

THE SLEEP PROCESS OF RABBITS EXPOSED TO LOW INTENSITY NON-IONIZING ELECTROMAGNETIC RADIATION. I. DEVELOPMENT OF METHODOLOGY

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ABSTRACT

Data have shown brain electrical activity associated with sleep states to be biologically constant for any given species. Further, nonionizing radiation exposure has been shown to alter brain electrical activity. The sleep process is characterized by cyclic fluctuations between alertness (W), slow wave (SW), and paradoxical (REM) stages. Rapid eye movement sleep (REM) is a very active state metabolically and is functionally defined as that period of sleep in which cortical activity paradoxically reveals low voltage, fast activity characteristic of the awake phase, concurrent with rapid eye movement, and atonus of neck muscle. Chronic recordings of electrocorticogram (ECoG), electro-oculogram (EOG), and electromyogram (EMG) were obtained daily from adult male rabbits on a 24-hour basis following daily 2-hour pulsed microwave exposure at 3.7 GHz (1000 pps, 60% duty cycle, 10 mW/cm²) for sixty days. The surgical implant assembly was designed to allow chronic post-irradiation recording while allowing no metallic elements to be associated with the subjects during microwave exposure. A unique application of logic circuitry allowed the investigator to quantify both the frequency and duration of REM stage episodes. The sleep process is viewed as a potential index of CNS adaptation to prolonged EMR exposure.

INTRODUCTION

Alterations in synchronization, desynchronization, and voltage amplitude have been used to assess altered EEG patterns of animals exposed to electromagnetic radiation [1, 3, 10]. Such measures, while descriptive, are highly variable, difficult to quantify, and as such, constitute poor dependent variables. Suggestions as to the relationship between altered EEG patterns in animals and electromagnetic radiation are inconclusive.

The sleep process, measured by conventional means, requires the assessment of not only an organism's EEG patterns, but also requires the assessment of concomitant electrophysiological variables such as the electromyogram (EMG), and the electro-oculogram (EOG). Together, these measures operationally define phenomena which are quantifiable in both frequency and duration of occurrence.

Further, there is a great deal of information currently available regarding biochemical, anatomical, and behavioral correlates of sleep [5, 6, 7]. Such correlates, derived from a variety of approaches, are in fair agreement. An understanding of possible sleep cycle alterations as a function of microwave exposure could offer the possibility for "translation" of generalized EEG

anomalies into a broader conceptual framework involving biochemical and/or anatomical relationships. An example of such relationships is illustrated in figure 1.

Michael Jouvet, a leading sleep researcher, has combined lesion technique with biochemistry, and shown a three-way correlation between discrete sleep states, their respective brain foci, and their respective transmitter substances [6]. Evidence supporting Jouvet's scheme is overwhelmingly convincing, and for years lured many investigators into predictions based upon a "one state, one transmitter" hypothesis. However, more recent work involving single cell recordings from raphe, locus coeruleus, and FTG neurons suggest Jouvet's scheme to be an oversimplification. Rather, conservative researchers are currently stressing the possible reciprocity of interaction between neurons of these discrete brain structures [4]. (Monoamine oxidase content of locus coeruleus and dendritic spine counting are presently being performed on the brain tissue of the animals that participated in this experiment.)

Our interest in the sleep process with regard to electromagnetic radiation exposure can be summarized as threefold:

1. The sleep process offers a quantifiable alternative to EEG phenomenology.
2. Possible EMR effects may be understood within the broader context of biochemical, anatomical, and behavioral relationships known to subserve the sleep process.
3. Sleep, according to several investigators, represents a constant biological rhythm for any given species [6]. If we are to assume that electromagnetic energy causes adaptive changes to an otherwise homeostatic organism, it seems desirable to examine a biological process known to have as little initial variability as possible.

We are particularly interested in the possible effects of microwave energy on the frequency and duration of occurrence of paradoxical sleep episodes. Operationally, paradoxical sleep has been defined as that period of sleep associated with low voltage, fast cortical activity, phasic eye movements, and atonus of neck muscle in small animals. Paradoxical sleep has been associated with increases in glucose utilization, and cerebral blood flow [5]. Further, this stage of sleep has been shown important for the facilitation and consolidation of learned behaviors [2, 8, 9, 11]. Paradoxical sleep is contrasted to synchronous, high amplitude, low frequency cortical records typically referred to as slow wave sleep. The hypothesis is advanced that prolonged exposure of an organism to microwave radiation will decrease the frequency and duration of REM stage sleep.

MATERIALS AND METHODS

Methodologies, unique to this paper, offer solutions to problems associated with an examination of the sleep process with regard to electromagnetic radiation exposure:

1. The use of an implantable, all-plastic electrode assembly avoids the problems of "electrode artifact" associated with permanently implanted electrodes and electromagnetic radiation exposure.
2. The use of logic circuitry for the control of long term sleep studies, while not unique to this paper negates the need to analyze volumes of polygraph paper, and allows more precise control and measurement of specific portions of the sleep record.

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3. Video equipment simultaneously records the sleeping animal and its polygraph
(BASELINE) record, thereby serving to verify the relationship between the signal processed
and the sleeping animal.

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The Implant Assembly

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Options to "electrode artifact" within the microwave context are limited.
Figure 2 illustrates our implant components consisting of an all-plastic assembly
which when implanted allows simultaneous recording of ECoG, EOG, and EMG. Exter-
nal guide tubes are directed toward their respective sites in neck muscle, ante-
rior and posterior extraocular muscle, and occipital cortex of the rabbit. An
internal tube is then inserted through which either a metal electrode or stylet
is ultimately passed. The electrodes are fabricated from 0.008 inch diameter
15 coated stainless steel, and all components are supplied by Plastic Products
Company of Roanoke, Virginia. Figure 3 represents a typical rabbit implant. It
should be noted that the electrodes are not connected to the animal during the
irradiation procedure. Our study was designed to assess either transient or
20 long term adaptive changes in the sleep process, both before and after, but not
during radiation exposure. While the sleep literature indicates traditional
use of rodent and feline models, it has been pointed out by several investigators
that neither model is adequate for purposes of extrapolation to human sleep
phenomena. The sleep cycle of rabbit may not be any more appropriate in this
25 regard, yet its use throughout the neurosciences community is becoming more
prevalent.

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Instrumentation

Figure 4 illustrates the manner in which ECoG, EOG, and EMG signals were
processed. Signals were taken from two animals simultaneously and displayed at
the polygraph pens in the usual fashion. However, logic circuitry was designed
35 to control activation of chart drive, pens, and the video tape recorder. Schmidt
triggers (BRS/LVE, Tech Serv Inc.) were set to discriminate both cortical and
neck muscle voltage amplitudes for each animal. Either subject could activate
the equipment only if the conditions of paradoxical sleep were met. That is,
either subject was required to demonstrate both low voltage, fast cortical ac-
40 tivity, and the total absence of neck muscle activity. A high speed printer
(Franklin Electronics Inc.) was used to record the frequency and duration of
paradoxical sleep episodes, as well as the time of day such episodes occurred
for each animal. A Sony Special Effects Generator was introduced in the video
system to allow simultaneous display of the sleeping animal and its associated
45 polygraph record.

Figure 5 illustrates the irradiation chamber and associated equipment. A
Hewlett Packard (HP616A) UHF signal generator was used in conjunction with a
TWT amplifier (model 10482, 100 watt, Microwave Cavity Lab, La Grange, Ill.) to
50 deliver pulsed microwave radiation at a frequency of 3.7 GHz and a pulse repe-
tition rate of 1000 pps (60% duty cycle) for two hours daily. Power density
was adjusted to an average of 10 mW/cm² within a 50 cm x 61 cm x 33 cm styrofoam
box placed in the far field. A calibrated Narda isotropic probe (model 8321)
was used to make this measurement. The styrofoam box was designed to allow
55 dorsal irradiation of two animals simultaneously (in separate compartments) in
the far field condition. Sham irradiation was accomplished on control subjects
by the use of an RF switch (Microwave Associates, Inc.) in line with a dummy
load. Irradiation and sham irradiation were carried out under double blind
conditions. Ambient temperatures were monitored and maintained at approximately
25°C (±1°C) within the chamber, and core temperatures were monitored (Yellow-
spring Inst. Co.) on all subjects both before and after irradiation procedures.

Of those animals examined, core temperature varied between 0.1 and 0.2°C for both experimental and control animals. We interpret these core temperature changes as indicative of the absence of thermal stress on the animals which might otherwise be attributable to environment or treatment conditions. Rather, minor variations in core temperature are to be expected in accordance with the manner in which such measurements were obtained. External fans were used to provide adequate ventilation to the chamber.

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Experimental Design

Eight male Dutch rabbits, 6-9 months of age and weighing approximately 1.8 kg (obtained from S.B. York, Jr., Richmond, VA), were maintained on a twelve hour light/dark cycle throughout the study. The animals were allowed two weeks post operative recovery from surgery prior to being fitted with an electrode cable. The animals were then allowed one week adaptation to their cables, environmental chamber (BRS/LVE Tech Serv Inc.), and handling conditions. No recordings were made during adaptation. Figure 6 illustrates the irradiation schedule which was continued for sixty consecutive days for all animals. Experimental animals were irradiated daily, and recorded from in pairs for convenience. Following actual irradiation or sham irradiation, the animals were treated in three possible ways: 1) they were recorded from, 2) they were placed in pre-recording status, or 3) they were returned to their home cage. The pre-conditioning box (PCB) consisted of an environmental chamber, (24 cm x 30 cm x 28 cm), similar to the recording chamber, within which the cabled animal was allowed to stay 24 hours prior to a recording session. All treatments were counterbalanced so as to allow equal treatments across animals, and to insure an equal probability that each animal would be recorded from following irradiation.

The person responsible for analyzing sleep records had no knowledge as to the identity of control or experimental animals. Irradiation procedures were carried out between 11 am and 3 pm daily, while all post irradiation conditions occurred between 4 pm (same day) and 10 am of the following day.

RESULTS

Figure 7 illustrates a typical record from a single animal. Part 1 illustrates low voltage, fast cortical activity characteristic of arousal in the awake animal. Part 2 illustrates a "drowsy" animal, while Part 3 illustrates slow wave sleep. Part 4 illustrates the combination of low voltage, fast cortical activity with the total absence of neck muscle activity characteristic of paradoxical sleep. Eye movements also occur in conjunction with paradoxical sleep in this record. Illustrated from video tape in figure 9 is the sleeping animal with an associated paradoxical sleep record. In contrast, the same animal appears in figure 8 representing its awake state. Preliminary data indicate that paradoxical sleep recorded from control subjects is fairly reliable between subjects. Approximately 25-35 minutes of the animal's total sleep time was exclusively paradoxical sleep. Paradoxical sleep for these animals was intermittantly dispersed throughout an 18-hour recording session in 3 to 8 minute segments. We observed that of those control animals examined, each had a propensity for blocks of sleep between 4 pm and 6 pm and between 4 am and 8 am the next day. We interpret these preliminary data as suggestive that our animals are adapting to laboratory routine. As our records are currently being scored and processed, it is too early to comment as to the possible relationships between electromagnetic radiation and paradoxical sleep. Hopefully, the techniques and methodologies discussed will lead toward an expanded understanding of altered brain electrical phenomena associated with electromagnetic radiation exposure.

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ACKNOWLEDGMENT

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The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. The animals used in this study were handled in accordance with the provisions of Public Law 89-54 as amended by Public Law 91-579, "Animal Welfare Act of 1970", and the principles outlined in the "Guide for Care and Use of Laboratory Animals", U.S. Department of Health, Education and Welfare publication no. (NIH) 73-23. The animals were maintained in an animal care facility fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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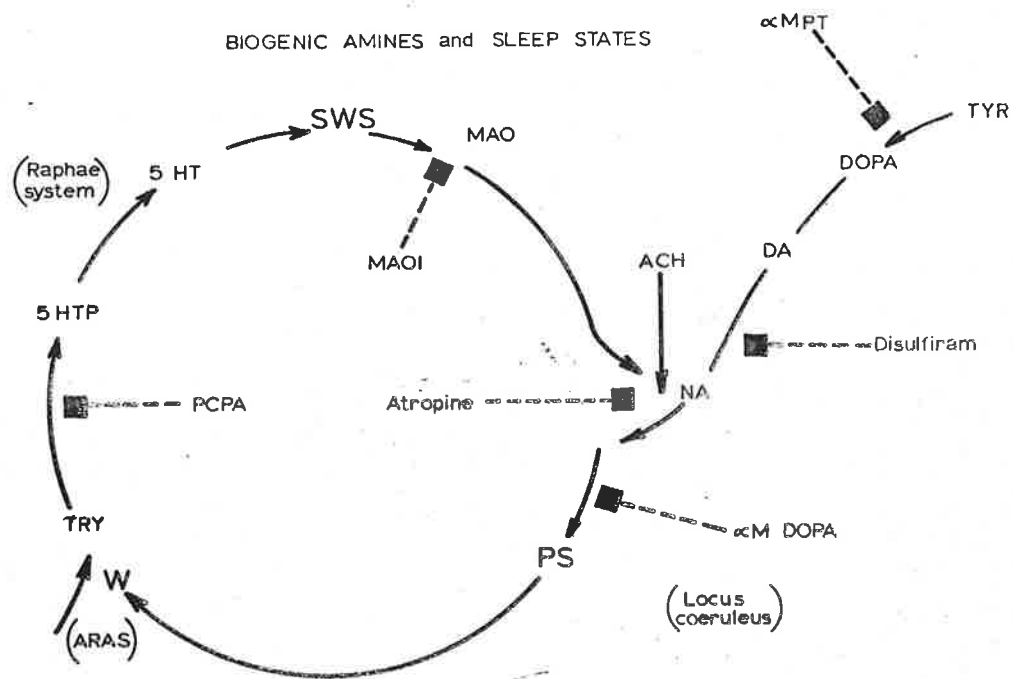
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Fig 1 (Manthel)

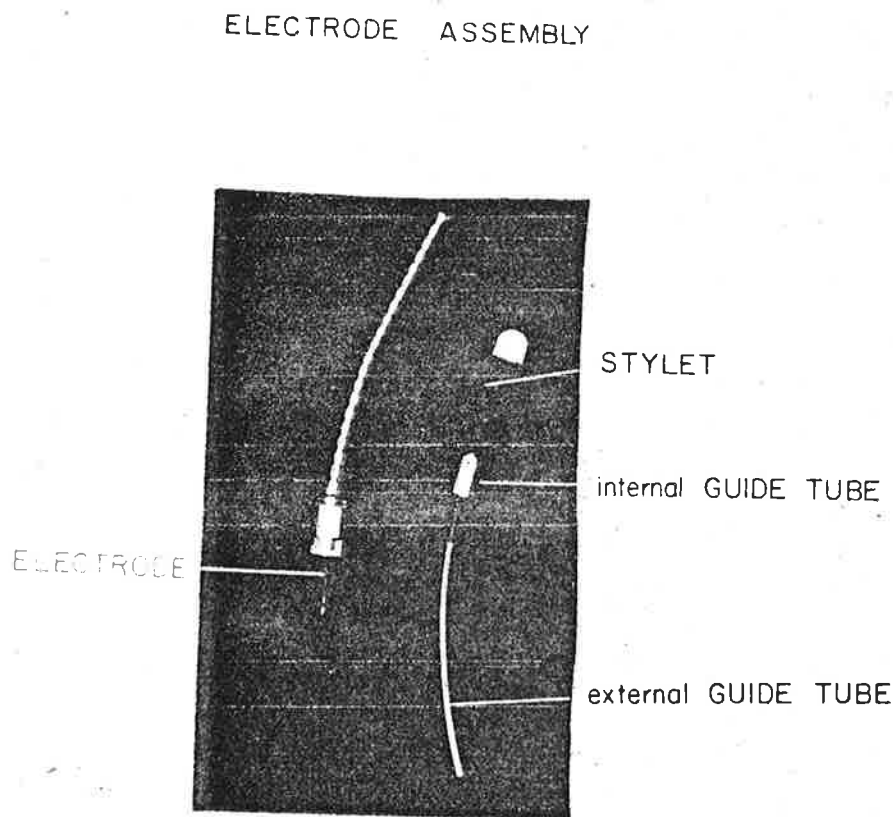


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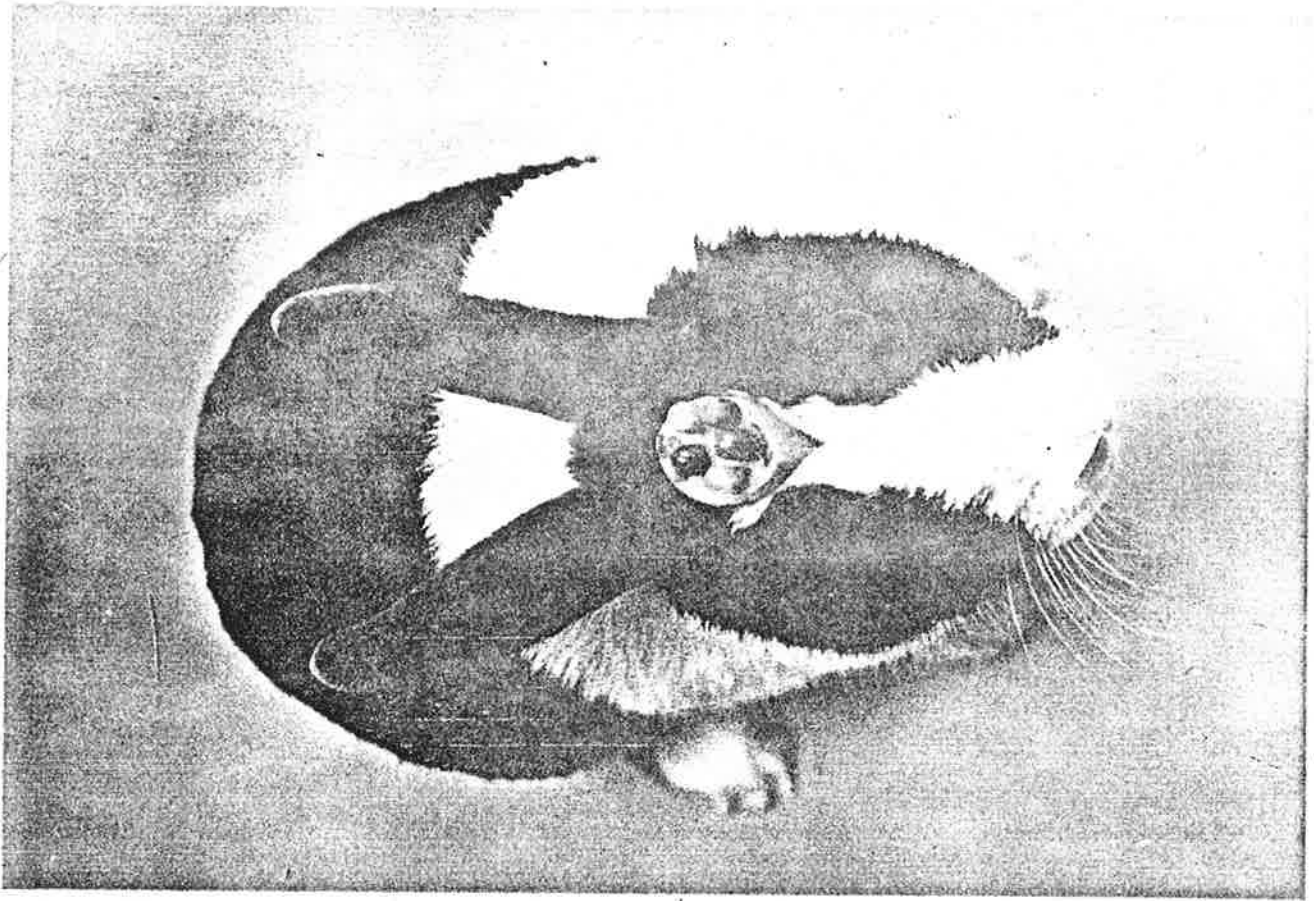


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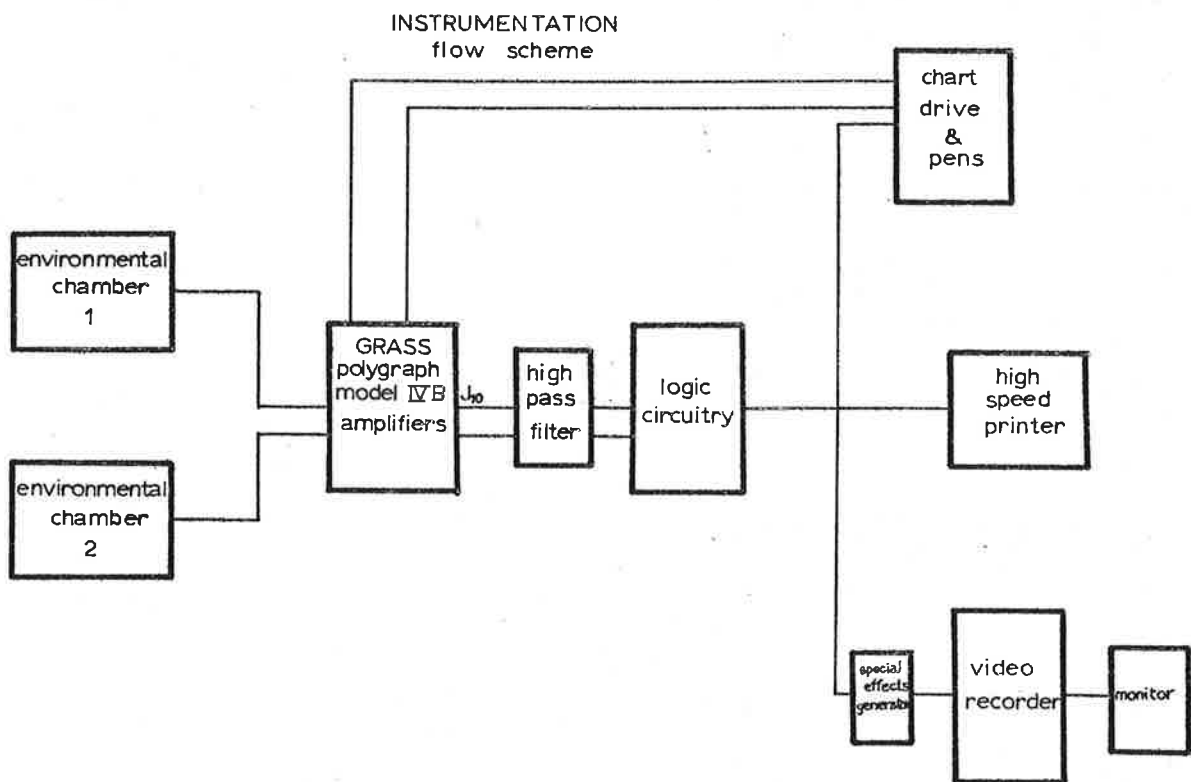


Fig 4 (Mantle)

IRRADIATION SCHEDULE

	day 1	day 2	day 3	day 4
group I 2 controls	SHAM HC	SHAM HC	SHAM PCB	SHAM REC
group II 2 controls	SHAM HC	SHAM PCB	SHAM REC	SHAM HC
group III 2 experi- mentals	Irradiated PCB	Irradiated REC	Irradiated HC	Irradiated HC
group IV 2 experi- mentals	Irradiated REC	Irradiated HC	Irradiated HC	Irradiated PCB

HC - HOME CAGE

PCB - PRE-CONDITIONING BOX

REC - RECORDING

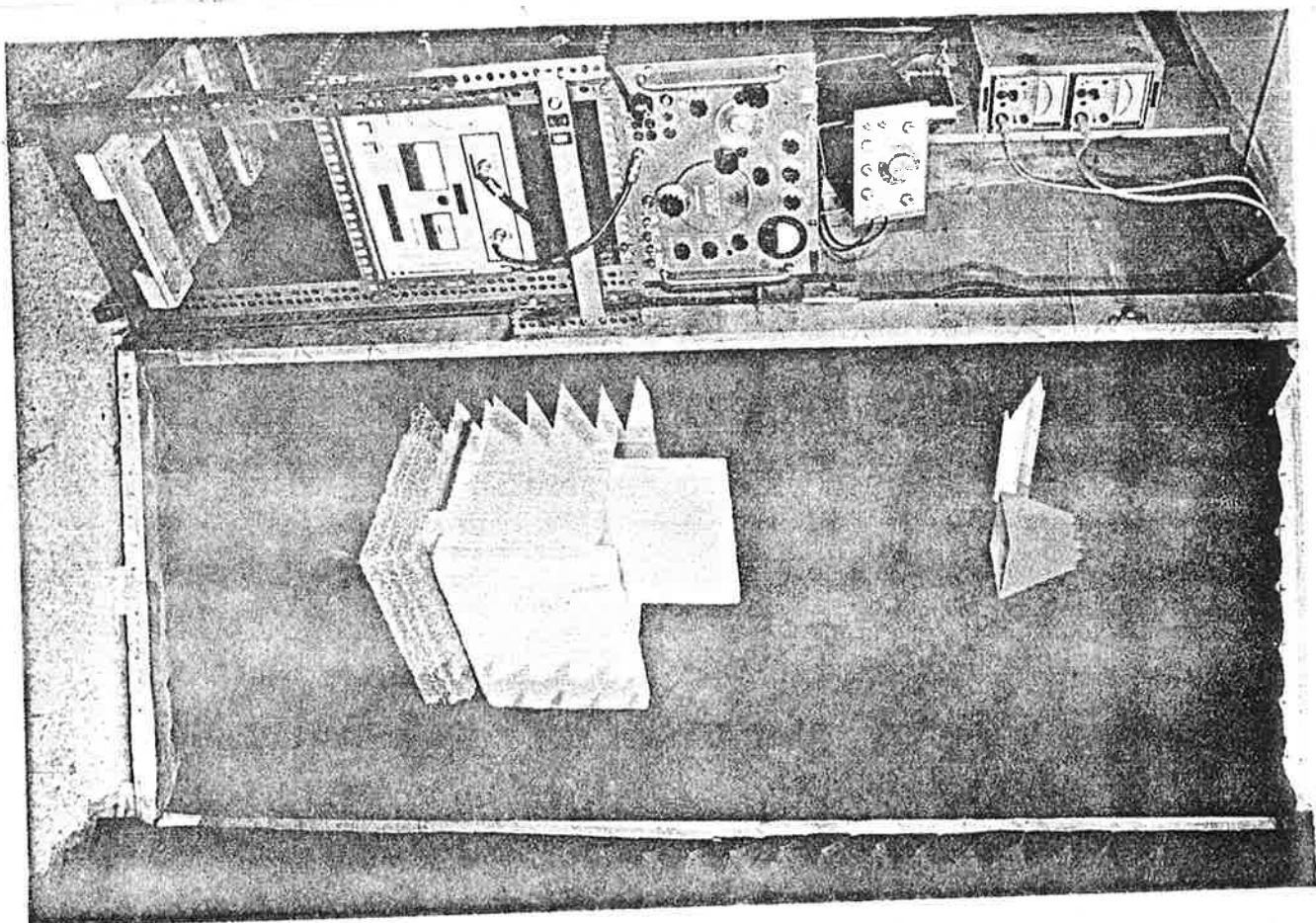


Fig 6 (Maathai)

Fig 5 (Maathai)

POLYGRAPHIC RECORDINGS OF ADULT DUTCH RABBIT

• brackets indicate 50 μ V deflection

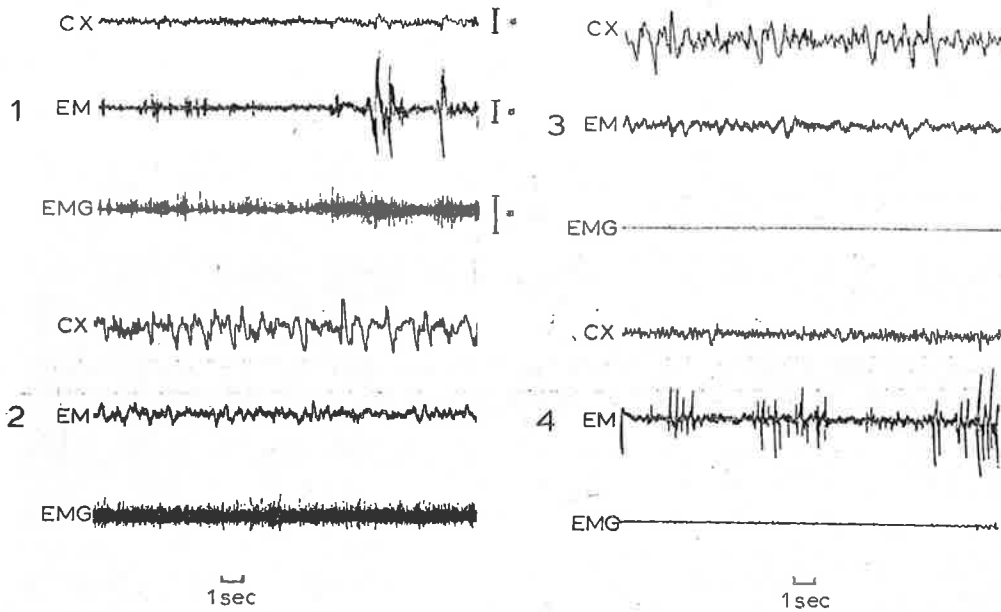


Fig 7 (Manthei)

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