SHORT TERM EFFECTS OF EXTERNAL ELECTRIC FIELDS

ON ELECTRICAL ACTIVITY OF THE PINEAL GLAND

IN RATS

THESIS

Presented to the Graduate Council of the

University of North Texas in Partial

Fulfillment of the Requirements

For the degree of

MASTER OF SCIENCE

By

Hung Q. Vu, B. S.

Denton, Texas

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Vu, Hung Q., <u>Short term effects of external electric fields on electrical activity of the pineal gland in rats.</u> Master of Science (Biology), May, 1996, 43 pp., 1 table, 12 figures, bibliography, 28 titles.

The effects of short term exposure (5 minutes) to EEFs at relatively high dosages (10, 25, 39, kV/m) on the electrical activity in rat pineal glands was studied. Daytime and nighttime recordings were taken from an implanted microelectrode in the gland. The data show that (1) both the activity and frequency were enhanced when the animals were exposed to EEFs at 39 kV/m continuously and discontinuously; (2) the later condition yielded a sustained increase (36%) whereas the former a brief (10 sec) increase. This enhancement was statistically significant under both conditions (day and night). The effects observed were thought to be due to membrane alterations either in the pineal gland itself or in the neural inputs to the gland.

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CHAPTER I

INTRODUCTION

Over the past few decades, there has been a persistent controversy over whether external electric fields (EEF), such as those formed under and near overhead high power lines, are potentially dangerous to one's health [Miller et al., 1978; Marino et al., 1977]. In the 1980's numerous epidemiological reports indicated that the incidence of cancer was significantly higher in humans and in particular, children who resided near overhead high power lines. Behavioral and CNS changes during [Lott et al., 1973] and following exposure to EEF have also been studied in depth [Hjeresen et al., 1980; Gavalas et al., 1970].

Several workers have calculated the EEF directly under a 765 kV power line and have found it to be about 10,000 V/m [Marino et al., 1978]. Earlier evidence of EEF's effects on humans came from Russian studies involving electric utility personnel working around 500-700 kV/m substations. Headaches, malaise, abnormal fatigue and sleepiness were reported from a majority of the workers; Some young men even complained of reduced sexual potency [Korobokova et al., 1972]. As a consequence, a restriction area under power lines and safety regulations was established. In addition, Wever's experiment [1970] involving humans indicated that,

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in an underground bunker where subjects were isolated from environmental cues, an EEF at 300 V/m shortened and desynchronized the circadian periods.

The pineal gland in mammalians is the end organ of the visual system, and produce primarily a neurohormone, melatonin (MT) that has been involved in regulating circadian rhythms. Not only do MT levels but also spontaneous electrical activity in the pineal gland exhibit a circadian rhythm (i.e. they increase at night and decrease during daytime [Reuss et al., 1984; Reiter, 1991]). Several reports have shown that exposing rats to EEF interferes with the usual increase of nocturnal MT levels in pineal glands. In 1981, Wilson [1981] exposed rats to a 65 kV/m electric field in a grounded system for 30 days; he found that the normal rise in nighttime pineal levels of MT and the activity of N-acetyltransferase (NAT), the enzyme involved in MT synthesis, were significantly suppressed. In 1986, Wilson et al. [1986] again exposed rats to a lower EEF. They observed the same effects of reduction in nocturnal MT and NAT activity. Reiter [1988, 1993] exposed rats to 60-Hz EEFs of 10, 65 and 130 kV/m from conception to 23 days of age. He observed that all electric field strengths decreased the nocturnal level of MT. Later, Reiter and Grota [1994] performed another experiment in which rats were exposed to a 65 kV/m field for 30 days. The MT in the blood serum, the pineal gland and activity of NAT and Hydroxyindole-o-

methyltransferase (HIOMT) were then analyzed. They discovered that the EEF did not reduce the normal rise in MT levels, NAT and HIOMT activity in pineal glands. Other investigators [Lerchl et al., 1990; Wilson et al., 1990; Lerchl et al., 1991] also studied the effects of both magnetic field (MF) and EEF on MT production in the pineal gland and came up with contradictory results.

Ruess [1987] in his review described the electrical activity of the pineal gland under varying conditions and listed several factors that influence pineal activity. Such factors include superior cervicle ganglia input, age, the habencular nuclei, the paraventricular nucleus (PVN) of the hypothalamus, sciatic nerve stimulation, acoustic stimuli, light and magnetic stimuli. This study will involve another possible external stimulus: an external electric field.

Most of the recent work on EEF effects on the pineal gland had been biochemical in nature and have involved long-term exposures (weeks/ months) at relatively high dosages (10 - 100 kV/m). The literature regarding the effects of short-term exposure to EEFs on the electrical activity in the pineal gland is currently sparse if not non-existent. The general scope of this study, therefore, was electrophysiological in nature since the electrical activity of pineal glands was recorded from microelectrodes implanted within the pineal gland in rats.

The specific purpose of this study was to measure the

electrical activity of rat pineal glands before, during and following short-term exposure to varying dosages of EEF (10, 25, and 39 kV/m), and under varying conditions (e.g day versus night; continuous versus discontinuous exposure).

CHAPTER II

MATERIALS AND METHODS

A total of 120 (60 males and 60 females) Sprague-Dawley
rats with average weights of 200 grams were used in this
study. In this weight range the brain size does not vary.
The age of the rats ranged between 8 to 11 weeks old. This
number excludes those animals used in developing the
experimental technique or those that died, or those in which
the electrodes were incorrectly implanted. They were housed,
one per cage, given lab chow and water ad libitum, and were
kept on a 12 hour light/dark cycle (light on at 6 AM, off at
6 PM). Room temperature was kept at approximately 26 degrees
Celsius. Every effort was made to minimize acoustic,
olfactory, air currents and motion cues to the animals
during the experiments. The experiments were carried out
between April and November 1995

The experiments were divided into two major series,
night and day. Each series consisted of six groups; each
group included 10 rats (5 of each sex).

I. Daytime Series

A. Sham-controls (15 minutes)

B. 10 kV/m - 5 minutes continuous exposure

C. 25 kV/m - 5 minutes continuous exposure

D. 39 kV/m - 5 minutes continuous exposure

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E. 39 kV/m - 5 minutes discontinuous (on-off at 5 second intervals)

F. Sham exposed - blindfolded (15 minutes)

II. Nighttime Series

A. Sham-controls

B. 10 kV/m - 5 minutes continuous exposure

C. 25 kV/m - 5 minutes continuous exposure

D. 39 kV/m - 5 minutes continuous exposure

E. 39 kV/m - 5 minutes discontinuous (on-off at 5 second intervals)

F. Sham exposed - light on (15 minutes)

Electrode preparation and implantation

Preparation of the electrodes for implantation into the pineal gland was carried out in the following manner. A liquid insulator (Epoxylite Corp., El Monte California) was used to insulate the stainless steel microelectrodes (#00, Clay Adams Co., New York), as outlined by Hines [1985]. Before implantation, the diameter of the electrode was about 0.4 mm and the distal end was scraped so as to expose 0.5 mm of the tip.

Prior to implantation, the subject was anesthetized by intraperitoneal injection of sodium pentobarbital (10mg/100 g body weight). The animal was then placed in a stereotaxic apparatus (Model-SMA-1 Baltimore Instrument Co., Baltimore, Maryland) and a midline incision on the surface of the skull was made. The skull was exposed and positioned so that

bregma and lambda were on a level plane (Fig.2). The coordinates used for positioning the recording microelectrode in the pineal gland were taken from the text, Rat Brain in Stereotaxic Coordinates [Paxinos et al., 1986]. A small hole (0.7 mm in diameter) was drilled 8.3 mm posterior to bregma on the midline to accommodate the recording electrode, and another small hole was drilled 4.0 mm posterior to the bregma to accommodate a small jeweler's screw. The plastic capped electrode was then lowered through the hole in the skull to a distance of 2.0 mm. The electrode opening in the skull was surrounded with soft acrylic. When the electrode was in place, the acrylic connection between the electrode cap and the skull was allowed to dry. The small jeweler's screw was used to maintain the rigidity of the electrode and the acrylic cap. When the cap was firmed, the recording electrode was clipped at about 1.5 mm above the plastic cap and the animal was allowed to recover for at least two days prior to experimentation.

Experimental

A photo of the room with the entire laboratory assembly is in Fig. 1 and its schematic set-up is in Fig. 4. A diagram of the Faraday cage ensemble with dimensions is depicted in Fig. 3. The purpose of the copperized Faraday cage was to negate as much as possible any stray electric fields produced by other electrical items in the room. The Faraday cage itself was grounded to stabilize the electrical

recordings from the implanted electrode. The suspended anode electrode (2.5 mm x 38 mm) consisted of a thin piece of light metal completely covered with a coating of Bakelite, a hard resinous insulating material. Therefore, the smooth surface was entirely free of any pointed dirt particles that might produce corona discharge during the exposures.

Recordings

Recordings were always taken in the morning between 8:00 AM and 10:00 AM and at night between 12:00 AM and 2:00 AM. All experiments were carried out under relatively constant conditions (i.e. lighting, sound and temperature). In the daytime experiments, the light sources in the experimental room came from three overhead 100 W light-bulbs. In the night experiments, a photographic dark-room ruby red light was used. Schapiro [1971] and Reiter [1985] found that red was the only wave-length that did not affect pineal gland activity. The room temperature ranged between 24 and 27 degrees Celsius.

The electric field produced in the experimental chamber was determined from the charge put on the electrode by the field generator (Electrofields Inc. Miami, Florida) and the distance to the top of the wooden box. Calculation of the field was as follow:

$$Vf = V / d$$

Vf = Voltage desired

V = Voltage needed from generator

d = Distance from suspended electrode to the wooden

platform

Every experiment lasted 15 minutes and consisted of a 5 minute pre-test, a 5 minute test (exposure) and a 5 minute post-test (recovery) period. With this procedure, in each experiment the animal was allowed to serve as its own control. Moreover, such a set-up allowed uninterrupted recording, before, during and following exposure to the EEF.

For those experiments involving blindfolding the rat during the daytime and running the nighttime experiments in the light, the electrical activity was continuously recorded from the electrodes for a period of 15 minutes. Recording from these experiments involved simply allowing the rat to lie in the Faraday cage for the entire 15 minute period.

On the day of each experiment, the animal was anesthetized with the same dose of sodium pentobarbital as in implantation. When the animal did not show a corneal reflex following the injection of the anesthetic, it was assumed that the anesthesia was complete. A shielded alligator clip reference electrode was attached to the back of the neck and the Faraday cage was grounded. The rat was then placed on a wooden platform in the middle of the cage; therefore, the animal was "floating" inside the cage between the field generating anode and a copper cathode. Prior to the recording, an equilibration period of at least 20 minutes allowed the instruments and the animal to stabilize.

Test recordings were not began until trial tracings were stabilized and presented valid pineal gland electrical activity.

The electrical data obtained from the implanted microelectrode were first amplified (Cold Springs Instruments Corp., New York) before being fed into a Physiograph (Desk Model Type Dmp-4a: Narco Instruments Inc, Houston, Texas), and simultaneously into a digital integrating device (Model 23 EEG Integrator: Cold Springs Instruments Corp., New York) attached to a digital drive recorder (Digital Data Recorder: Cold Springs Instruments Corp., New York). These latter instruments were capable of integrating the areas under the electrical tracing over a given period of time and converting this activity into a numerical printout every 60 seconds throughout the experiment. Mean values were then obtained from this printout and plotted accordingly. The electrical data from the integrator unit were also fed into a pulse counting unit (Universal Electronic Counter, Model Simpson 7026: Simpson Electronic Co, Elgin, Illinois) where the response frequency was measured and plotted.
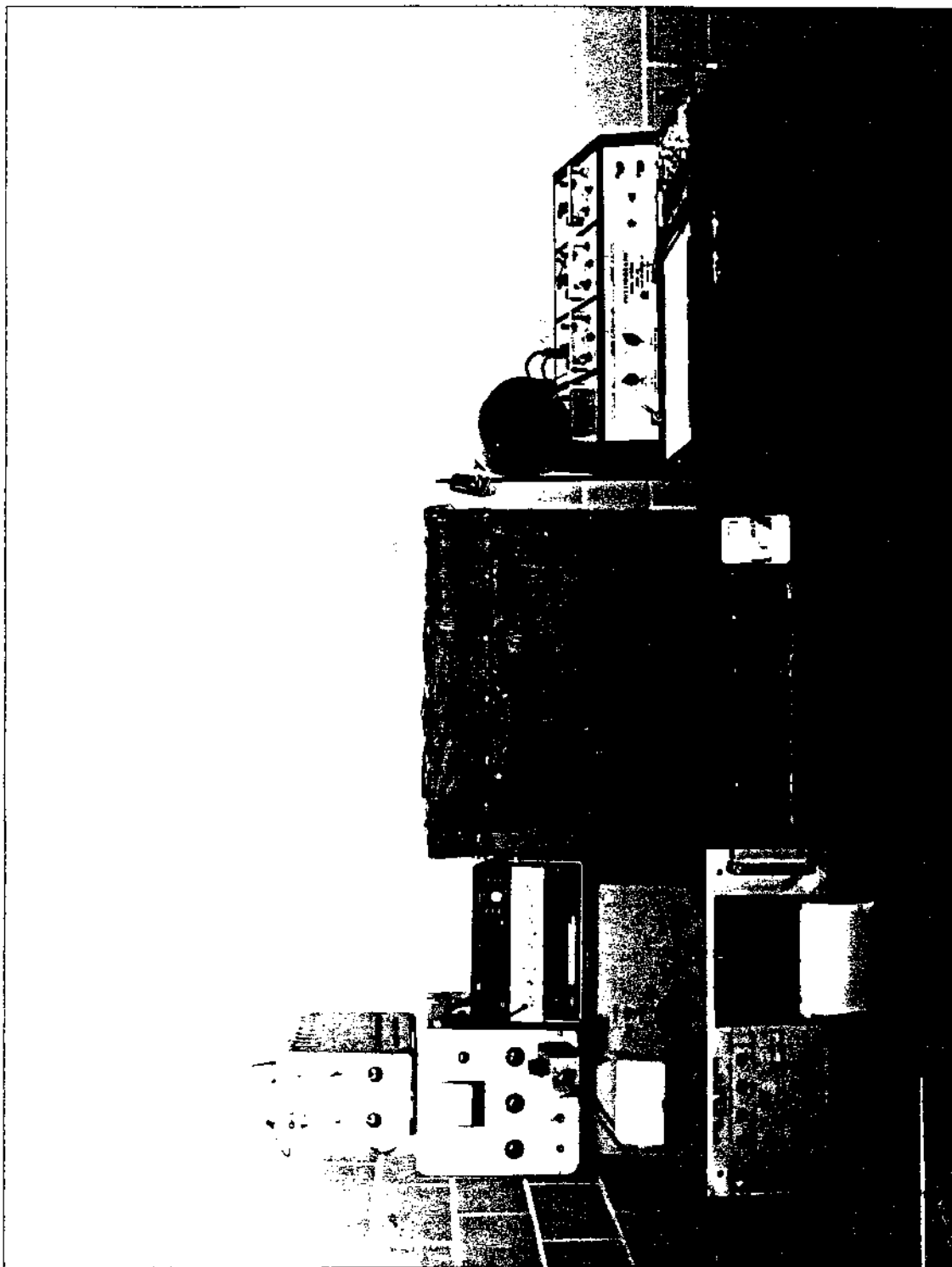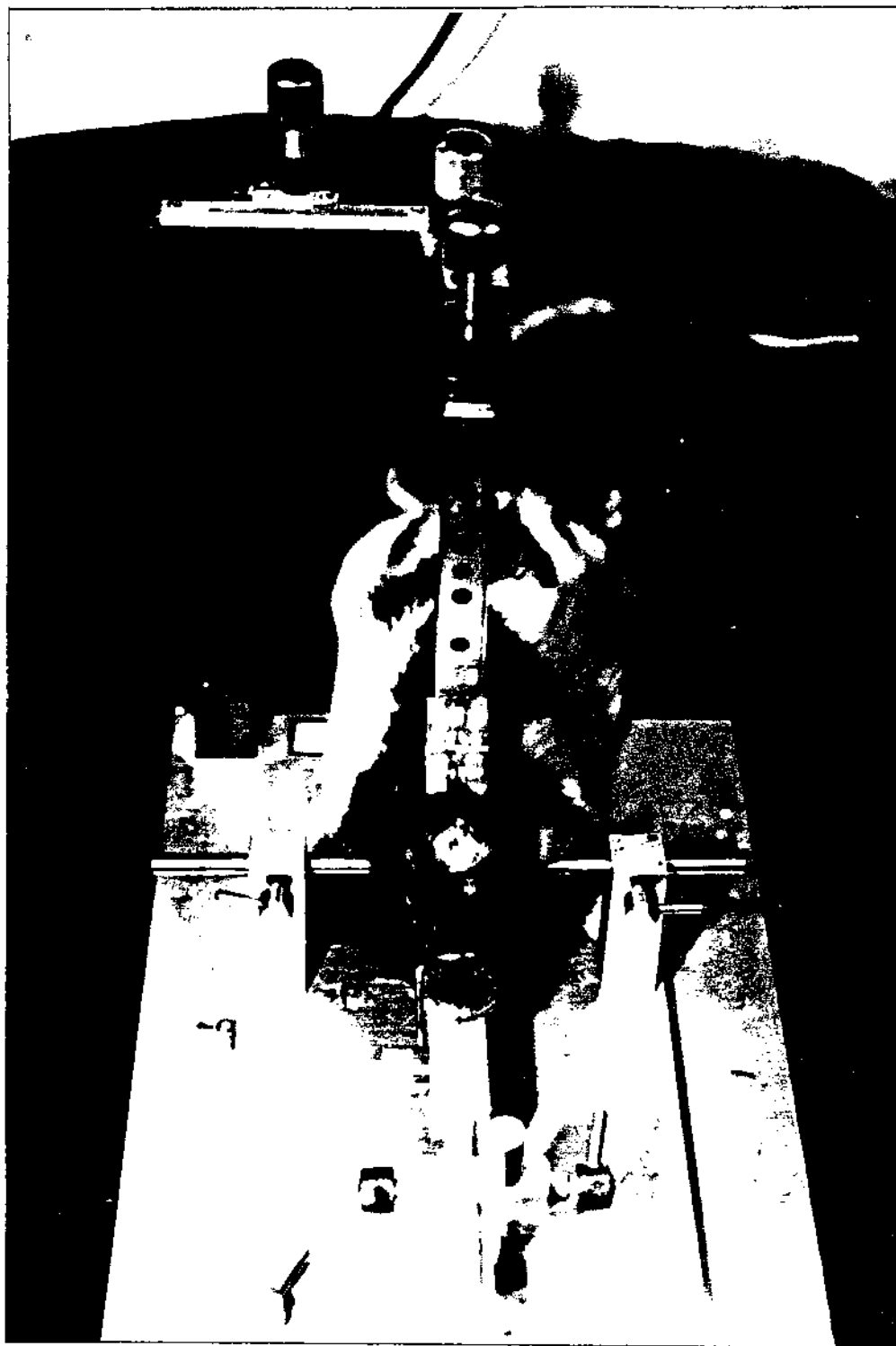
Figure. 1: Experimental set-up

Figure. 2: Stereotaxic Apparatus

suspended electrode
(anode)

copper mesh

acrylic platform
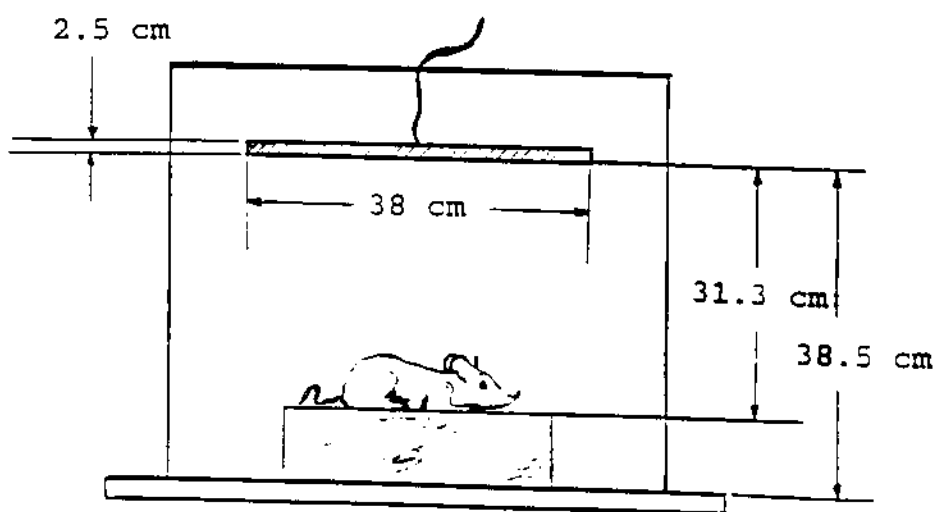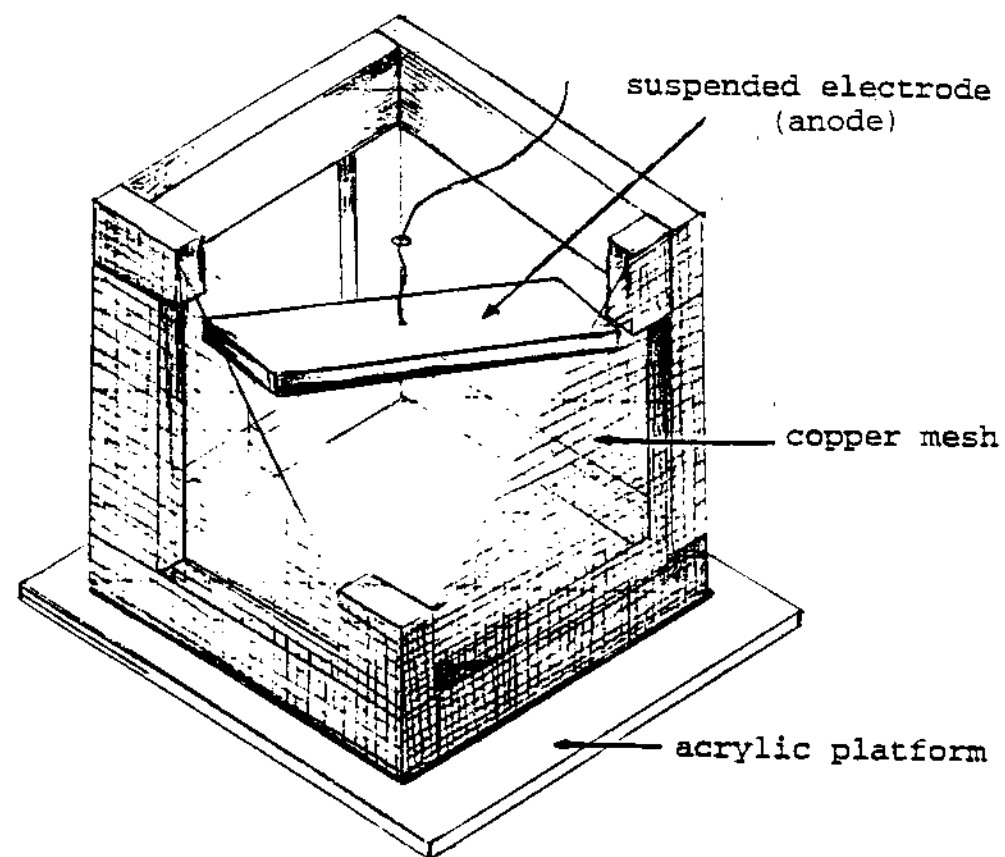
2.5 cm
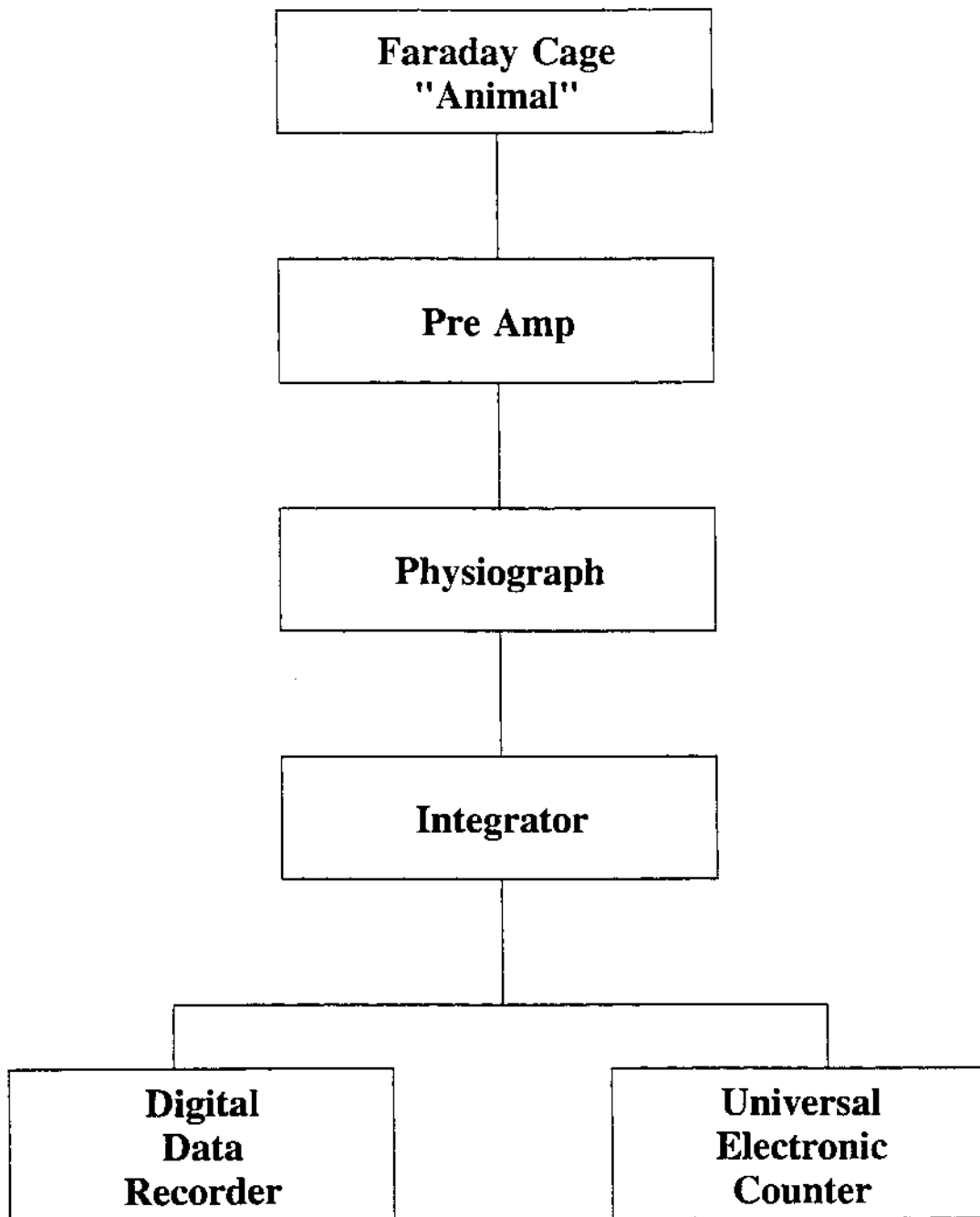
38 cm

31.3 cm

38.5 cm

Figure. 3: Faraday cage

Figure. 4: Diagram of experimental apparatus

CHAPTER III

RESULTS

The data presented here came from 120 male and female Sprague-Dawley rats, 8-11 weeks old, that were maintained in an environment in which light, temperature, food and water intake, sounds, humidity and handling were kept constant. The sham-control animals were implanted with pineal gland microelectrodes and recordings made in the absence of an EEF.

The data are presented in the form of 12 Figures and one Summary Table. The Figures contain curves that depict the electrical activity from the pineal gland before, during and after varying dosages of EEF (10, 25 and 39 kV/m). The EEF were applied continuously and discontinuously (off-on every 5 sec) for five minutes. The Summary Table contains the percentage change in response in terms of overall activity and frequency during pre-test (5 minutes), test (5 minutes), and post-test period of 5 minutes. Thus, each experiment was a 15 minute duration. The change between the mean pre-test and the mean test period were analyzed statistically using Student's t test. The placement of the electrodes was verified at autopsy.

Fig. 5A shows the mean electrical activity in ten sham-control rats during the day. It is evident that no

significant change in activity or frequency occurred in the
15 minute experiments. In Fig. 5B the mean activity and
frequency from 10 sham-control rats also failed to show
changes in the entire experimental period. There was a clear
difference in the level of activity and frequency between
night and day that was expected. The nighttime activity was
about 47% higher than the daytime activity and the frequency
at night was 50 % higher.

Fig.6 contain curves showing the effects of 10 kV/m
continuous EEF (5 minutes) on the mean electrical activity
of the pineal gland in 10 rats during the daytime A and
during the night B. There were no measurable effects in
either the daytime activity or the nighttime recording. The
overall records were similar to those in the sham-control in
both activity and frequency.

Almost identical findings were observed in Fig. 7A and
B, in those rats receiving 25 kV/m EEF continuously for five
minutes. In Fig. 8 the effects of 39 kV/m continuous EEF on
the pineal gland activity are depicted. In Fig. 8A, a
distinct but short-lived increase in both the mean activity
(12%) and frequency (51%) occurred during the daytime
exposure. As shown in Fig. 8B, there was a clear but short-
lived increase in both the activity (15%) and frequency
(46%) when 39 kV/m EEF was given continuously at night. As
shown in Fig. 8. Recovery was complete in all animals within
one minute.

Fig.9A and B contain actual physiographic tracings that show clearly the effects of exposing the animals to a discontinuous EEF of 39 kV/m (on-off at 5 sec intervals for 5 minutes) in the day and in the night. The effects were sustained during the entire period of exposure. Note the differences in the amplitude between the day and night recordings.

Fig. 10A and B show the effects of discontinuous (on-off at 5 sec intervals for 5 minutes) day and night exposure to 39 kV/m EEF. These findings were totally unexpected. As shown in Fig. 10A, exposure to 39 kV/m intermittent EEF during the day resulted in an increase of 36% in the mean overall activity and over 136% in mean frequency. Fig. 10B shows similar effects in the dark i.e. a 26% increase in the mean activity and a 81% increase in frequency. The observed increase in activity and frequency lasted the entire length of exposure (5 min) and recovery was slightly slower during the post-test periods.

Fig. 11A contains curves depicting the effect of blindfolding the animals during daylight but no EEF exposure. Recording was 15 minutes. No significant changes were observed in the activity nor the frequency. Fig. 11B curves also show no changes in activity or frequency when the animals were in a lighted room at night and in the absence of an EEF.

Fig. 12A curves show almost identical electrical

activity and frequency in male and female rat pineals either during the day or during the night (Fig. 12B).

Table. I A summary of the effects of short-term exposure to EEF on mean activity of pineal gland in rats. As shown in the table, the greatest response in both the mean activity and frequency occurred when the animals were exposed to 39 kV/m discontinuously, although a significant yet momentary response also occurred when the animals received the same dosage (39 kV/m) continuously. Moreover, in both the day and night discontinuously exposed group, recovery did not occur until the field was turned off. The differences in the activity in the interrupted field were statistically significant.

FIGURE. 5

Mean pineal gland activity in sham-exposed rats, day and night. (Each circle and square represent the mean electrical activity and frequency of ten animals).
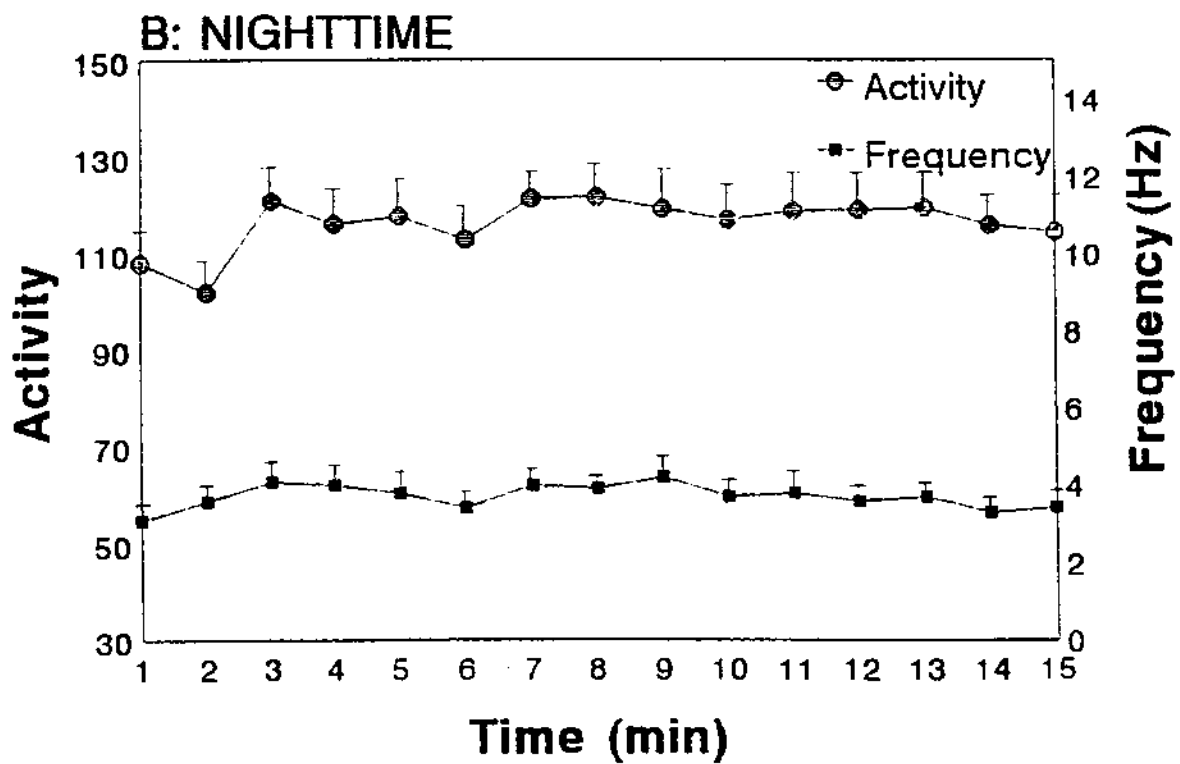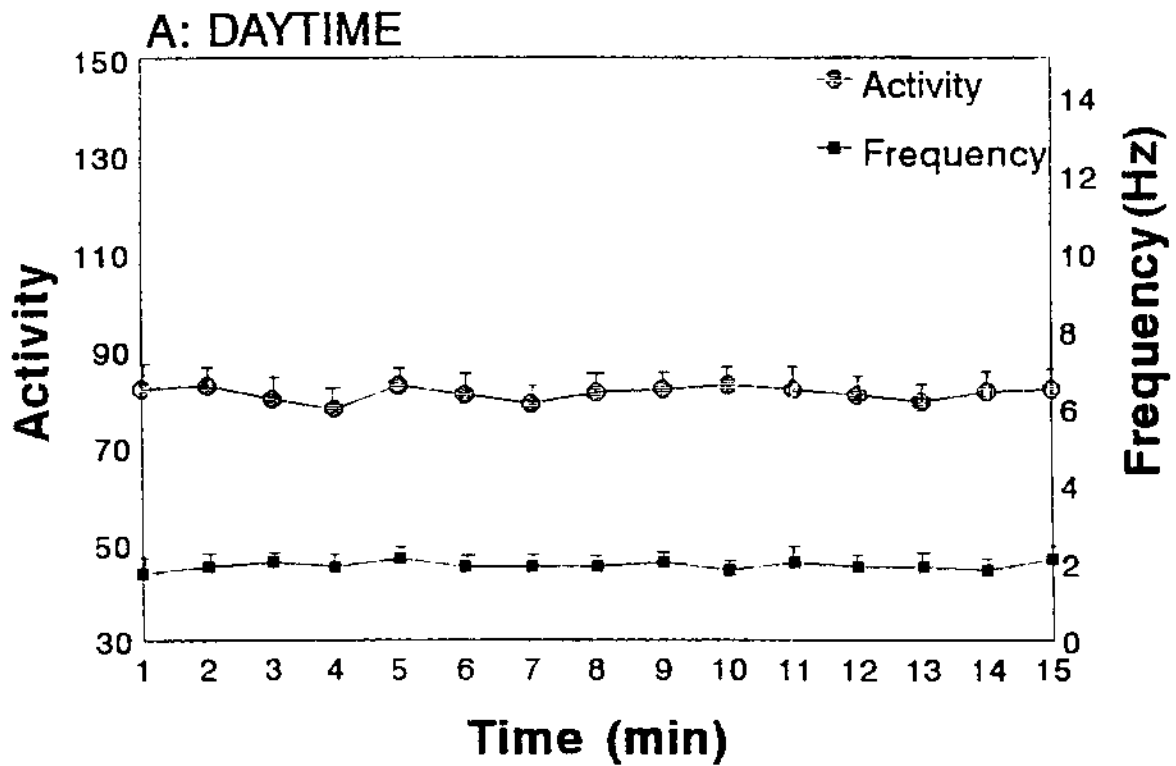
A: DAYTIME

B: NIGHTTIME

FIGURE. 6

Effects of 10 kV/m continuous EEF on the daytime and nighttime pineal gland activity in rats. (Each circle and square represent the mean activity and frequency in ten animals).
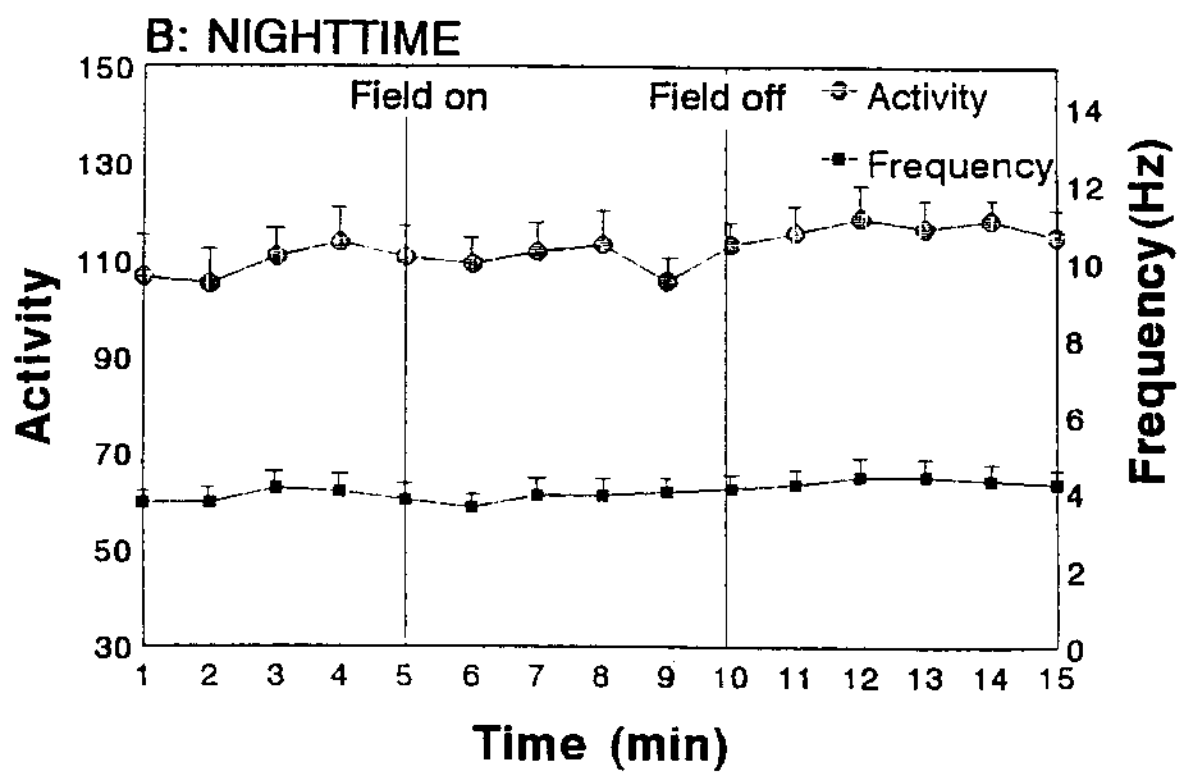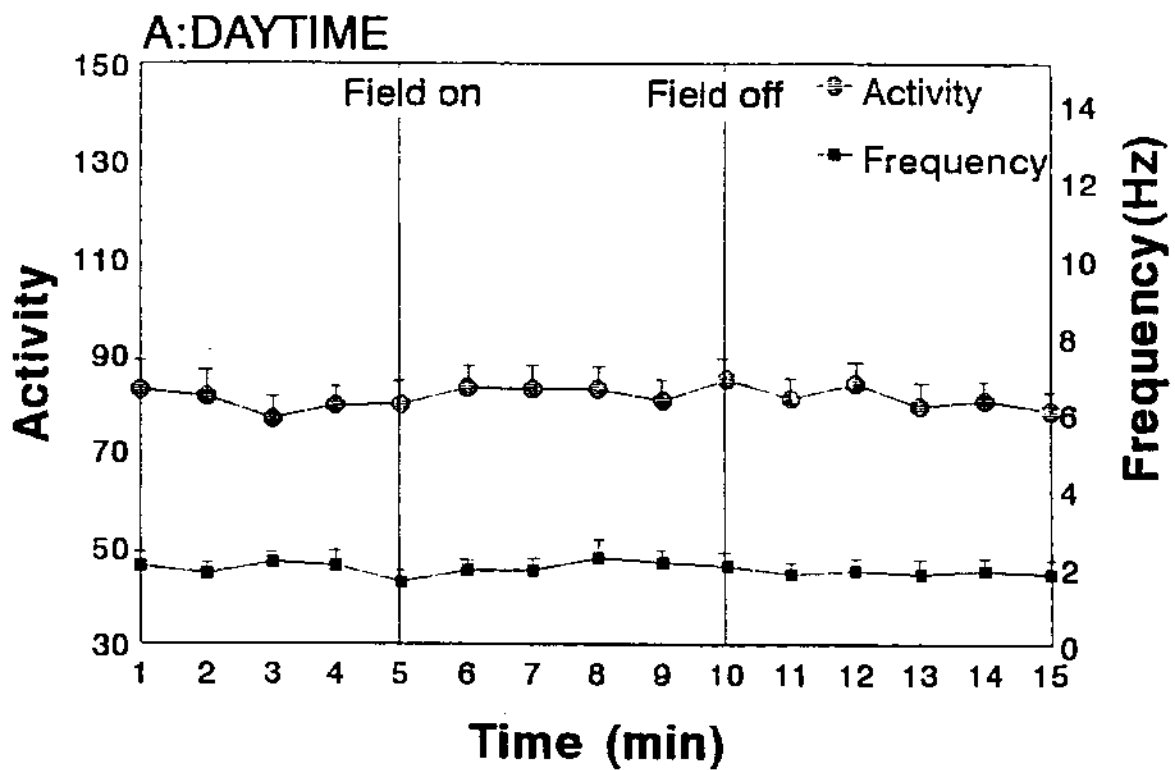
A:DAYTIME

B: NIGHTTIME

FIGURE. 7

Effects of 25 kV/m continuous EEF on the daytime and nighttime pineal gland activity in rats. (Each circle and square represent the mean electrical activity and frequency in ten animals).
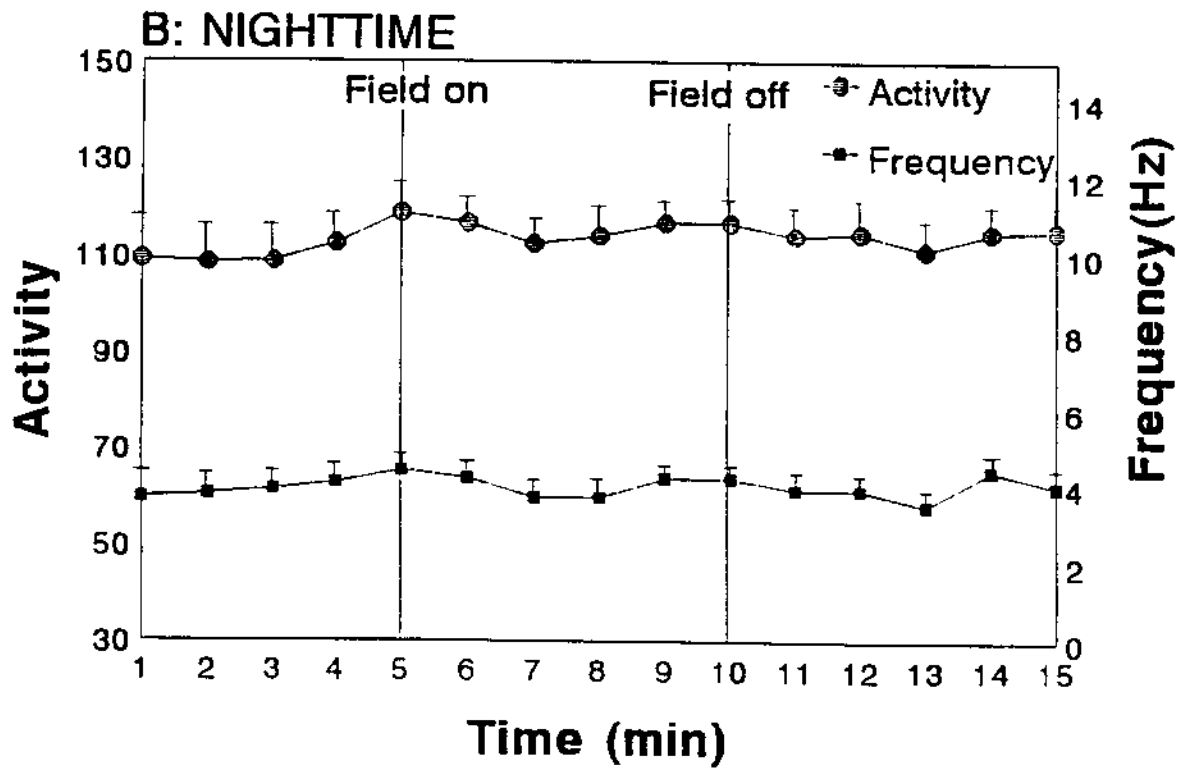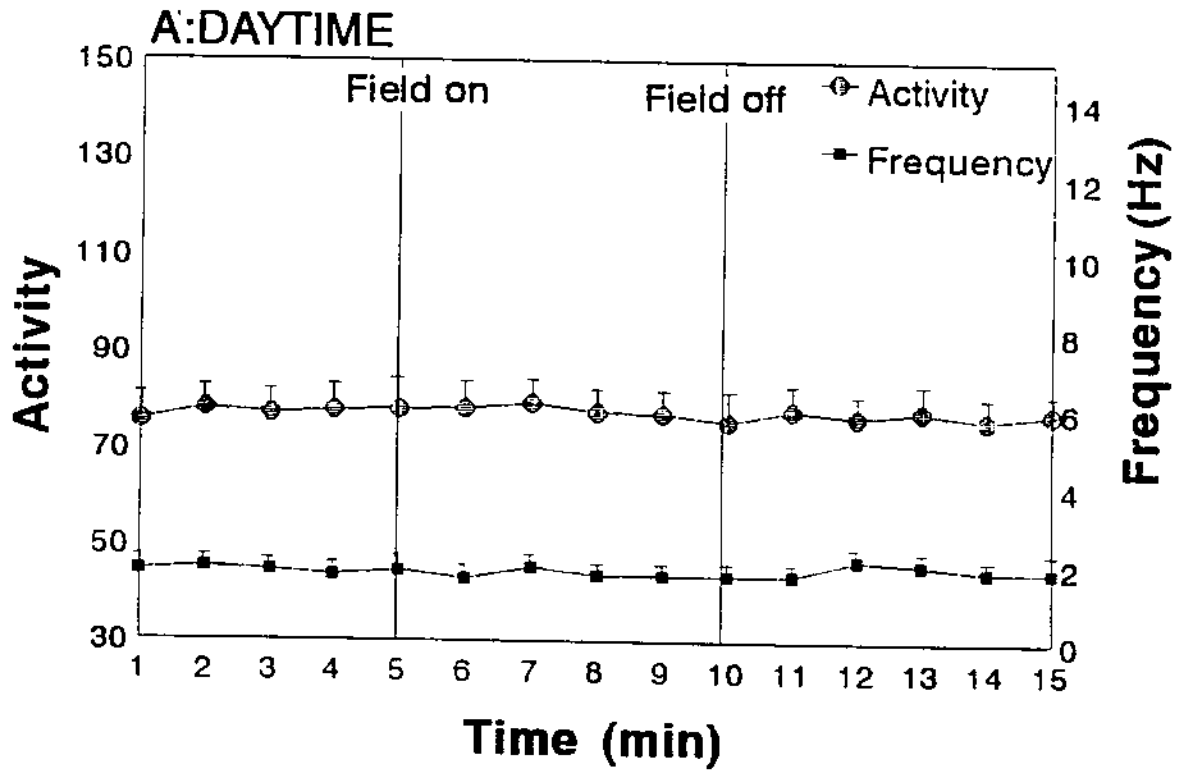
FIGURE. 8

Effects of 39 kV/m continuous EEF on the daytime and nighttime pineal gland activity in rats. (Each circle and square represent the mean electrical activity and frequency in ten animals).

A: DAYTIME

B: NIGHTTIME

FIGURE. 9

Typical tracing of electrical activity recorded from the pineal gland for day and night sham-control and exposed to discontinuous 39 kV/m field rats

ELECTRICAL ACTIVITY FROM A PINEAL GLAND

EXPOSED TO A DISCONTINUOUS

39 kV/M EEF RATS

A. DAYTIME

5 Sec   5 Sec

PRE-TEST   **FIELD ON**

B. NIGHTTIME

5 Sec   5 Sec

PRE-TEST   **FIELD ON**
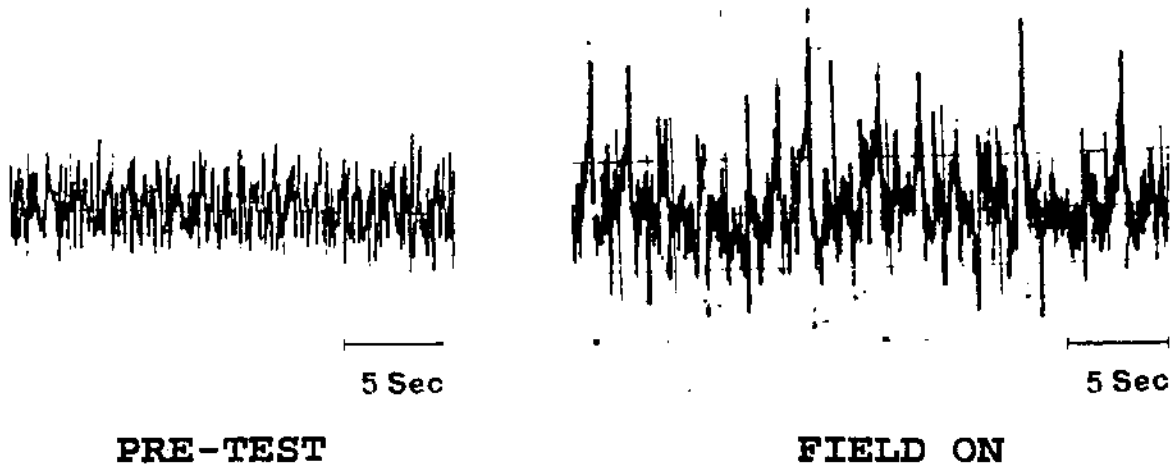
FIGURE. 10

Effects of 39 kV/m discontinuous EEF on day and night pineal gland activity (EEF interrupted 1/5 sec during exposure. Each circle and square represent the mean electrical activity and frequency in ten animals).

A:DAYTIME
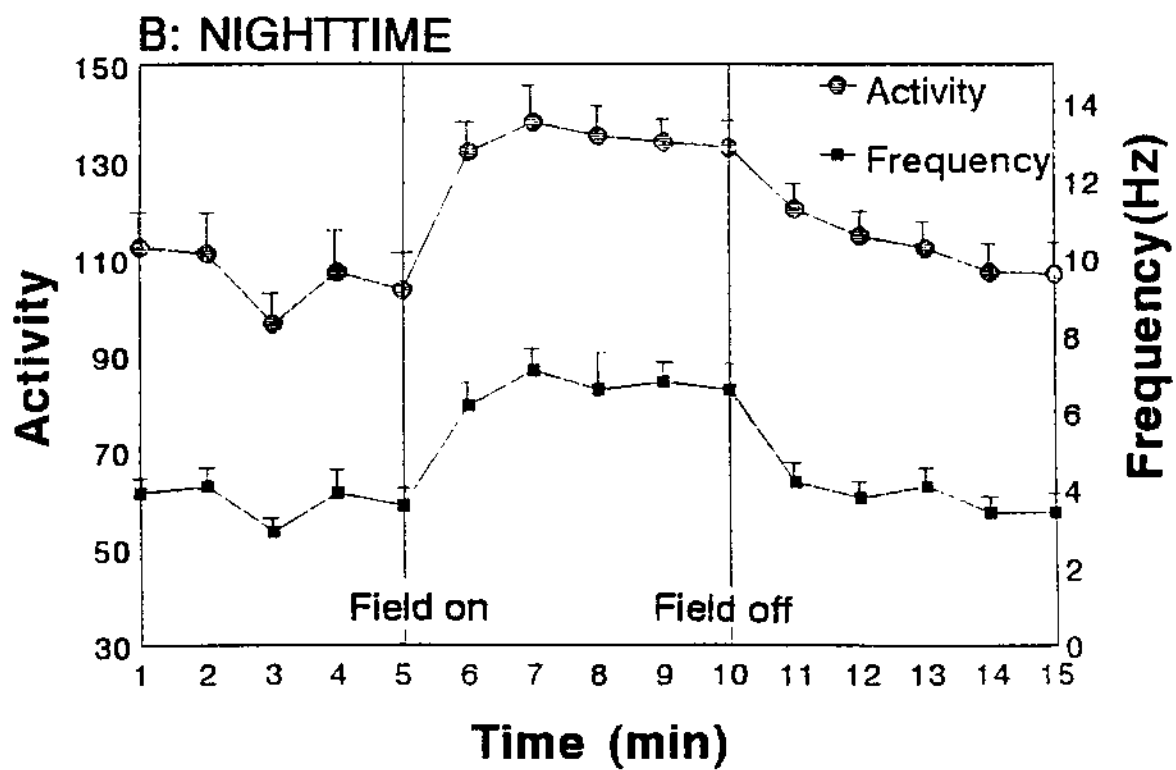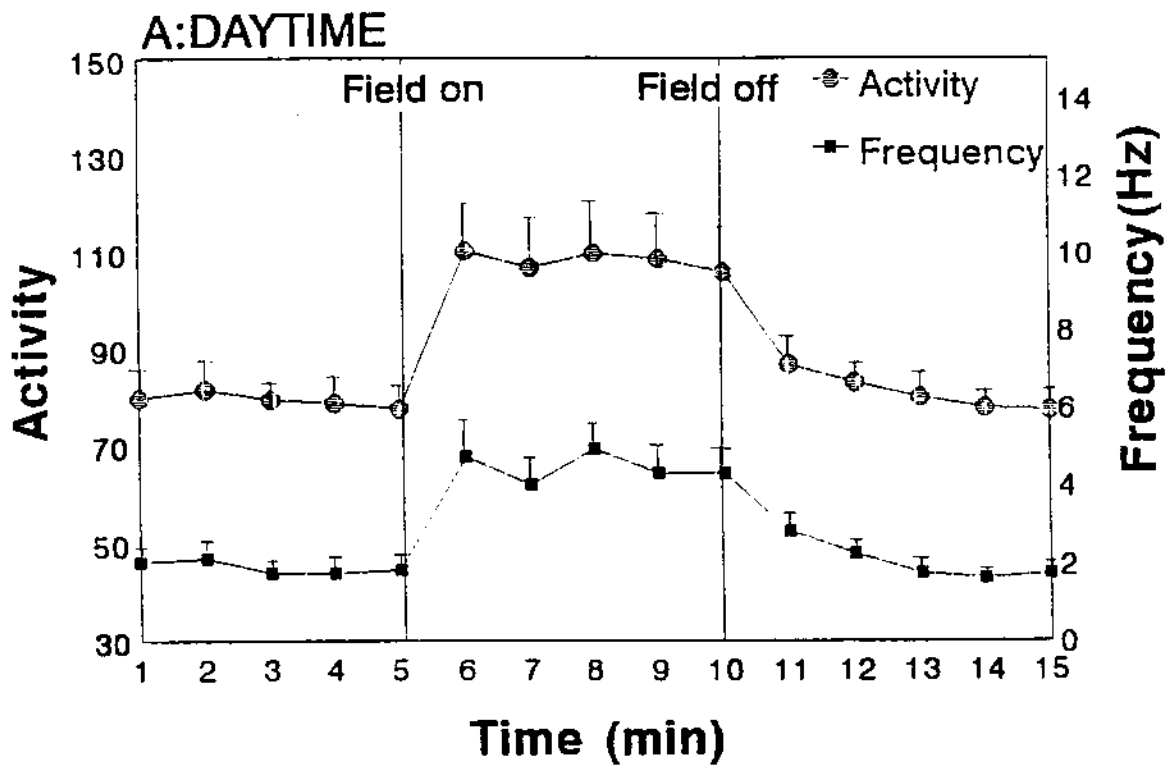
B: NIGHTTIME

FIGURE. 11

Effects of blindfolding on daytime and light on at nighttime pineal gland activity in rats. (Each circle and square represent the mean electrical activity and frequency in ten animals).
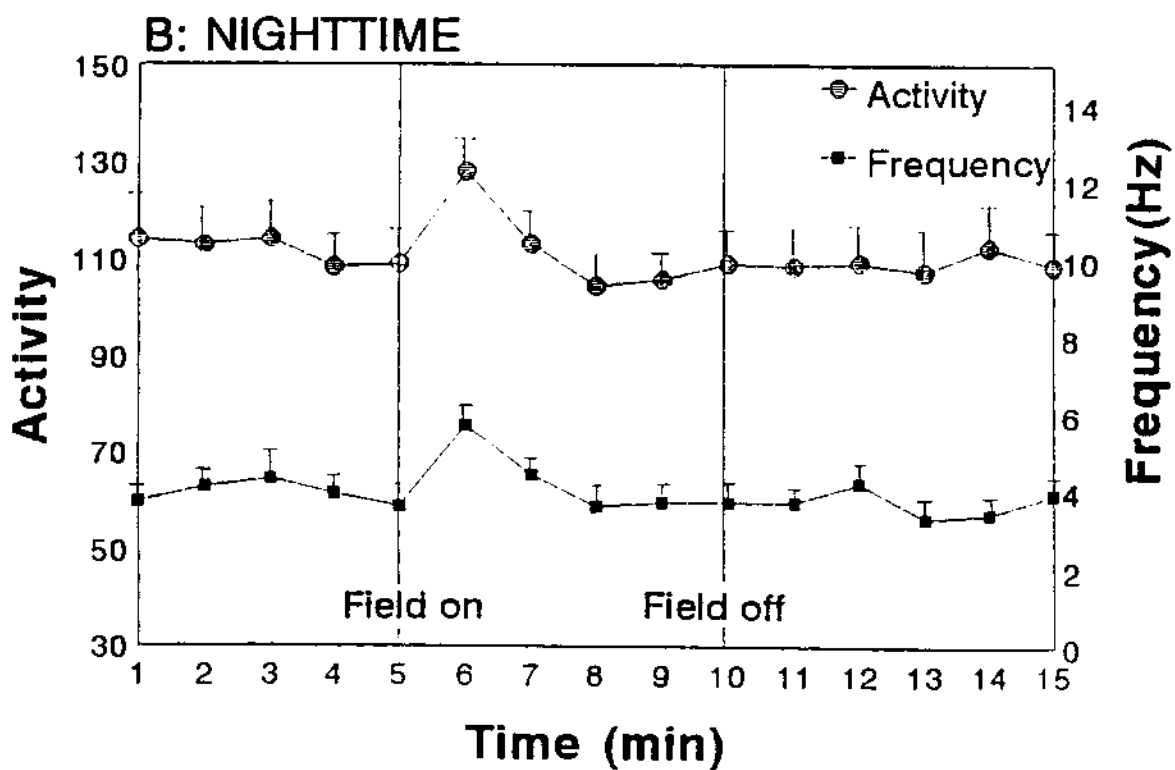
A:DAYTIME
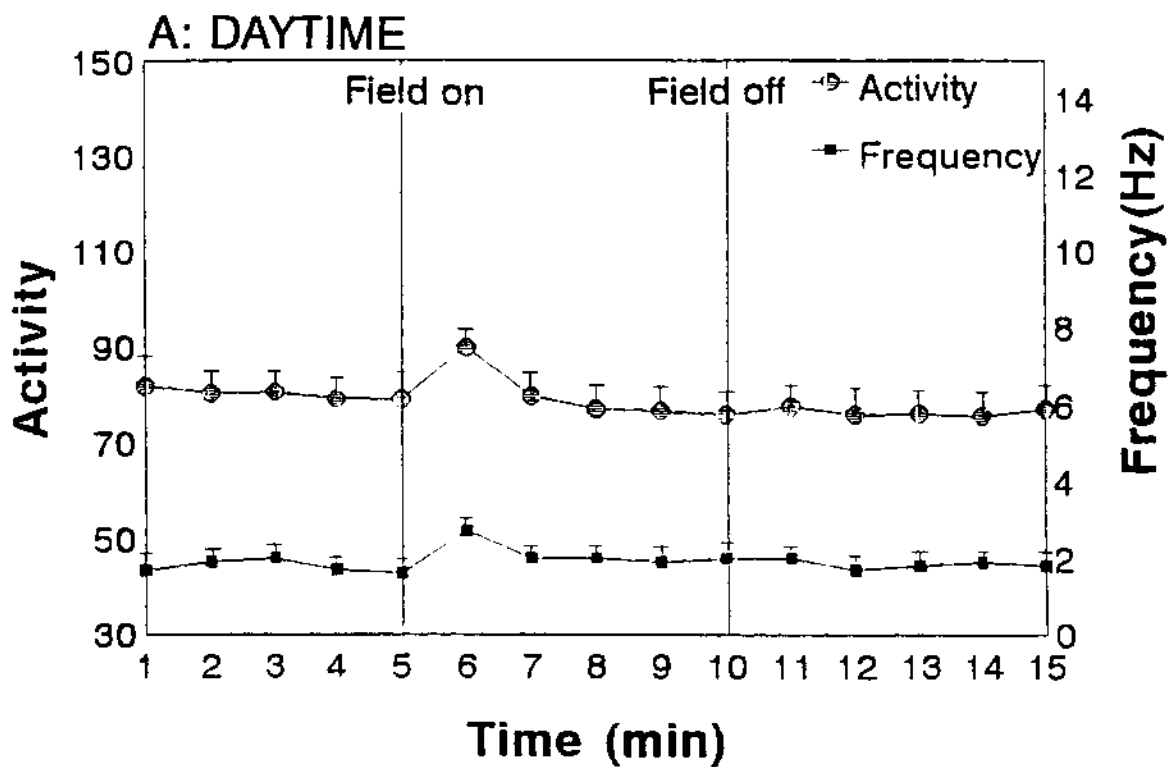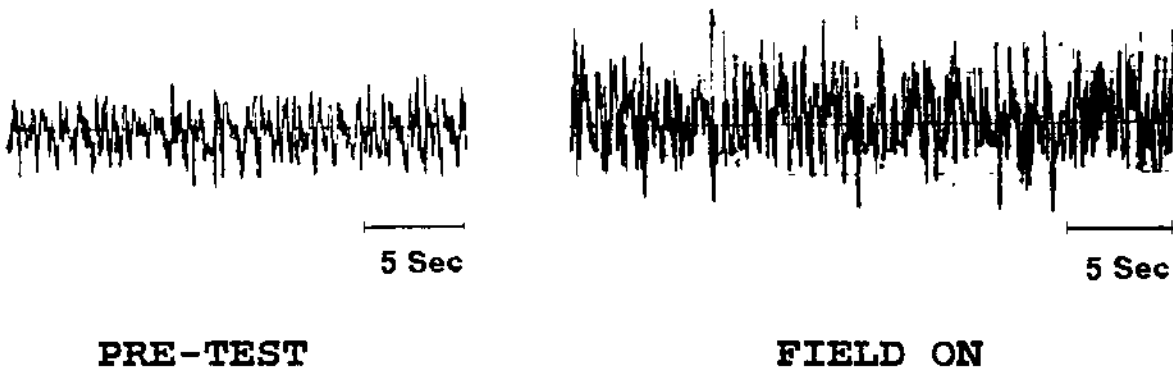
B: NIGHTTIME

FIGURE. 12

Daytime and nighttime pineal gland activity in sham-control male and female rats. (Each circle and square represent the mean electrical activity and frequency in five animals).

A:DAYTIME

Female activity
Female frequency
Male activity
Male frequency

Activity

Frequency(Hz)

Time (min)

B: NIGHTTIME

Male activity
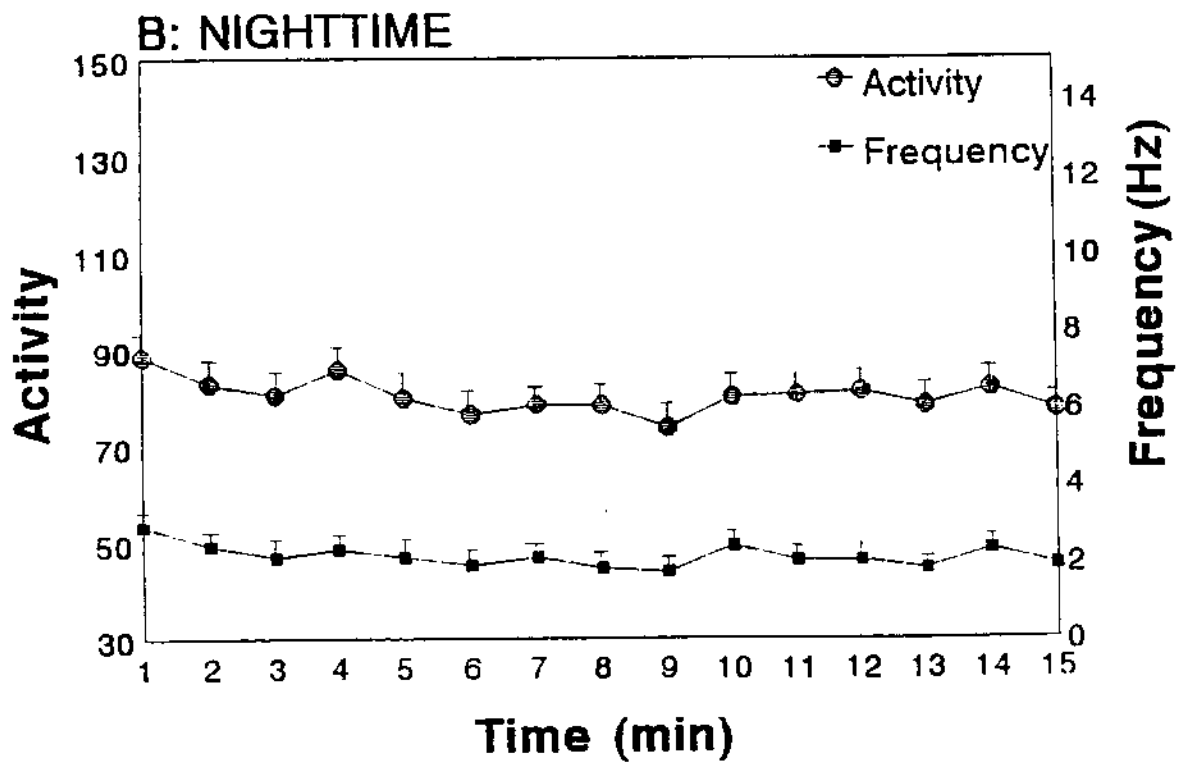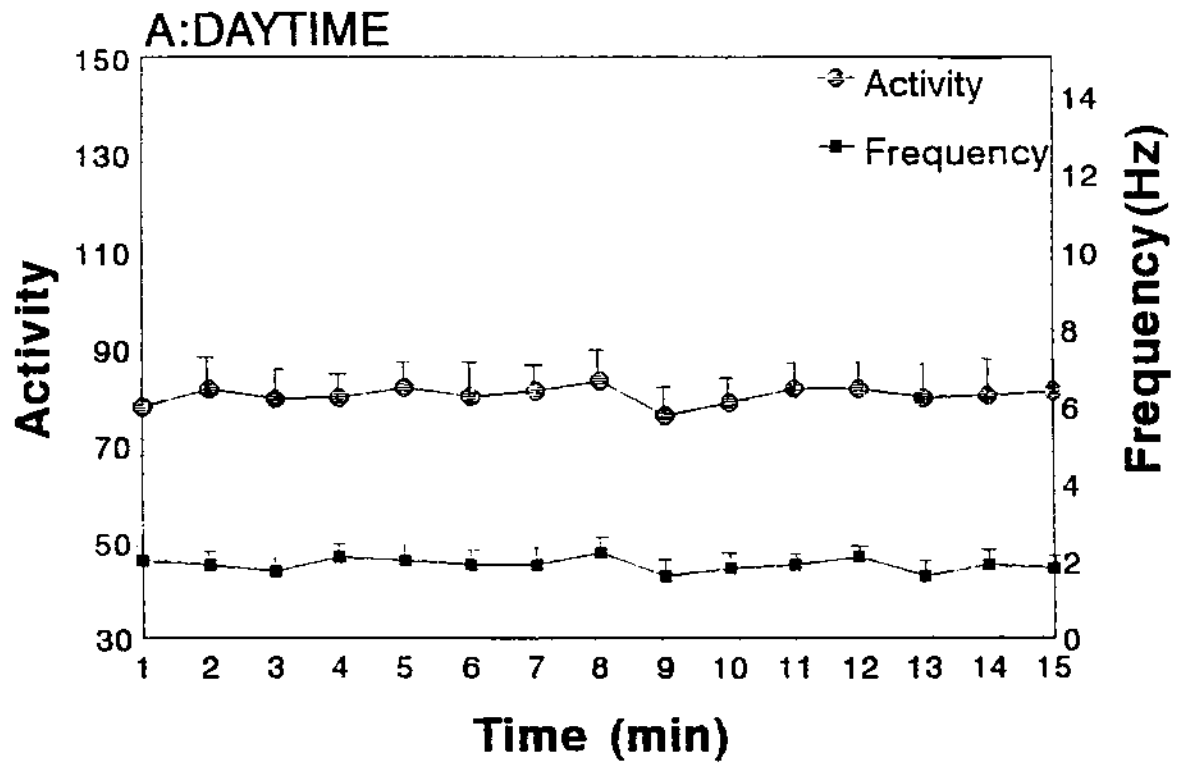Male frequency
Female activity
Female frequency

Activity

Frequency (Hz)

Time (min)
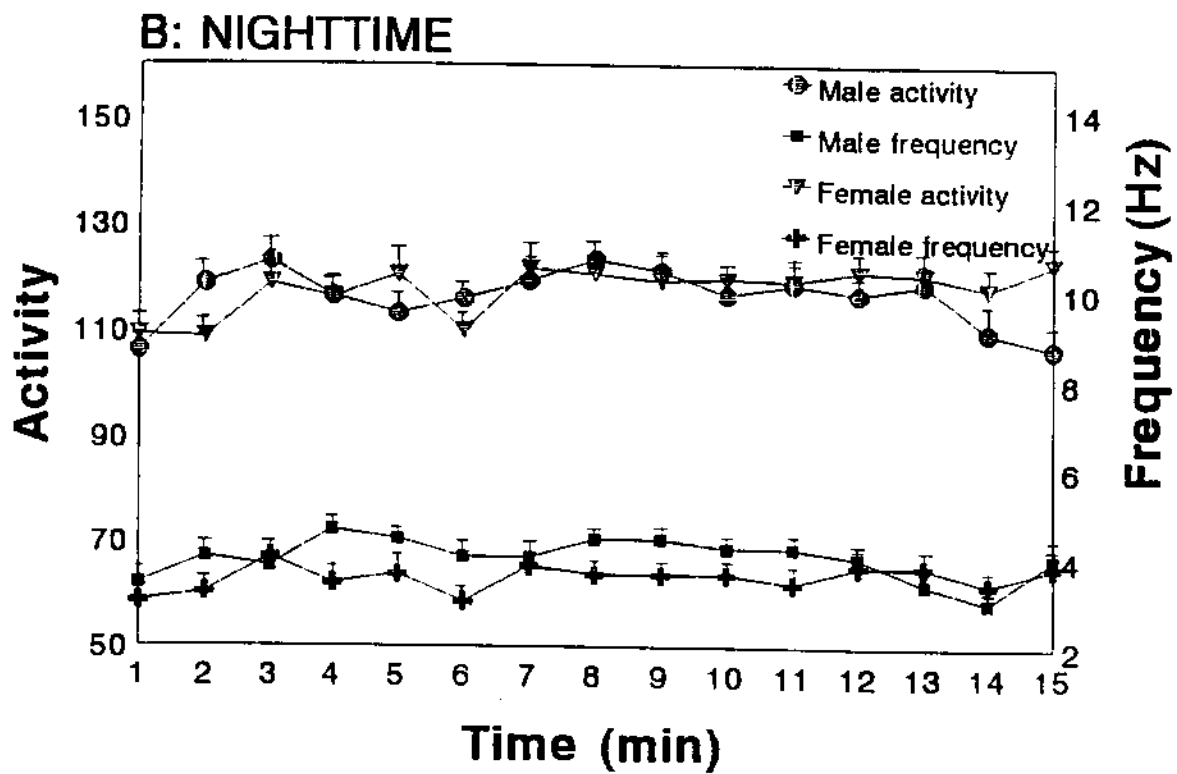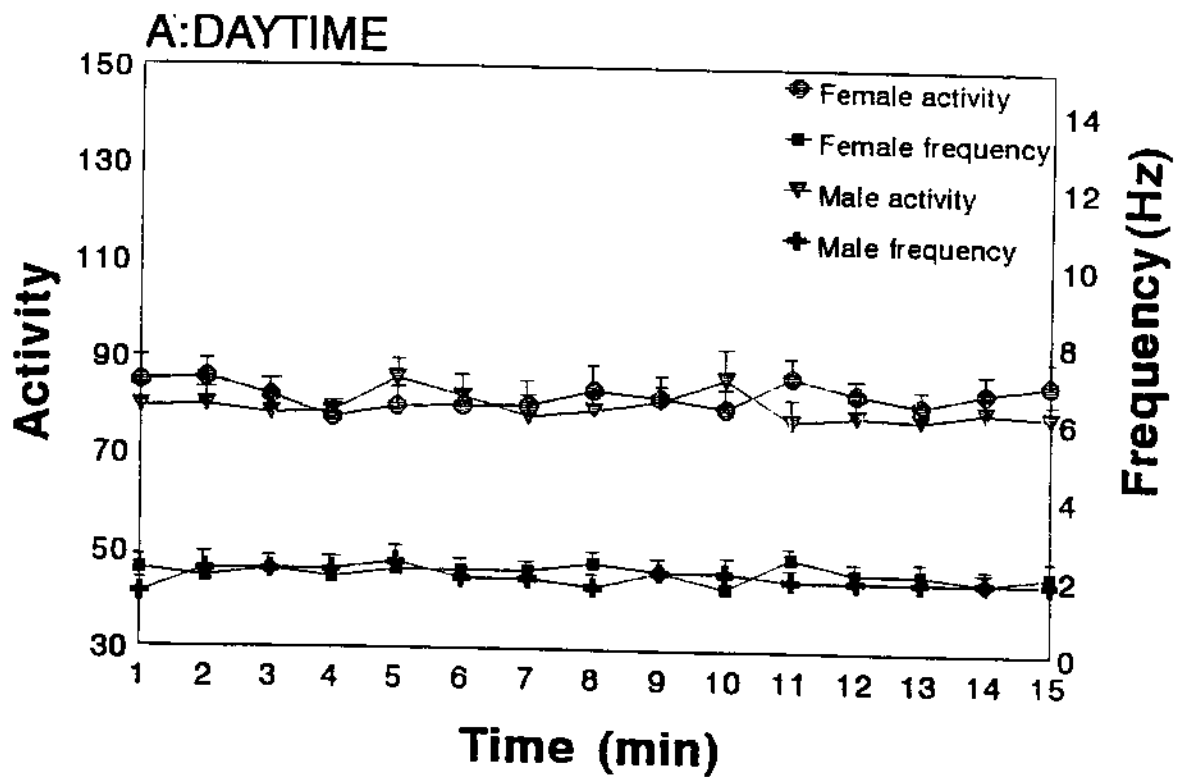
TABLE I

A summary of the effects of short term EEF on the mean activity in rat pineal gland.

I. Continuous fields (5min exposure)

A. Day

Animal	Condition	Response			
		%△Activity	Duration	%△Frequency	Duration
10	Sham	0	—	0	—
10	10 kV/m	0	—	0	—
10	25 kV/m	0	—	0	—
10	39 kV/m	10 %	1 min	30%	1 min

B. Night

Animal	Condition	Response			
		%△Activity	Duration	%△Frequency	Duration
10	sham	0	—	0	—
10	10 kV/m	0	—	0	—
10	25 kV/m	0	—	0	—
10	39 kV/m	15 %	1 min	46 %	1 min

II. Discontinuous fields(5 min on-off at 5 sec interval)

Activity

Animal	Condition	Pre-test	SEM	Test	SEM	%
10	39 kV/m Day	79.3	±1.3	108.6	±1.7	36 *
10	39 kV/m Night	106	±1.8	134	±1.6	26 *

Frequency

Animal	Condition	Pre-test	SEM	Test	SEM	%
10	39 kV/m Day	1.86	±.16	4.4	±.32	136 *
10	39 kV/m Night	3.68	±.42	6.66	±.3	81 *

△% = Control - Test/ Control x 100

* = P < .001

CHAPTER IV

DISCUSSION

During this study, several problems concerning arose.
A technical problem involved grounding of the various
electronic devices. Proper grounding was essential so as not
to form ground loops that would alter the extremely
sensitive physiographic tracings.

The effect of anesthesia was another problem. It was
very easy to overdose the animal with sodium barbital
(Nembutal); on the other hand, some animals had a high
tolerance for Nembutal, they recovered during the experiment
causing that experiment to be aborted. Accuracy in
electrode implantation was at times another problem. Some of
the electrodes would bend slightly upon entry and deviate
from desired location. Occasionally, the animal would on the
cage strike the electrode while walking around following
recovery. Another problem arose concerning how to position
the animal in the Faraday cage, i.e. whether to allow the
animal to be part of the ground or let it "float" on a non-
conductive wooden platform. Poznanick [1978] reported that
in some experiment by other workers, a free moving animal
was allowed to remain on the grounded copper mesh floor and
thus when it reached for a drink of water from a cup
attached to the copper wall, it received a "mini shock." To

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avoid this, it was decided to float the animal between the generating field anode and the grounded cage.

It was difficult to compare the data here with other workers regarding the effects of short term exposures to EEFs on pineal activity due simply to the fact, there were none to be found in the literature. Most if not all of the reported EEF data have concerned long-term or chronic exposures and at much higher dosages [Wilson et al., 1986; Reiter, 1988; Grota et al., 1994]. Moreover most if not all of these experiments were biochemical in nature rather than electrophysiological.

It appears that both EEFs and MFs reduce the overall nighttime activity as reflected by the reduction in circulation and pineal gland MT levels and the enzymes involved with MT production. No attempts were made in this study to run MT and enzyme analysis of the pineal gland nor plasma levels of these substances following the exposures. Therefore, only the neuroelectrical consequences of exposure to EEF will be discussed here.

First the data in this study confirmed the finding of others regarding the increased pineal activity at night over the daytime [Schapiro et al., 1971; Ruess et al., 1984; Ruess ae al., 1986]. Moreover the lower daytime activity was not changed when the animal was blindfolded during recording (Fig. 11A) whereas the higher activity at night was distinctly lowered by recording the activity in a lighted

room (Fig. 11B) which were in agreement with Taylor and Wilson [1970], Schapiro and Salas [1971], Reiter [1985].

Therefore, whatever mechanisms are involved, the pineal gland "knows" about the presence of an external light and its role as an external "cue."

Secondly, there were no indications of a gender factor in the results of this study. Male and female activity were almost identical both in the daytime and at nighttime (Fig. 12).

Third, the effective dosage of 39 kV/m was in agreement with that used in the biochemical works [Wilson et al., 1986]. Higher dosages were not used in this study due to the concern of producing corona discharge on the hairs of the animal or possibly creating acoustic cues [Kaune, 1981] to the animal. That the effects observed were not the result of current conduction in the recording electrodes coming from the animal: no shock artifacts were observed at the lower dosages. In addition, the dosage used had to be calculated rather than measured directly with a suitable field meter. Therefore, it is difficult to make any statements on what the real EEFs were in the cage. The shape of the target animal and the various current densities in the various body tissues are all the factors that prevent the investigator from obtaining an accurate measurement. The results reported here, however, were replicable as indicated by the relatively low SEM's in the data.

The distinct but brief increase in both activity and frequency in response to 39 kV/m applied continuously strongly indicate that the pineal gland is not only "magneto-sensitive" [Lerchl et al., 1991] but may be also "electro-sensitive."

Similar electro-sensitivity has been reported for the paraventricular nucleus (PVN) of the hypothalamus in rats [Lott et al., 1973]. Since there are many neural inputs into the pineal gland, including the PVN, one may conclude that the EEF had at least an indirect action on the pineal gland. The disadvantage of studying external factors on whole intact animals is that all of the complex response systems are intact. Therefore determining whether given external change such as an EEF has a direct or indirect action is difficult.

The distinct and prolonged response to the interrupted exposure to the EEF followed by a rapid recovery again indicate extreme electro-sensitivity when an effective dosage EEF was applied. One wonders what the real EEF threshold is in regard to pineal gland activity.

Moreover, the rapidity of the response and recovery suggested a distinct neural as opposed to biochemical changes action brought about an EEF. It would be interesting to know whether similar electrical responses also occurred in the optic nerve, the suprachiasmatic nucleus, and the superior cervical ganglia that form the major pathways

between the eye and the pineal gland that results in MT production.

In summary, then the data in this study indicate clearly that (1) external electric fields do alter the electrical activity in rat pineal glands as reflected by the observed increase in both activity and frequency when exposed to both continuous and discontinuous EEF, (2) the responses to the EEF are not altered by the light-dark periods, (3) there are no gender differences in the responses, (4) that interrupted exposure to EEF brought a more profound and prolonged increase in activity than continuous exposure, (5) that the dosage threshold for EEF was relatively high (39 kV/m), (6) that the nature of the EEF response was probably neural rather than biochemical, and finally (7) that the pineal gland in rats has a distinct electro-sensitive component.

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