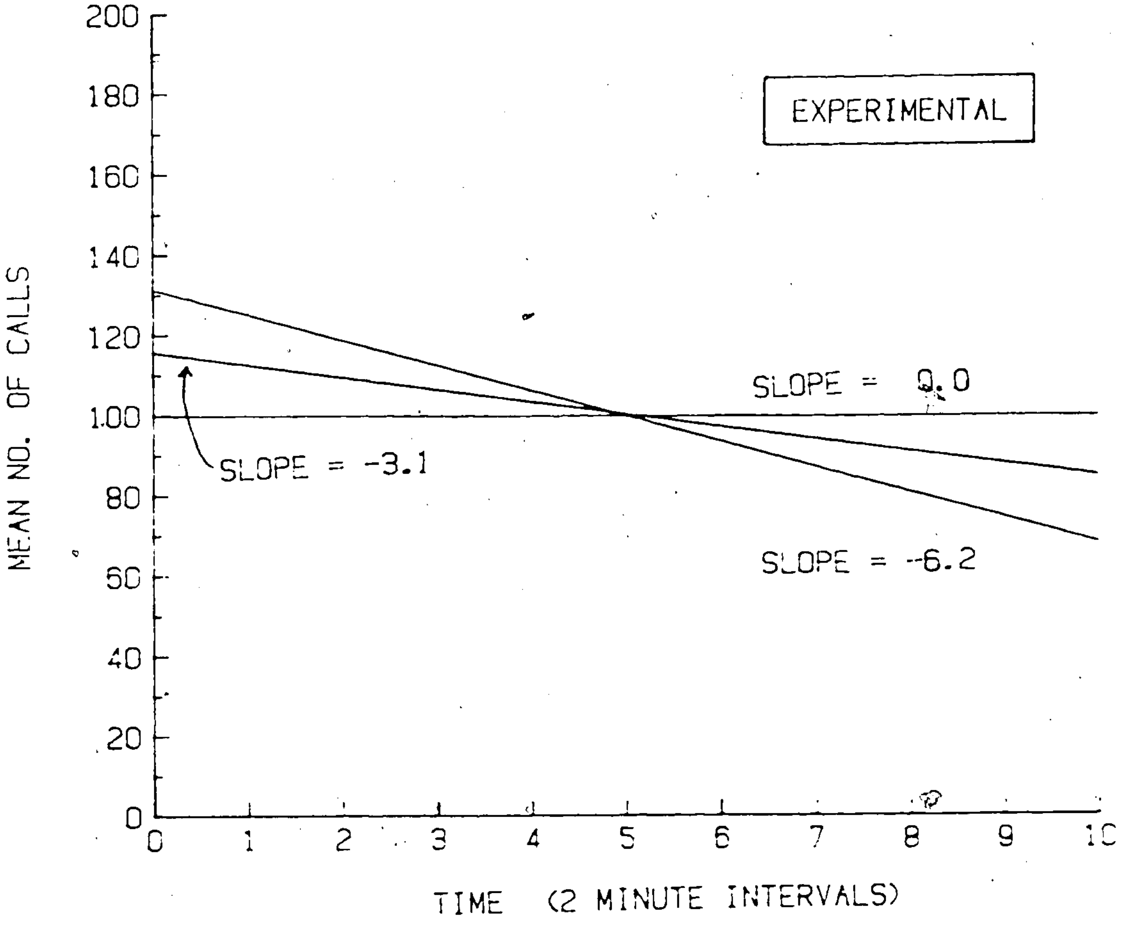


Figure 13. Normalized slopes of the number of calls for the experimental animals. The center line is the mean slope and the peripheral lines are one standard deviation away. All lines are standardized to intersect the point (5,100).

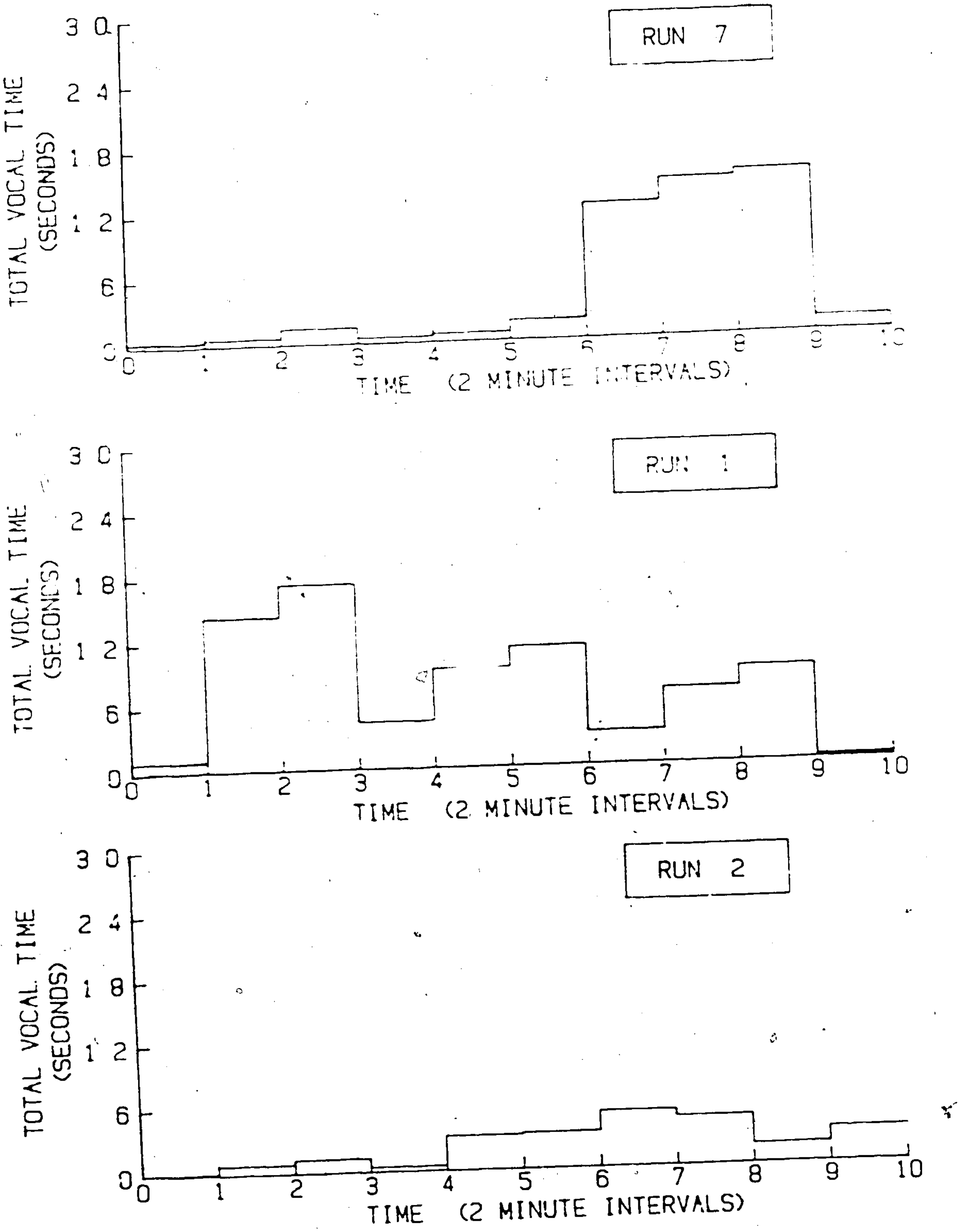


Figure 14. Total vocal time for control animals. The total vocal time is a measure of the time that an animal vocalizes during a 2 minute time interval. It is the duration of all the calls in an interval (Runs 1,2,7).

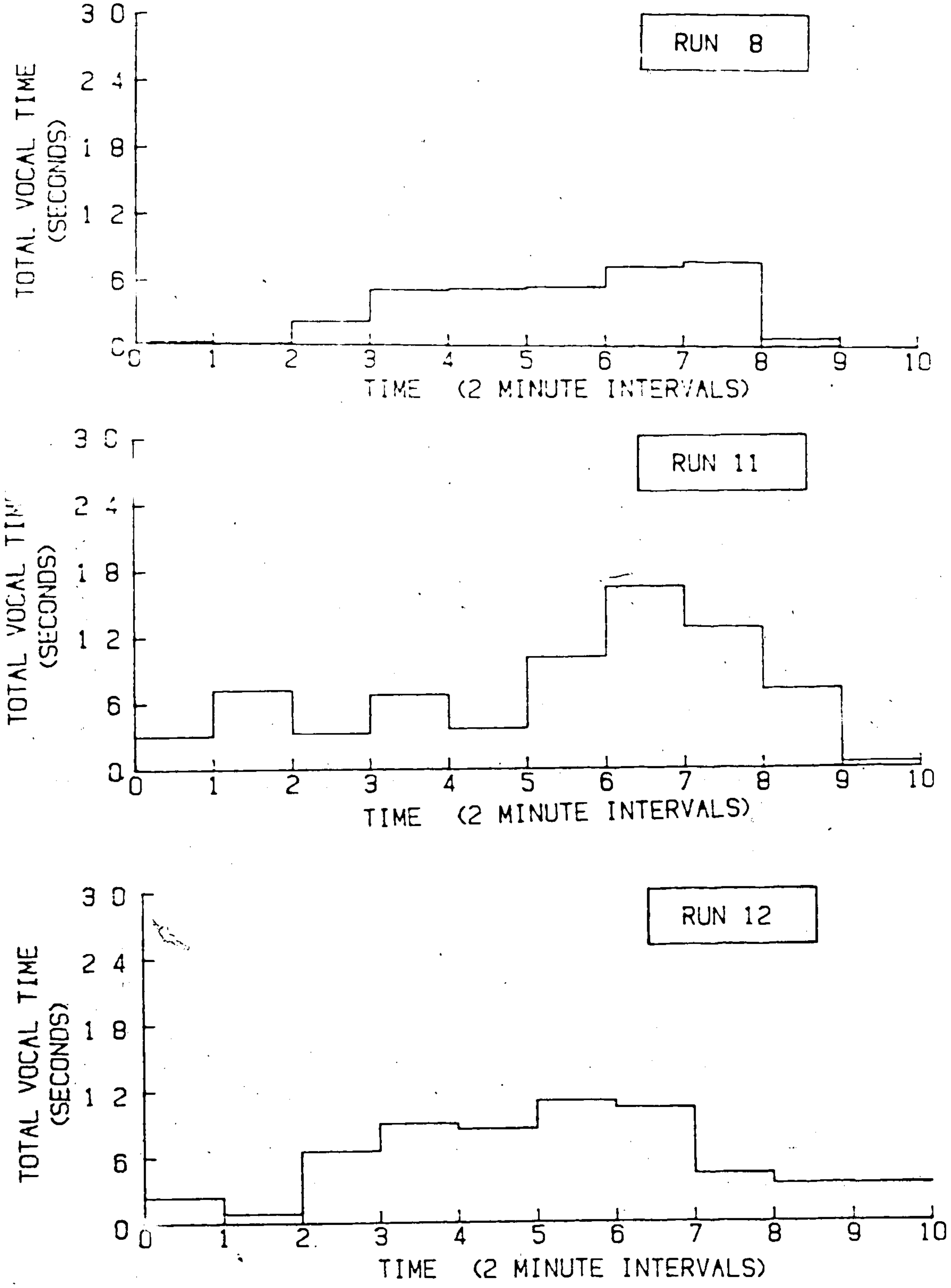


Figure 15. Total vocal time for control animals. The total vocal time is a measure of the time that an animal vocalizes during a 2 minute time interval. It is the duration of all the calls in an interval (Runs 8,11,12).

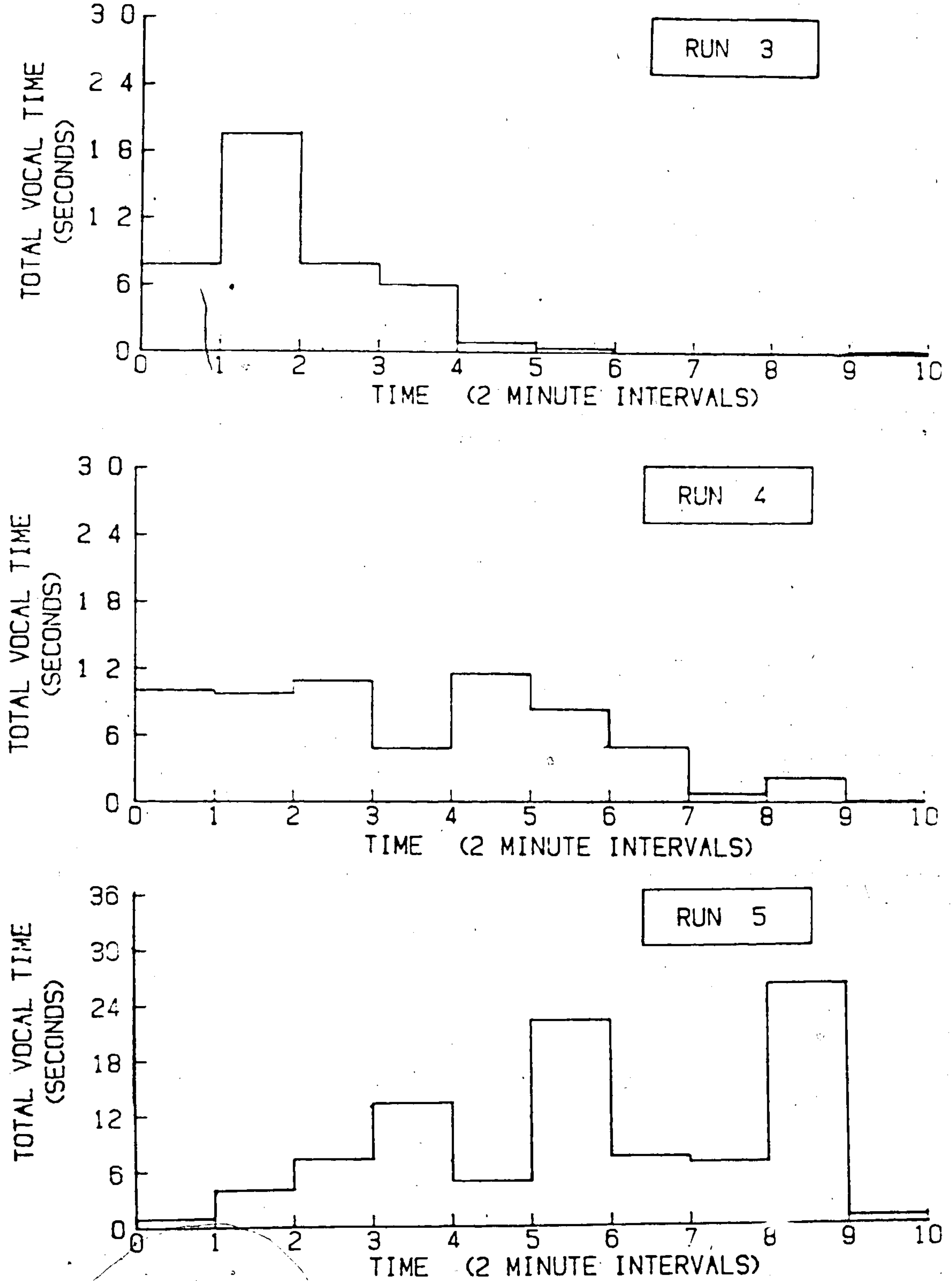


Figure 16. Total vocal time for experimental animals. The total vocal time is a measure of the time that an animal vocalizes during a 2 minute time interval. It is the duration of all the calls in an interval (Runs 3,4,5).

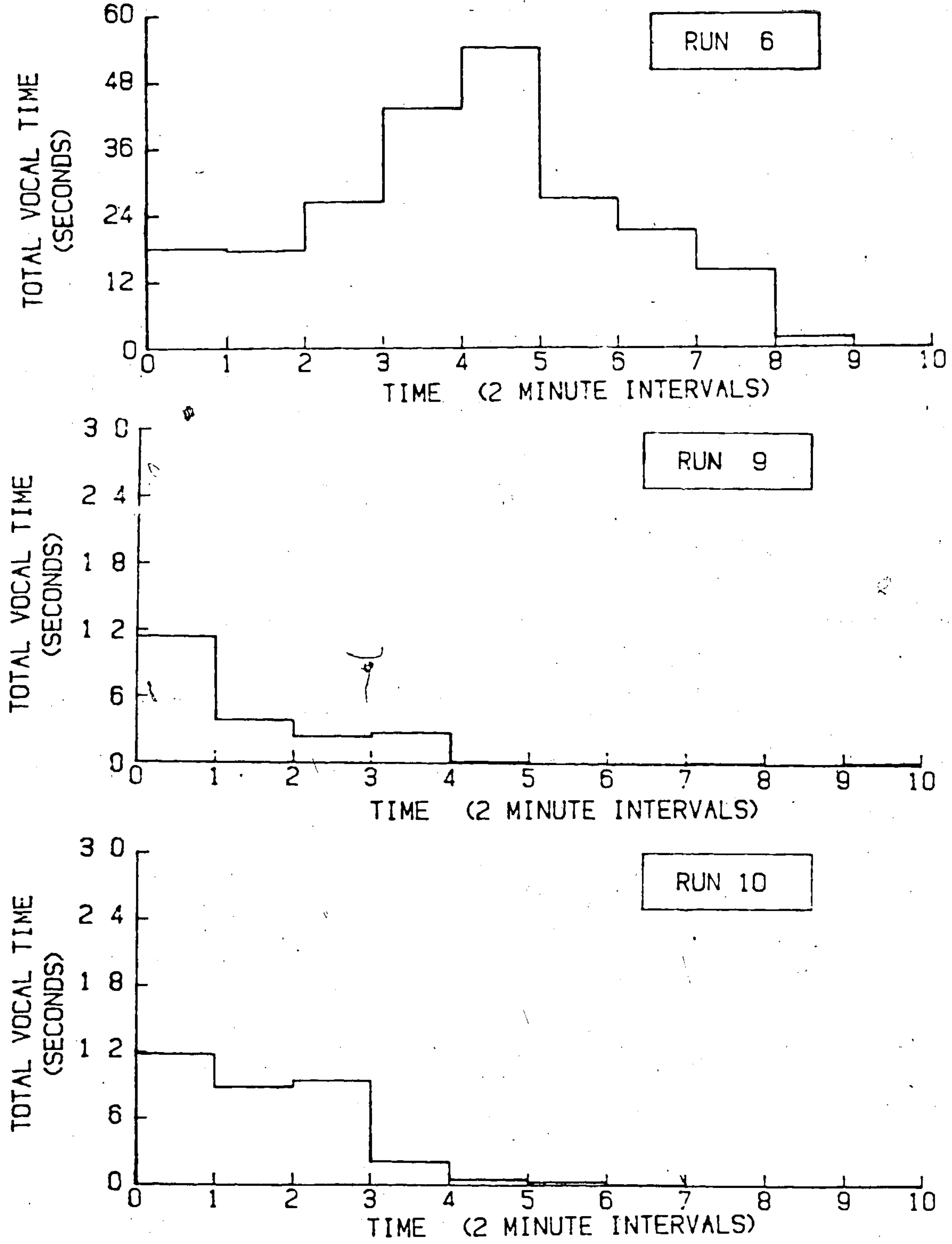


Figure 17. Total vocal time for experimental animals. The total vocal time is a measure of the time that an animal vocalizes during a 2 minute time interval. It is the duration of all the calls in an interval (Runs 6,9,10).

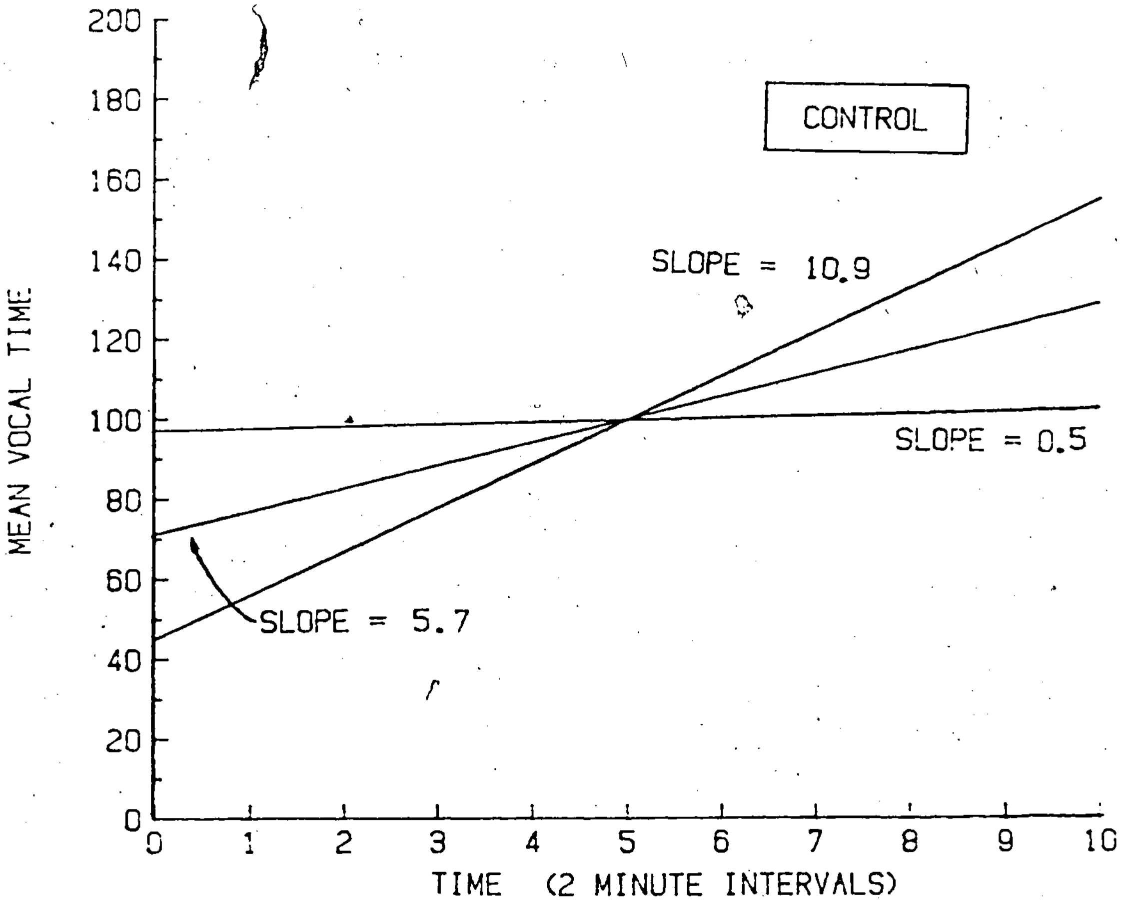


Figure 18. Normalized slopes of the total vocal time for the control animals. The center line is the mean slope and the peripheral lines are one standard deviation away. All lines are standardized to intersect the point (5,100).

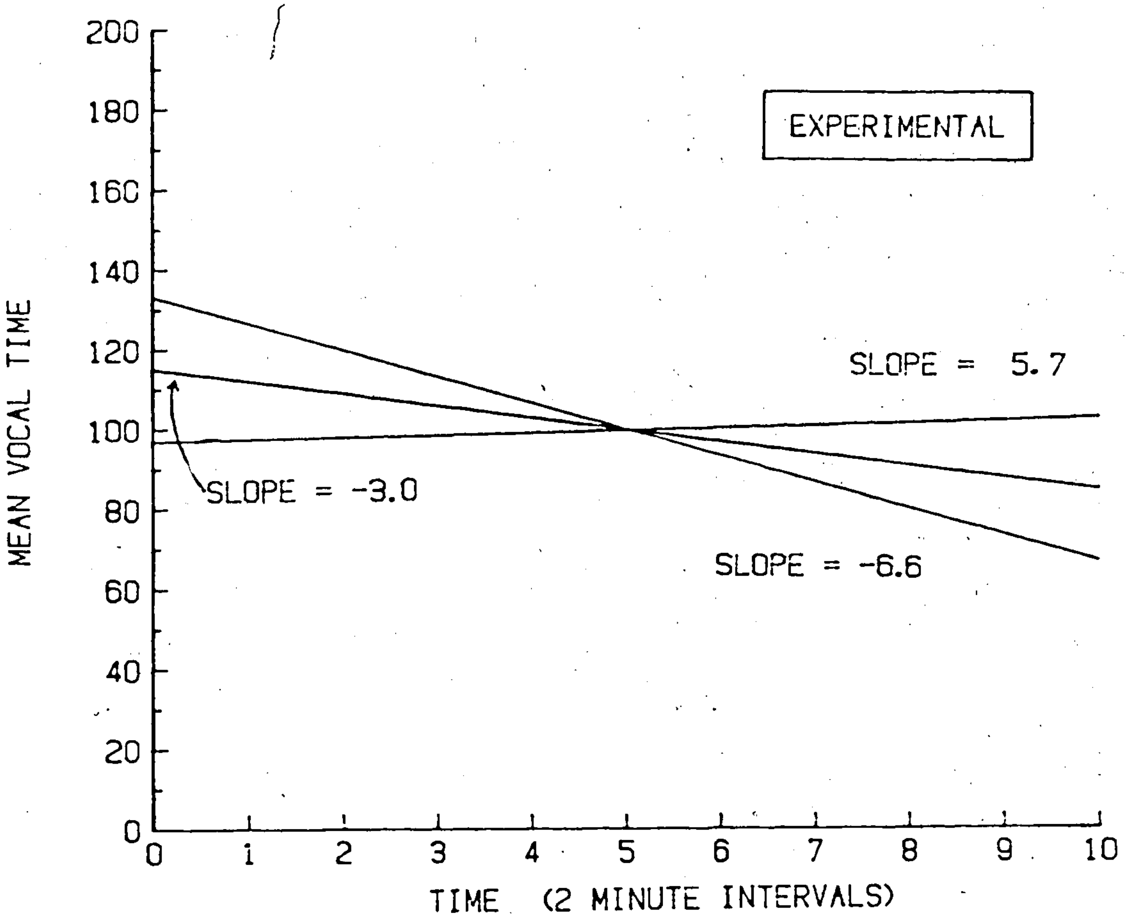
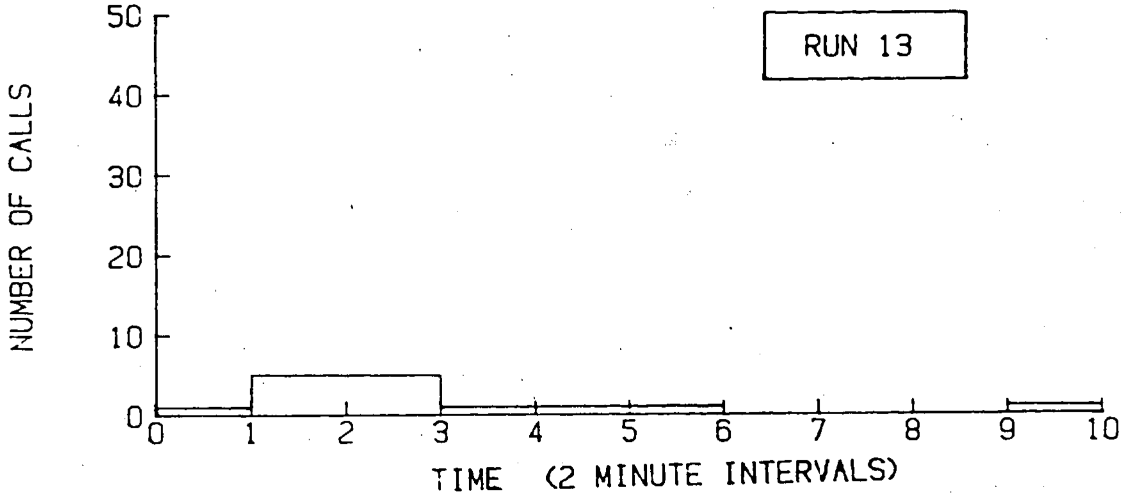


Figure 19. Normalized slopes of the total vocal time for the experimental animals. The center line is the mean slope and the peripheral lines are one standard deviation away. All lines are standardized to intersect the point (5,100).



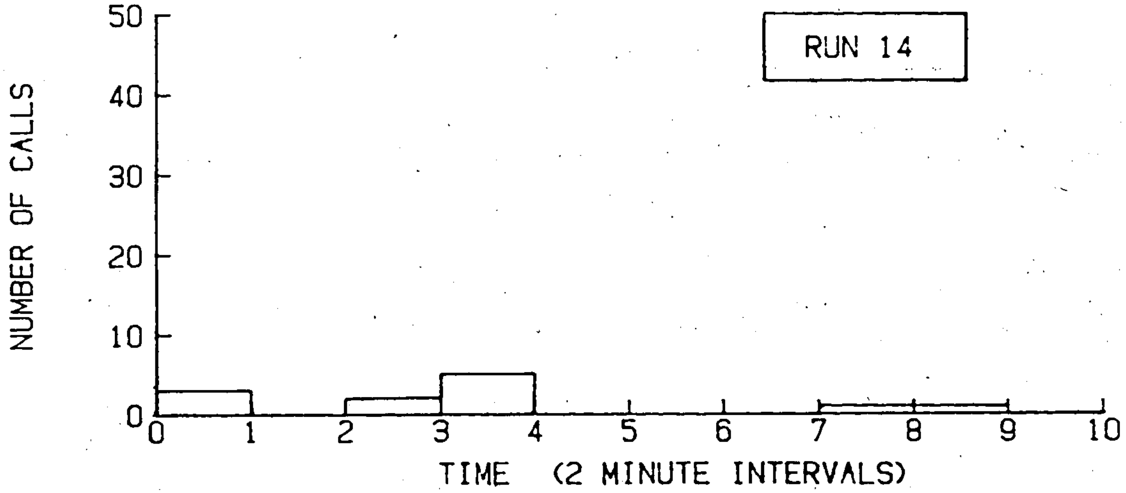


Figure 20. Number of calls for isolated animals. There are very few calls resigtered by isolated animals. Isolation from nest and maternal care is not a strong stimulus for vocalization (Runs 13, 14).

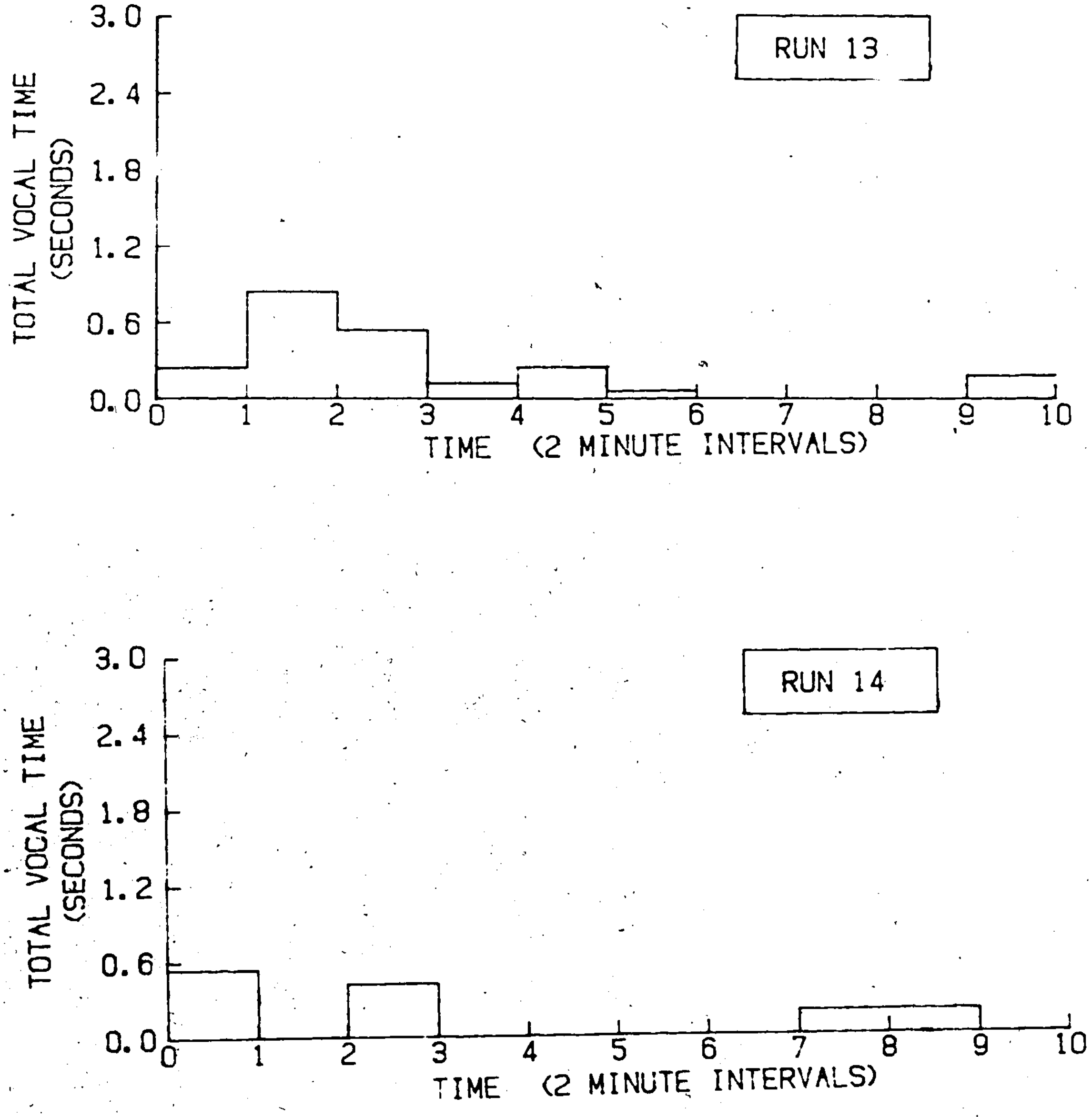


Figure 21. Total vocal time for isolated animals. The total vocal time is very small. Isolation from nest and maternal care is not a strong stimulus for vocalization (Runs 13, 14).

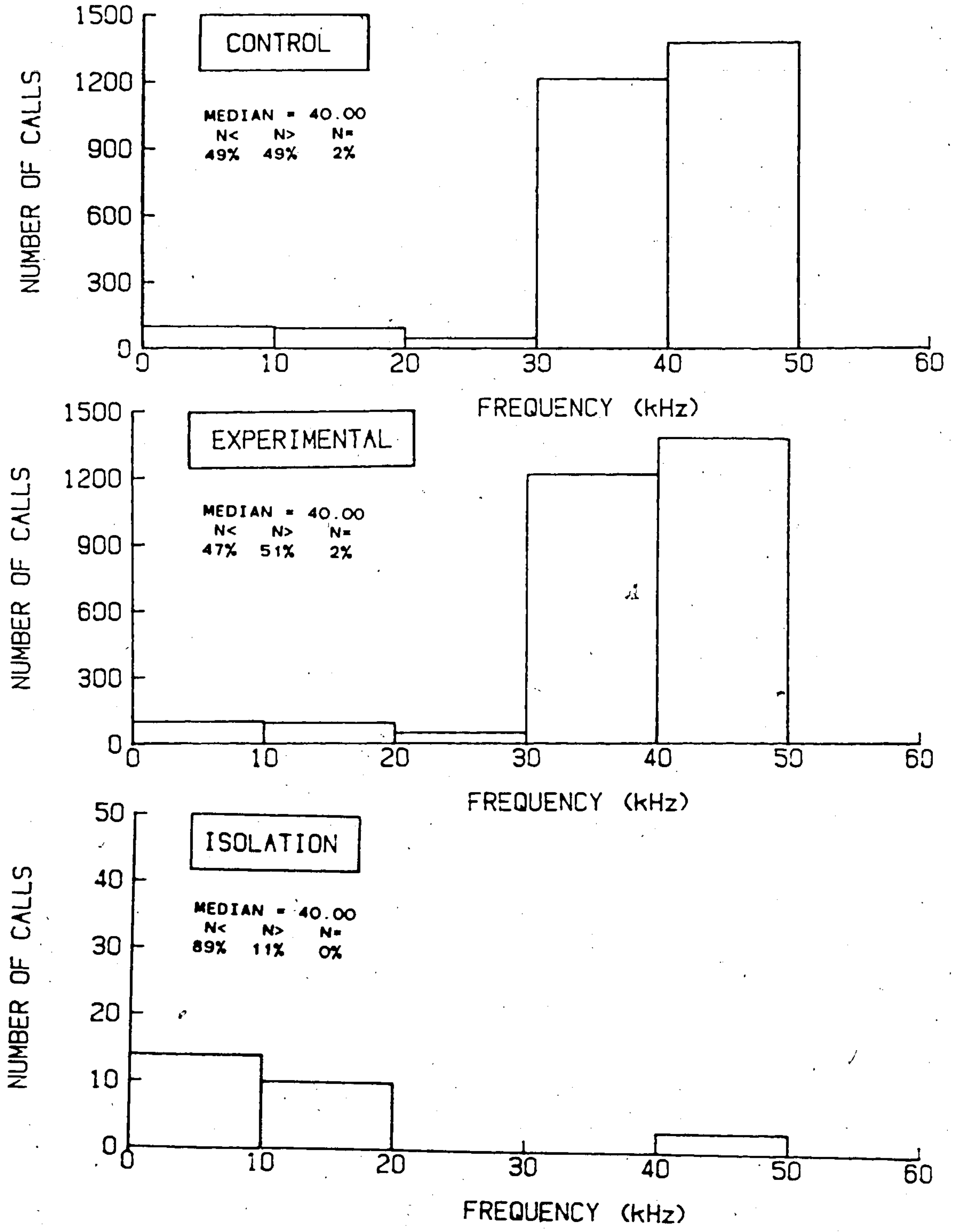


Figure 22. Histogram of the frequency of vocalization for each animal group. The median for all the calls is 40 kHz. Where N<, N> and N= indicate the percentage of the calls less than, greater than and equal to the median, respectively.

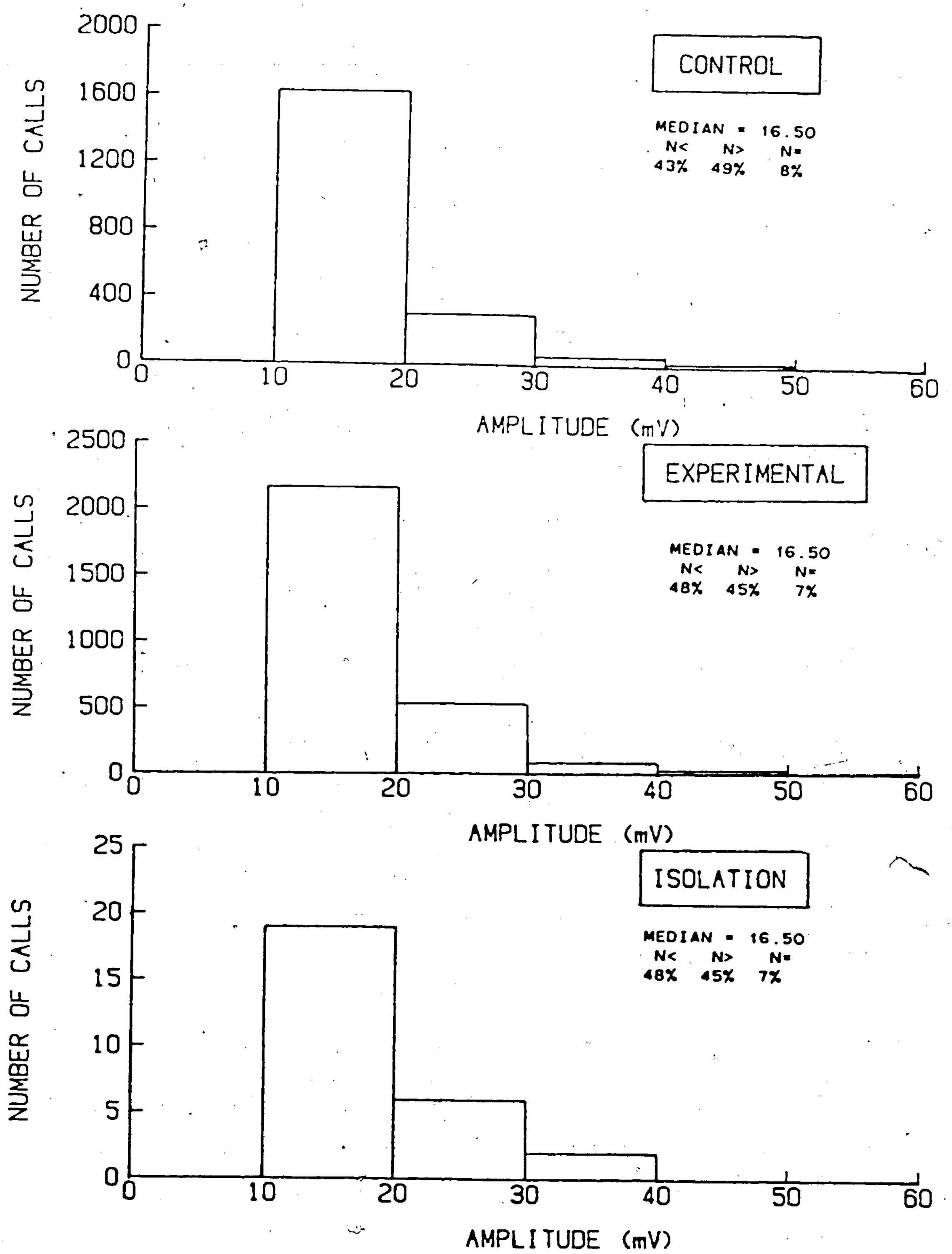


Figure 23. Histogram of the amplitude of vocalization for each animal group. The median for all the calls is 16.5 mV. Where N<, N> and N= indicate the percentage of the calls less than, greater than and equal to the median, respectively.

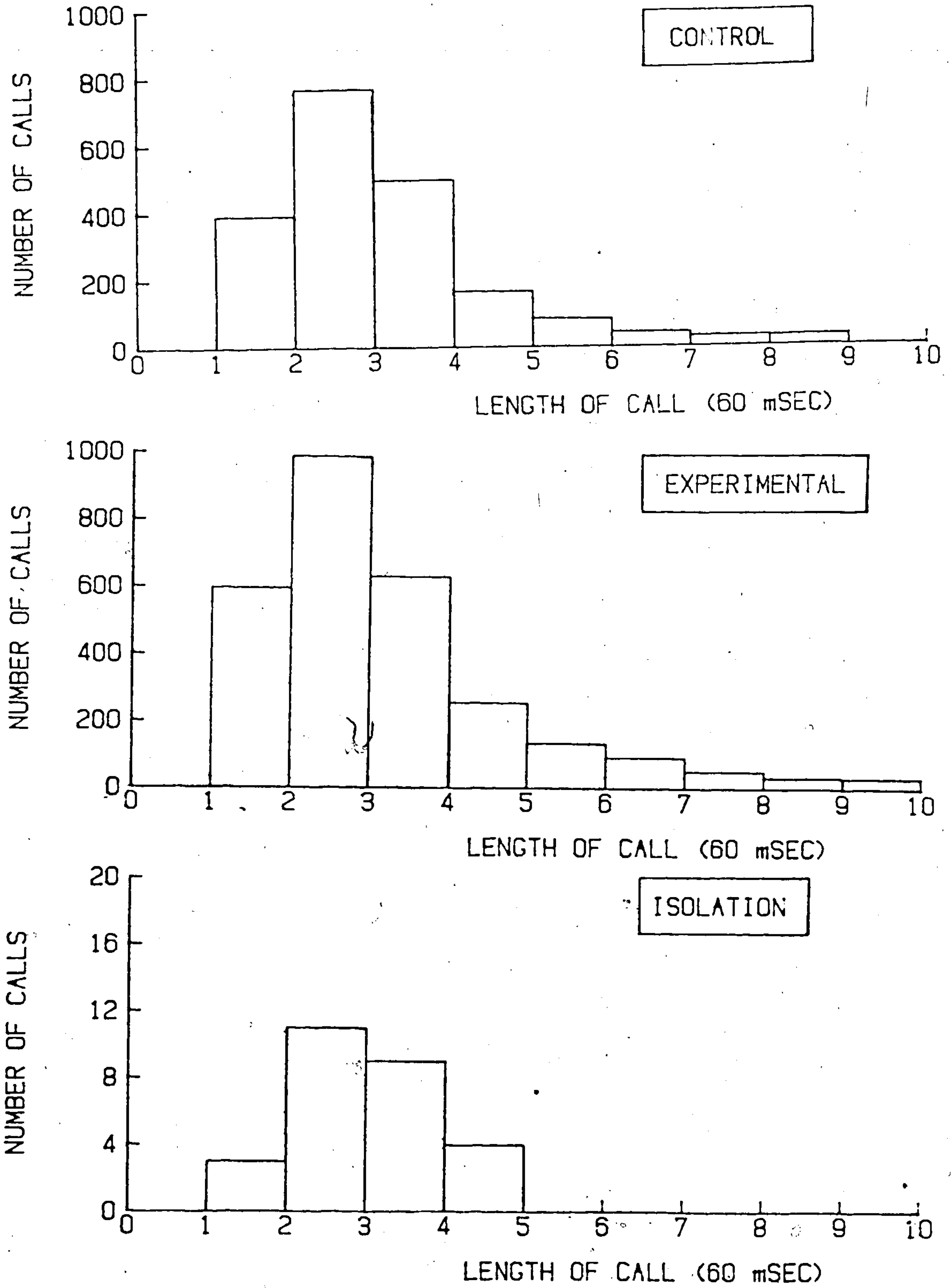


Figure 24. Histogram of the duration of calls for each animal group. The duration of the call is measured by testing for the presence of the call every 60 msec.

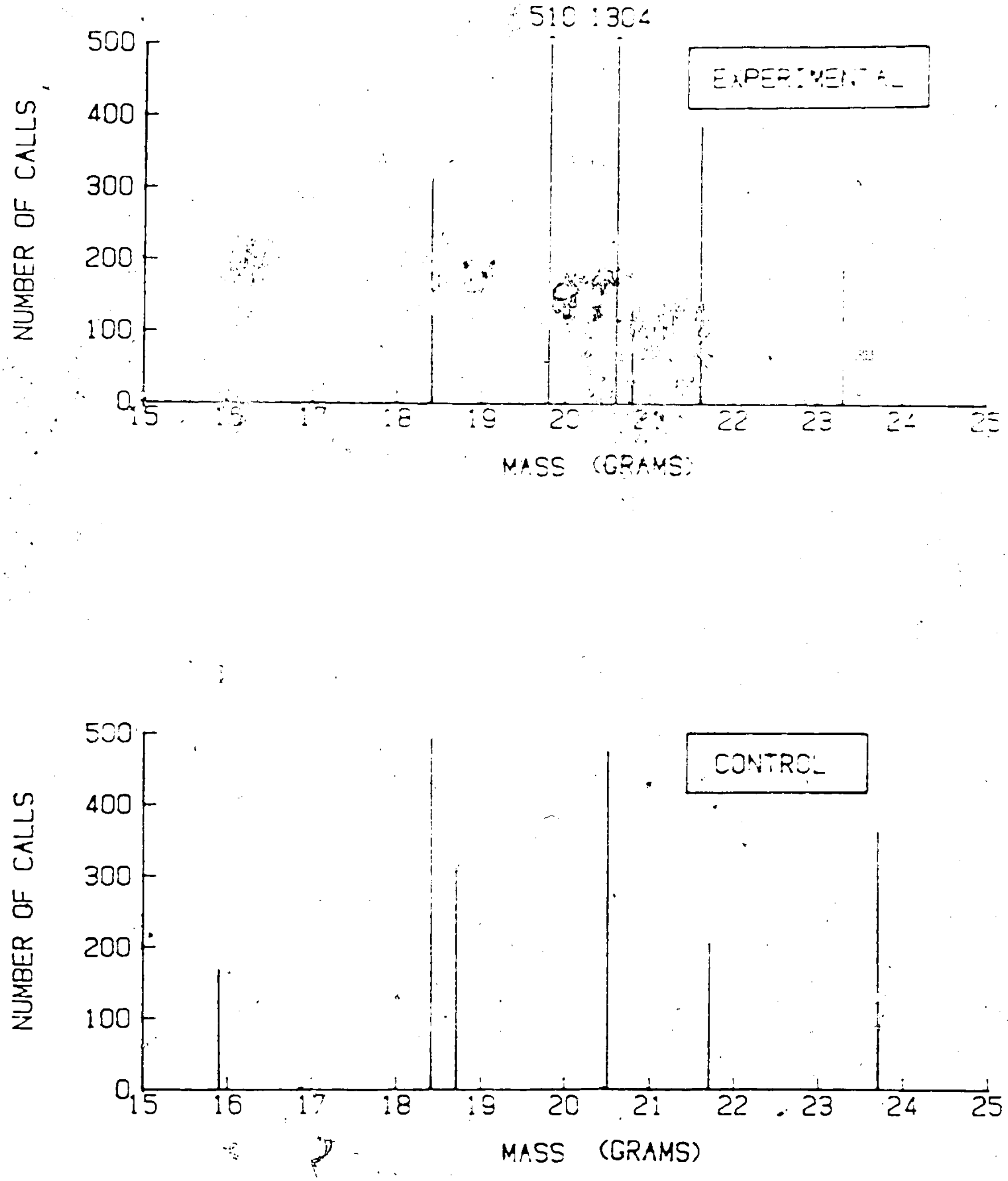


Figure 25. Number of calls per pup versus mass, for each animal group. The mass was measured by placing each rat pup on a balance after its recording session.

V. Discussion

Against the background of increasing exposure of human populations to EM radiation, studies into the biological effects of chronic, microwave irradiation are of great importance (Learner, 1980). The present thesis provides a technique to probe into this general problem. Preliminary experiments show that effects associated with the microwave irradiation of biological systems do exist. These experimental procedures are a precursor to the larger study of microwave induced neurasthenia in rodents. They confirm two basic ideas: (1) low power microwave fields are sufficient stimulus to induce changes in vocalization of rodents, and (2) the microprocessor-based, ultrasound recorder (which was constructed for this experiment) can identify the variations in the vocalizations of the animals. The results of the experimental procedures used are now discussed.

The digital ultrasound recorder does provide a technique for monitoring ultrasound signals. The recorder is able to measure the frequency and the amplitude of the input signal with an error of less than 5% and 10% respectively.

Data transfer between the recorder and the Amdhal 5860 computer was basically error-free. There were no instances where the rate of calling was sufficiently high to cause the local memory buffer to overflow. The output transfer routine operated at a rate of 1200 baud. At this operating speed, the 10 kilobyte local memory buffer would take 83.3 seconds to empty. This assumes that there are no calls during the transfer. A single call would have to be 300 seconds long to fill the buffer completely. Neither of these conditions is expected to occur.

The ultrasound recorder is able to collect and transfer salient information, about the ultrasound vocalizations of rat pups, without functional difficulties. It is shown that this detection system can define the differences in the calling patterns of thermally stressed rat pups.

The differences in calling cannot be attributed either to separation from the nest or lack of maternal care, because vocalizations of isolated rat pups are significantly less frequent than those from the control or experiment animals. The vocalizations occur only after a change in the body temperature of [illegible] rat. Therefore, the vocalization may be affected by a thermal stimulus, such as a radiant microwave field.

From an analysis of the results of these experiments it is found that there are significant differences in both the slope of the number of calls and the slope of the total vocal time. The detection system measures many other parameters such as amplitude, frequency and duration of calls. These parameters do not show drastic variation when rat pups are exposed to a thermal insult.

The frequency of vocalization does show some interesting effects in our experiments. The frequency histograms indicate changes in vocalization due to the thermal stimulus. There are calls in the 0 kHz - 20 kHz band and calls in the 30 kHz - 50 kHz band. Allen and Banks (1971) and Okon (1971) studied the vocal activity of rat pups as a function of temperature. Allen and Banks specified the 30 kHz - 50 kHz band as commonly used by thermally distressed pups. Okon showed that vocal activity is greatest during changes in the body temperature, and that after the core temperature stabilizes, vocalizations are not as common.

The amplitude of vocalization has a small variation between control and experimental groups. These variations can be the result of many factors. There may be a genuine difference in acoustic pressure, or the position and orientation of the vocalizing animal may change, relative to the microphone. The surroundings of the animals can cause changes in this reading. Echoes from solid objects can produce interference patterns, artificially increasing or lowering measurements.

There were no significant differences found in the length of individual calls. The duration of most vocalizations was two measurements long. The calls were not measured with great accuracy in this experiment. If the data collection rate were increased, it would be possible to measure call duration with a finer grading. It would then be possible to make a more detailed analysis.

Finally to review this study, a microprocessor-based, detection and datalogging system was designed and constructed to monitor rat ultrasonic vocalization. The system measures four basic parameters of the vocalizations: the amplitude, the frequency, the period of the calls, and the time that the call began. This system can store the information it collects in a file in the Amdhal 5860 computer (which operates under the Michigan Terminal System (MTS)). Statistical analysis of the data can be carried out on this computer.

The microprocessor system was used to collect data from the vocalizations of rat pups exposed to thermal stress. 10-11 day old rat pups were individually monitored by the system. The pups were removed from their nest and maternal care, and placed in an ice-bath for 10 minutes. They were then removed and placed in the animal holder of a microwave irradiation system. They were allowed to warm up under either sham-irradiation or a 2 W microwave field. During the 20 minute warming period, the ultrasound detection system was switched on and the vocalizations of the pups were monitored. Pups were weighed and then returned to their nest.

The detected vocalizations were stored in a file on the Amdhal 5860 computer. Using the MTS operating system and a Pascal program, the data was reformatted to allow analysis by the statistical software package, MIDAS. By applying recognized statistical techniques it was shown that there are significant variations in the data collected from control and experimental animals. From these experimental procedures it was also shown that the detecting system can record variations in the ultrasound of rat pups exposed to microwave and sham radiation.

The results of the experiments show that it is possible to determine statistically significant differences in the vocal activity of these rat pups. Further studies can now be based on these findings. There are three areas in which further work could be carried out. Most immediately, the analysis of data could be done in more complex ways. Secondly the detection system could be made more flexible by writing more software or by developing a new system, using the present one as a model. Finally, further animal experimentation would produce more data and would begin the study of microwave induced neurasthenia.

The analysis of data is, at present, done at the single variable level. That is, a particular variable is defined and studied to determine if its values have significant variation between control and experimental data. The analysis is directed without consideration of other variables that are measured at the same time. A multivariable analysis of variance may provide interesting avenues of study.

Similarly, but on a larger scale, data collected about a single call could be defined as a single point in a four space of amplitude, frequency, call duration, and time of call. If this compound reading technique were done, cluster analysis could be used (Wishart, 1978). Regions in the four space could be grouped and assigned specific meaning with regard to behavior or physiological stimulus. The calling patterns of rats in different experimental conditions, could be analysed under this framework.

Although the detection system, as defined, is fully functional in its present state, some improvements could be made in its basic operations. At present there is no method by which the system can collect more information. The system could be expanded to test more inputs each time it records data. The researcher could then cue information about the experimental conditions into the data. A facility to mark points of special interest in the data would be available. This technique could be used to trigger the system to perform some special function such as the transmission of data.

If a completely new system were to be built, a more powerful CPU, operating at a higher frequency, would allow higher rates of data collection. The operating system could be improved to make the recorder easier to use.

At the time of development, the necessary digital technology was not available to allow a FFT to be carried out on the data as they were being collected. A new unit operating at higher speeds and using the most up-to-date components may be able to achieve this. This would ally fears that noise signals could obstruct a frequency reading based on the zero-crossing rate.

Many animal experiments will have to be carried out before a reference data-base can be developed. Rats are known to vocalize under many different situations. Mating, fighting, handling of infants, dominance and submission are some examples in which ultrasound vocalizations are normally produced.

The analysis of the vocalizations of depressed rats will be very important to the long term study of exposure to microwave radiations. Since the development of neurasthenia has been thought to be associated with microwaves, this chronic depression may be manifested in ultrasonic vocalizations.

Under the conditions of this study the following limitations are recognized:

1. Laboratory rats of the Sprague-Dawley strain were used in this study.
2. The only manifestation of stress measured was the ultrasonic vocalizations of rat pups.
3. Only severe short term stress from cold was monitored.
4. The recorder detects only single frequency tones. It can only be used for studying animals that vocalize in this way.

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Appendix I - An analysis of hypothermal stress

When a rat is placed in a cold environment it, quite naturally, loses heat to its surroundings. An adult rat will increase its metabolic rate in order to maintain a normal body temperature. Rat pups, which are less than 21 days of age, do not have fully developed thermo-regulatory systems. When pups are exposed to cold temperatures, their core temperature drops rapidly. In a study of the relationship between temperature and vocalizations of infant hamsters and rats, Okon (197[illegible]) monitored the core temperature of pups exposed to 2-3 °C. His findings for 6 day old Wistar rat pups indicate that core temperature drops to 16 °C in [illegible] minutes, to 8 °C in 20 minutes and to 6.2 °C after 60 minutes.

In the present experiment the rat pups were 10-11 days old. The rate of cooling in these pups was examined by measuring the changes in core temperature of two pups exposed to cold. A copper/constantine thermocouple was inserted into the rectum of the pups after they were removed from the nest. The end of the thermocouple was taped along the tail of the animal to ensure that the tip of the thermocouple remained in place. The pups were placed in a closed ice-bath at a temperature of 1-2 °C. The cooling conditions were identical with those used for control and experimental animals. Figure 26 shows the change in core temperature for each pup (measurements taken once every minute). Room temperature at the ti[illegible]of testing was 24 °C.

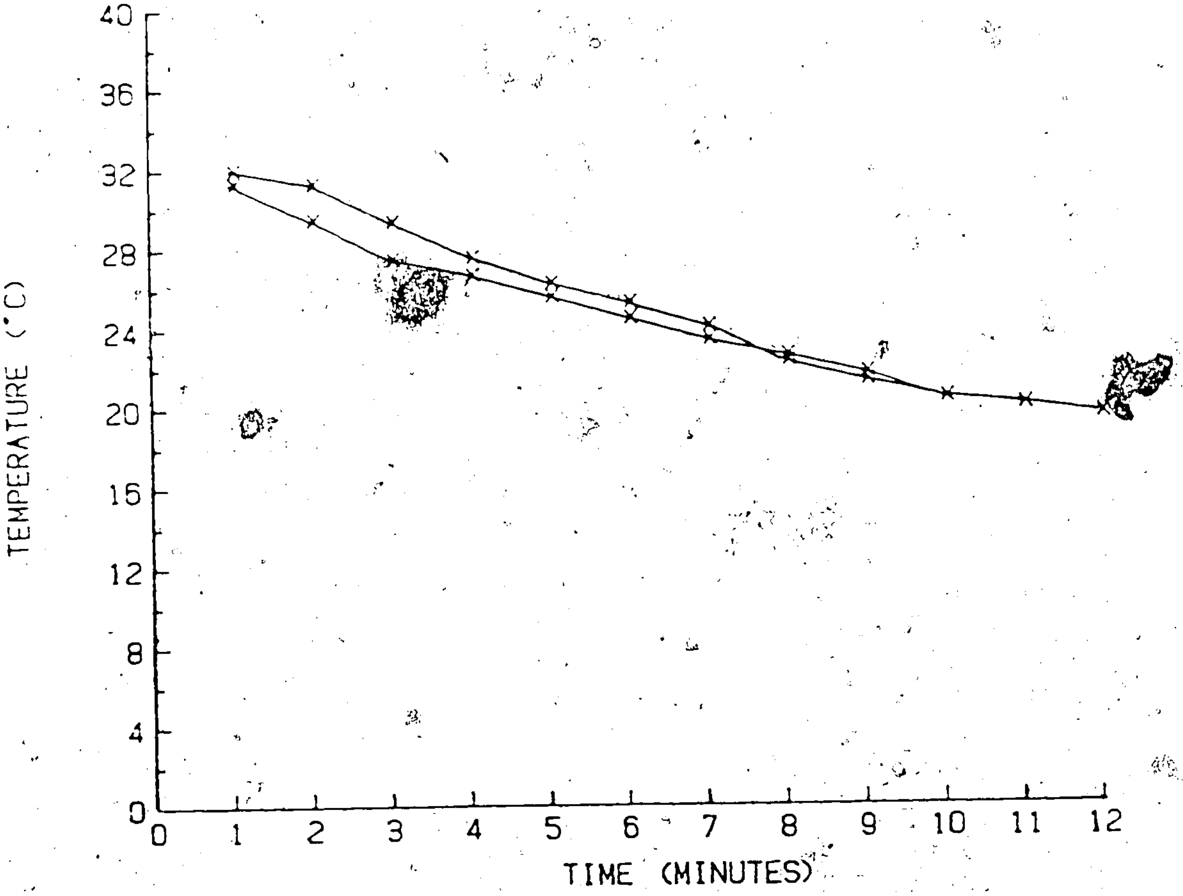

Figure 26. Core temperature of rat pups when placed in a 1-2 °C ice-bath. A copper/constantine thermocouple was inserted into the rectum of the pups to make measurements.

The results show that in 10 minutes these animals dropped to an average temperature of 20.2 °C. There is a 4 °C difference between Okon's result and this value. This higher reading is understandable in light of the pup's age. Assuming that the normal body temperature for these pups is 37 °C, they experienced a 17 °C drop in core temperature. The average mass of these animals is 20.5 grams. Therefore they lose 348.5 calories or 1519.8 Joules of energy.

Appendix II - Absorption of circularly-polarized EM waves

A nonstationary object, such as a rat, absorbs power in a relatively uniform way in a circularly-polarized EM field. A physical argument to explain this absorption follows from the definition of the radiation field. A circularly polarized electromagnetic field can be defined as the sum of two plane polarized fields 90° out of phase in both time and space. The two fields are defined $Q_1(E_1,H_1)$ and $Q_2(E_2,H_2)$. E_1 is parallel to H_2, and E_2 is parallel to H_1.

It is well known that for plane wave radiation the highest rate of energy deposition occurs when the electric field is polarized along the longest dimension of the body ($E\|L$) (Gandhi et al., 1977). In the other orientations, $H\|L$ and $K\|L$, the rate of energy deposition is approximately half.

A rodent in a circularly-polarized field has three possible orientations with respect to the field. It can either be parallel to H_1 and E_2, parallel to E_1 and H_2, or perpendicular to E_1, E_2, H_1 and H_2. No one position will result in the rat absorbing a significantly larger amount of energy. In two positions it is parallel to both the E field and the H field, and in the third position it is perpendicular to all fields. The drastic variations from parallel to E, to parallel to H, are always avoided. Therefore, the fluctuations in power absorption are minimized.

Appendix III - Pascal and Midas computer programs

Midas computer program

```
read file=datafile format=i5,2i3,3i4 var=1-6 label=run, expo, intr,call,ampl,fre cases=1-14000
tran v1=v1-3 l=*
code var=1-4 results=1-4 function=* label=*
trans v100=linear v=1,3,4 rel=100000.,1000.,1. l=*
code v101=ordinal(v100) l=levels
trans v200=linear v=1,3 rel=100.,1. l=*
code v201=ordinal(v200) l=nlevels
compute @new strata=v101 var=1-4,101,201 result=1-4,101,201 function=* l=*
compute @new strata=v101 var=5,6 result=5,6 function=mean l=c mamp, c mfre
set @data strata=none
code strata=v101 v66=v5>0 l=cperiod
compute @new strata=v101 v7=count(v66,1) l=vperiod case*all
set @data strata=none
compute @three strata=v201 var=1-3 result=1-3 function=* l=*
compute @three strata=v201 var=4 result=4 function=max l=tcall
set @new strata=none
compute @three strata=v201 v=7 result=v5 function=sum label*vtime
write @three v=all strata=none file=* fo=*
set @data strata=none
trans  v50=linear v=6 rel=.3333 l=freq
describe byst st=run v=2,3,4,5,50
describe byst st=intr*expo v=5,50
histogram byst strata=expo[illegible]50 i=(0,)/10 o=leftend,hist%.nonempty
histogram byst strata=expo[illegible]5 i=(0,)/10 o=leftend,hist%.nonempty
set @new
trans v50=linear v=6 rel=0.3333 l=c mfreq
histogram byst strata=expo v=50 i=(0,)/10 o=leftend,hist%.nonempty
histogram byst strata=expo v=5 i=(0,)/10 o=leftend,hist%.nonempty
describe byst st=intr*expo v=7
oneway v=1 strata=none option=marg
twosample v=5,50 st=expo:1,2
twosample v=5,50 st=expo:1,3
twosample v=5,50 st=expo:2,3
set @three strata=none
compute strata=v1 v=5 result=v7 function=sum label=vtime
compute strata=v1 v=4 result=v6 function=sum label=tcall
tran strata=v1 v8=v4/v6 l=ftcall
tran strata=v1 v9=v5/v7 l=fvtime
set @three strata=none
compute @four strata=intr:(1-5)*run v=8,9 function=sum result=v10,v15 label=sfTcall, sfvtime
write v=all strata=none fo=* fi=*
set @four strata=none
tran strata=run v11=1.-v10 l=*
tran strata=run v12=v10-v11 l=cslope
tran strata=run v16=1.-v15 l=*
tran strata=run v17=v15-v16 l=vtslope
write v=all strata=none fo=* fi=*
student  v=12 st=expo
student  v=17 st=expo
finish
```

Appendix IV - Circuit design of the digital ultrasound recorder

The recorder is constructed from three main circuit boards and various connectors and plugs. Two of these boards hold wire wrapped IC's and the third is the Motorola MEK D5 circuit board. The three boards are interconnected by a 60 wire ribbon cable. A chip layout of the two wire wrapped boards follows.

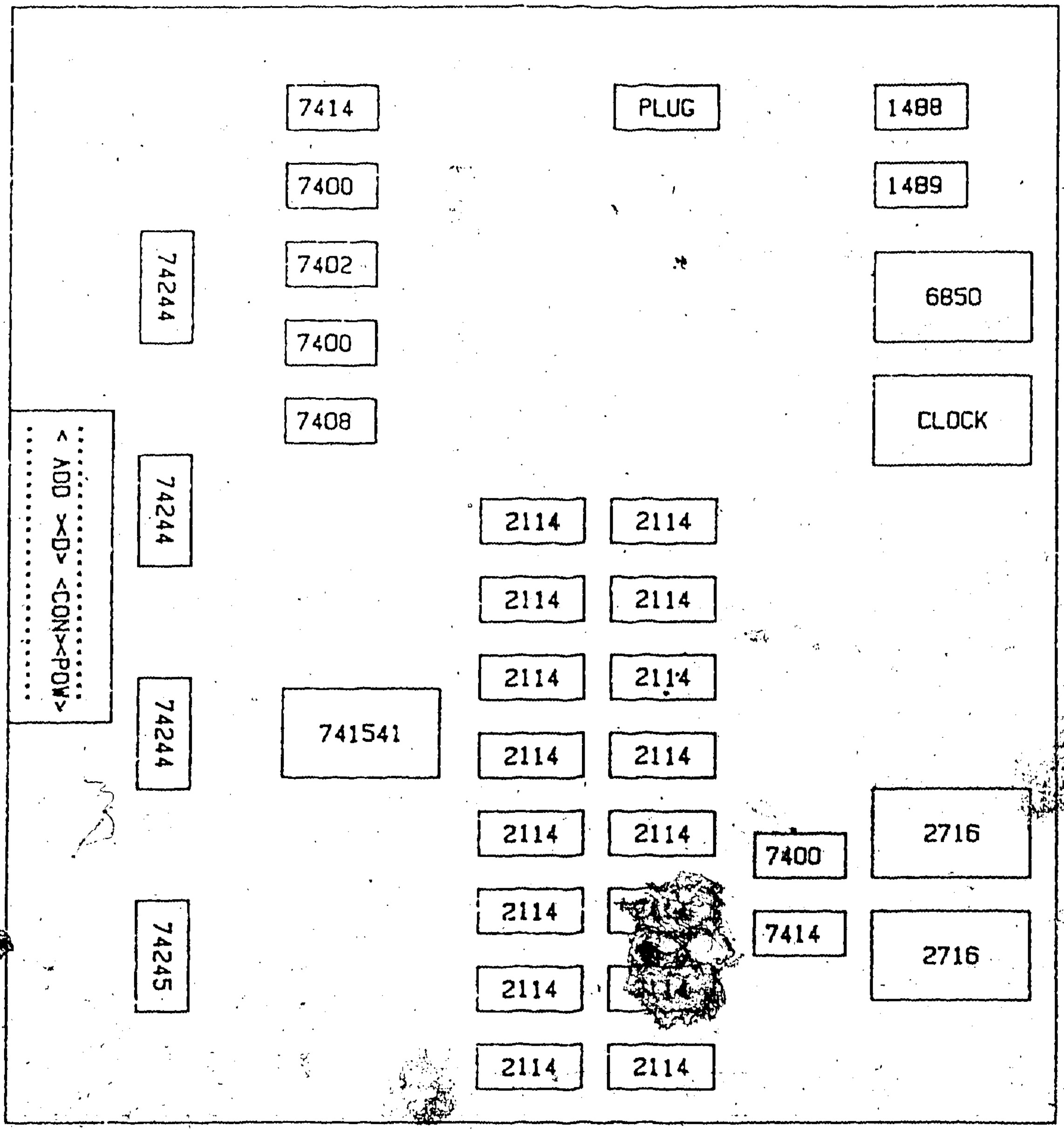

Figure 27 Circuit Board 1 - Memory and RS-232 interface.

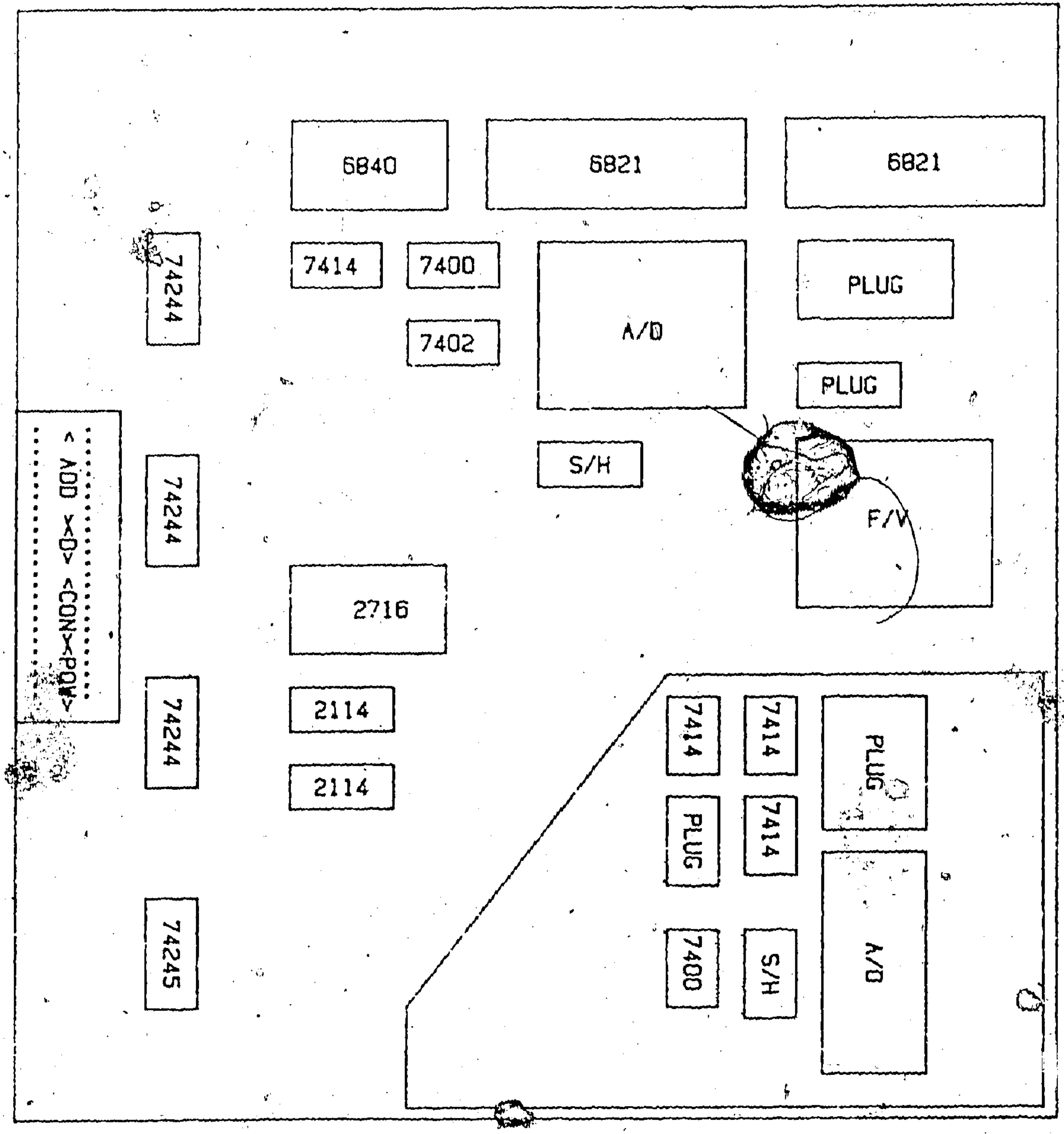

Figure 28 Circuit Board 2 - Digital conversion.