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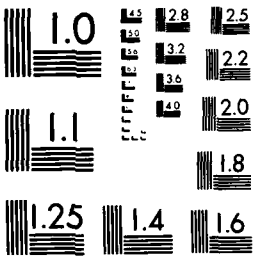

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We studied the effects of the electric and magnetic components of a Loran-C type waveform on three biological systems. Neurochemical assays of brain neurotransmitter substances indicate field-related changes in the levels of norepinephrine in the hippocampus and in the number and affinities of the opiate receptors in the cortex. Behavioral data showed that rats trained in an operant conditioning task did not reliably detect any electric field stressor [illegible] Biochemical data demonstrated that the Loran-C field did not modify basal activities [illegible] decrease activity in primary brain areas.

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ELECTROMAGNETIC FIELD PARAMETERS AND INSTRUMENTATION. A. R. Sheppard, R. A. Jones, M. E. Stell, W.R. Adey.

1. WAVEFORM CHARACTERISTICS

Following consultation with ONR (Marron, 1984, personal communication) the Loran-C waveform was chosen as a model for the test waveform. The simulated waveform is a pulse-modulated 100 kHz carrier with 9 pulses per burst, each pulse of 1 ms duration. The interpulse interval is 1.5 ms and the interburst interval is 40 ms. The pulse envelope is approximately square. Pulse risetimes of 40 to 200 us are determined by power amplifier and load considerations for each apparatus. Table 1 summarizes these parameters in comparison with actual Loran-C waveforms.

2. EXPOSURE APPARATUS

The apparatus for these experiments was custom-designed and constructed in our laboratory as previously described (Renewal Proposal, January, 1986). The systems are a parallel plate system (producing electric fields) for the behavioral studies, a solenoidal apparatus (producing combined electric and magnetic fields) for the neurochemical studies, and an in vitro exposure system (producing electric fields) for the cell culture studies.

3. FIELD MEASUREMENTS

Commercial instruments for E- and H-field measurements in air at 100 kHz were not available for measurements over the frequency and amplitude ranges of this study. Thus, we designed, constructed and calibrated suitable instruments for field calibration and for the mapping of field uniformity within the exposure apparatus.

Electric fields in the solenoidal system were measured using a small probe based on the principles developed for small 60 Hz probes at the National Bureau of Standards (1). A pair of hemispherical brass shells are capacitively-coupled to the field. Electronics within the shells amplify the current and transmit this datum as a frequency-encoded infra-red signal through a fiber optic guide to a receiver located outside the field region. Within the region of the solenoid in which the animals were housed, (fig. 1), the electric field varied about ± 10% from the nominal value. Electric field strengths in the parallel plate behavioral apparatus were determined from the voltage on the plates and the plate separation. Previous calibration of that apparatus at 60 Hz indicated field uniformity of ± 20% over the cage region. Waveforms were observed on the oscilloscope and showed no discernible harmonic distortion.

Magnetic field strengths were measured by a low-capacitance, low inductance search coil connected by magnetically shielded wire to a remote high impedance voltmeter. The search coil was calibrated in a Helmholtz Coil which in turn was cross-calibrated at zero and extremely low frequencies to a Hall Effect Probe, Bell FTB1-0415. The calibration techniques evaluated frequency-dependent changes in the performance of the calibration instruments and coils, especially because unintended resonance conditions could greatly affect the measurements. The magnetic field of the solenoid apparatus was uniform to ± 10% over the cage region (fig. 2).

Electric field strength in the in vitro apparatus will be mapped directly by a pair of electrodes in a medium of reduced conductivity. Electric field strength is routinely determined from total current in the dishes and the depth of the physiological medium (i.e., area through which the current flows).

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NEUROCHEMICAL STUDIES. B.J. Vasquez, S.M. Bawin, and W.R. Adey

1. OBJECTIVES

The goal of this research is to monitor brain neurotransmitter substances after single and repeated in vivo exposure to combined electric and magnetic fields. Although there is a body of evidence showing that exposure to electric and magnetic fields can alter synaptic function (1) and by consequence nervous system integrity, the neurohormonal correlates of these changes have not yet been systematically studied. The availability of neurotransmitter substances as well as the number and sensitivity of their pre- and postsynaptic receptors are of main importance in the sequence of neural transmission (2). To address the question of the combined effects of the electric and magnetic components of the Loran-C waveform, we have begun to study the biogenic amine and opiate receptor systems in rat brain tissues. These systems are correlated with endocrine control of biological rhythms and responses to environmental stresses.

2. METHODS AND PROCEDURES

2.1 Exposure apparatus

The studies are conducted in a solenoid driven by a power amplifier and custom-built resonant transformer that creates both electric and magnetic fields. Each solenoid contains 1 acrylic chamber; each chamber holds 8 rats in two tiers. The maximum electric field strength is about 3.3 kV/m rms, maximum magnetic field, 53 uT rms. The apparatus is contained in a cubical shielded room 2.06 m on each side. Two identical shielded enclosures and solenoids were constructed and either may be selected as the sham or test chamber. The enclosures are located in a temperature and humidity controlled room in the vivarium facilities. In between exposure periods, animals are housed in a nearby room under the same environmental conditions. The light are on a 12:12 cycle (lights on at 0600h).

2.2 Animals

Male albino rats (Sprague-Dawley) (Bantin & Kingman, Fremont, CA) were used. The experiment was started after 5-7 days of habituation to the vivarium facilities. For three days prior to exposure animals were handled and were then placed in the deactivated exposure system for one hour. Food and water was available ad libitum except during the time the animals were in the exposure apparatus.

2.3 Exposure Schedule

Previous results from this laboratory have shown no reliable electric field-related effects on the brain biogenic amines from animals exposed to 60 Hz (14 kV/m) fields when samples were obtained at only one time during the day. However, examination of the same biogenic amines at different times during the day (in animals exposed to 39 kV/m) allowed us to detect effects on the daily rhythms of norepinephrine (NE), dopamine (DA) and its main metabolite (DOPAC). Based on these data we have chosen three times of the day for single or repeated exposure of rats to the Loran-C fields (9AM, 12PM, and 3PM). The animals were housed in the exposure apparatus at the designated times and were habituated for 30 min before the field was turned on. They were exposed one hour per day, five days a week, for up to four weeks.

2.4 Neurochemistry

Following treatment the animals were immediately killed by decapitation and the brains were dissected into 6 regions: striatum, hypothalamus, hippocampus, anterior and posterior cortices, and brainstem. Adrenal glands were also removed to test for possible changes due to generalized stress. The tissues were immediately submerged in liquid nitrogen and later stored at -70 C until assayed. Biogenic amines were estimated in the striatum, hypothalamus, hippocampus, anterior cortex and adrenals using standard techniques for HPLC with electrochemical detection. For the opiate receptor studies, homogenates of brain stem and posterior cortex were used to obtain Scatchard plots with one of six concentrations of [^{3}H]naloxone ranging from 0.125 to 4 nM. Calculations provided the number (Bmax) and apparent affinity (Kd) at each time point.

3. RESULTS

We have completed exposures at 9 AM and 3 PM. Each shift included 48 (24 sham and 24 exposed) rats field-exposed for one day, 1 week or 4 weeks. Data were analysed by two-way analysis of variance (ANOVA) and "a posteriori" comparisons by Newman-Keuls tests with one step between means.

3.1 Biogenic Amines

The data obtained in this experiment are generally in agreement with results from a previous study where rats were exposed for four weeks to a 60 Hz electric field (3, 4).

Daily changes (circadian or ultradian) in the levels of biogenic amines have been observed in our laboratory (3). In the tissues from animals exposed to the Loran-C fields we have found significant differences in the amine levels at the two times of day tested so far. These changes were independent of the length and type of treatment. A field effect was detected only in the levels of NE in hippocampus after four weeks of exposure [ANOVA: $F(1,28) = 5.75$; $p = 0.0234$]. Tests of group comparisons ($n = 8$) determined the difference to be significant only at 9AM (fig. 3a), with field-related increases in the levels of NE. Levels of NE, epinephrine (E), and DA in the adrenal glands were not altered by field exposure. This is evidence that the treatment did not produce any stress response.

3.2 Opiate Receptors

Brain stem:

a. Time of day significantly increased the apparent affinity of the receptors showing lower Kd values in the PM groups.
b. No field-related effects in the Bmax or Kd were observed.

Posterior cortex:

a. Time of day produced a significant change in the number and affinity of receptors with higher values in the PM group.
b. The field produced a borderline effect on Kd [ANOVA: $F(1,19)=4.08$; $p=0.0576$]. A field-related decrease in the Kd values for the PM group ($n = 6$) indicated an almost significant increase in the receptor affinity (see fig. 3b).

As in our previous study with 60 Hz electric fields (4), the opiate receptor system in the brain stem (a deep brain structure) was found to be insensitive to the Loran-C field. Conversely, the same system in the posterior cortex (a peripheral brain structure) seems to be more easily affected by the electromagnetic fields. A replication of the experiment including a third time of day group (12PM) is now underway at our laboratory.

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BEHAVIORAL STUDIES. M.E. Stell, P.M. Sagan, G.K. Bryan, and Adey W.R.

1. OBJECTIVES

The objective of this study is to determine if behavioral changes are produced by a 100 kHz electric field with a Loran-C type waveform. The biological effects of this frequency have not received extensive investigation. However, the rationale for suspecting that there might be behavioral changes comes from the observation that there are changes produced by electromagnetic radiation at frequencies both above and below 100 kHz. For example, in the the ELF range numerous behavioral effects have been demonstrated at 60 Hz (e.g. 1-4). At microwave frequencies behavioral changes have also been demonstrated (e.g. 5-6). We therefore undertook to investigate effects at this intermediate frequency, 100 kHz.

Experiment One

METHODS

The first experiment was a psychophysics experiment for the following two reasons: 1) rats can detect 60 Hz electric fields, and 2) the Loran-C type waveform has frequency components in the ELF range. A test chamber was constructed that would produce a relatively pure electrostatic electric field using a 100 kHz carrier, pulse modulated at the Loran-C frequencies. The system was capable of producing fields from 0 to 9 kV/m (rms when the carrier was unmodulated).

Rats were were taught to indicate the presence or absence of a stimuli by pressing levers in an operant chamber. Initially the stimulus was a tone. After sufficient training the Loran-C type field became the stimulus; and the rats were required to indicate the presence or absence of the electric field.

RESULTS

Two rats were trained on this procedure. Neither could reliably detect any field strength used. The psychometric function for one rat is shown in fig. 4. If the rat could detect the field then the figure should display a positive slope, this is because as the field strength increased the subject's ability to detect it (and the resultant discrimination ratio) should increase. In fig. 4 the subjects' performance did not improve as the field strength increased.

Experiment Two

METHODS

Since the previous two subjects appeared not to detect the field we used a slightly different method. In this experiment the goal was to make the chances of a successful detection of the field as likely as possible. Two changes were instituted. First, the subjects pre-trained on both a tone and then a 60 Hz electric field in order to maximize the training the subjects received, and to make the transfer to the 100 kHz field as easy as possible. And, secondly, only two intensities of field were used; full field during stimulus trials (50%) and no field during no-stimulus trials (50%).

RESULTS

Two rats used in this modified procedure could detect the 60 Hz electric field, as has been demonstrated before. Thus, the training procedure, equipment, and the subjects appeared to be performing normally. However, once again, no evidence of detection of the Loran-C type field was obtained.

Experiment Three

The third experiment in this series, currently ongoing, is a replication of

experiment one using four additional subjects, to verify that no detection can be demonstrated.

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BIOCHEMICAL STUDIES. C.D. Cain, A.R. Sheppard, and W.R. Adey.

1. OBJECTIVES

The electric field strength induced in the pericellular space by Loran-C signals is small compared to the electric field across a cell membrane. This suggests that nonlinear and nonequilibrium forces are likely to be involved in amplifying these signals if they are to have biological effects. Enzymatic systems within the cell membrane offer tremendous amplification of signals on the cell surface and could reflect interactions with electric fields. Ornithine decarboxylase (ODC) is a highly regulated enzyme and its activity can be controlled by external signals such as hormones and growth factors. Its activity is therefore an excellent marker to monitor membrane signal transduction. ODC's importance in cellular growth and proliferation is another reason for its appropriateness as a measure of field interaction. ODC is the rate limiting enzyme in polyamine biosynthesis and is absolutely required for cellular growth in all eukaryotic cells. Field effects on ODC activity can be indicative of changes in cell growth and proliferation. The reproducibility and ease of assaying ODC make it an excellent marker enzyme to observe field effects at the cellular level.

2. EXPOSURE SYSTEM

The agar-bridge system was designed to expose cells in tissue culture to electric fields of well-defined fields strengths and current densities. Current was passed through growth medium in petri dishes (9 x 9 cm) connected in series by glass bridges. Bridges were made from two concentric glass tubes having different diameters (28 mm and 41 mm). The tubes (8 cm long) were sealed at the ends and cut in half lengthwise to make two bridges. A solution of 1% agar in phosphate-buffered saline (10 ml) was then poured into the space between the walls to form a hemi-cylindrical conducting bridge. The resistance of a bridge (150 ohms) was comparable to the resistance of the medium across the length of a petri dish (170 ohms). The electric field was produced by a constant-current source gated by a microprocessor-based Loran-C waveform generator.

3. METHODS

Bone cells were collagenase digested from mouse cranial bones and then grown for 6 days (1). Confluent cells in 9 x 9 cm petri dishes were exposed to the field for one hour. ODC activity in the cell homogenate was determined at various times after field exposure by measuring the amount of $^{14}CO_2$ released from [^{14}C]ornithine (1).

4. RESULTS

Since we previously found ODC activity in primary bone cells was sensitive to low-frequency pulsed electromagnetic fields, we first tested the effects of a 60 Hz field on ODC activity in the same cells (1). A one hour exposure to a 10 mV/cm (rms) field with an associated current density of 160 uA/cm^2, produced a two fold increase in the ODC activity when assayed 10 minutes to one hour after field exposure. The magnitude of the fields used in vitro is similar to the endogenous fields that are measured on the bone surface (2). The second test used the Loran-C waveform also at 10 mV/cm (rms) field strength. Under the same protocol as the 60 Hz field, the Loran-C field did not modify basal ODC activity in primary bone cells (fig. 5).

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CHARACTERISTIC	UNIT	LORAN C ANTENNA	LABORATORY
Source		Antenna & feed lines	Solenoid (0.77 m dia.) or Parallel Plates
Frequency	kHz	100	100
Pulse Modulation			
number/burst		8, MS: 9	9
pulse duration	us	~250	1000
interpulse	ms	1	1.5
final MS[1] interpulse	ms	2	1.5
burst duration	ms	8, MS:10	21.
interburst interval	ms	40-100	40.
rise/fall times[2]	us	~50/150	200/200 (Sol.) est.
E-field			
max. in air	kV/m	2.7 rss[3,4]	3.3 rms (Sol.) est.
		0.6 rms	9.0 rms (Par. Plate)
typical		<0.2 rss	
		<0.05 rms	
orientation		near field	perp. to spine
H-field			
max	uT	33. rss[3,4]	50. rms (Sol.)
typical		0.1 rss, rms	0 (Par. Plate)
orientation		near field	parallel to E_o, perpendicular to E_{ind}

1) MS ==> Master Station
2) Pulse shape envelope function: $t^2 e^{-2t/65}$, t in microseconds.
3) Values cited are maximum or typical values from McEnroe, 1980 who surveyed the Nantucket Loran C Station. Maximum values occurred at 5 feet from the tower base.
4) rss indicates square root of the sum of the squares; rms indicates square root of the mean squared for the unmodulated carrier.

TABLE 1

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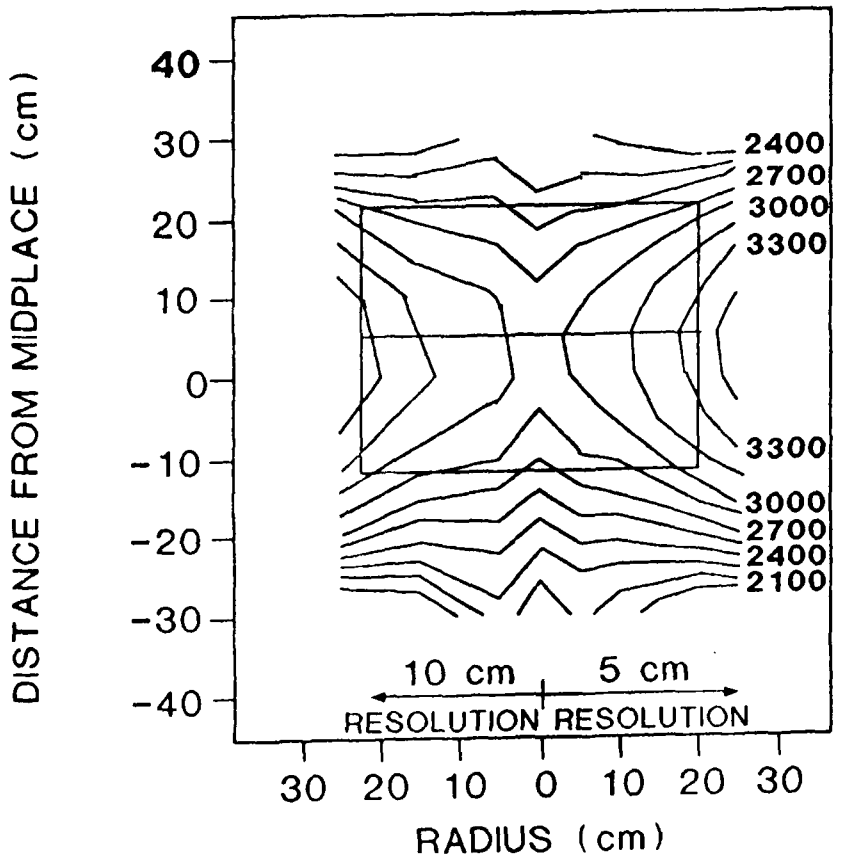
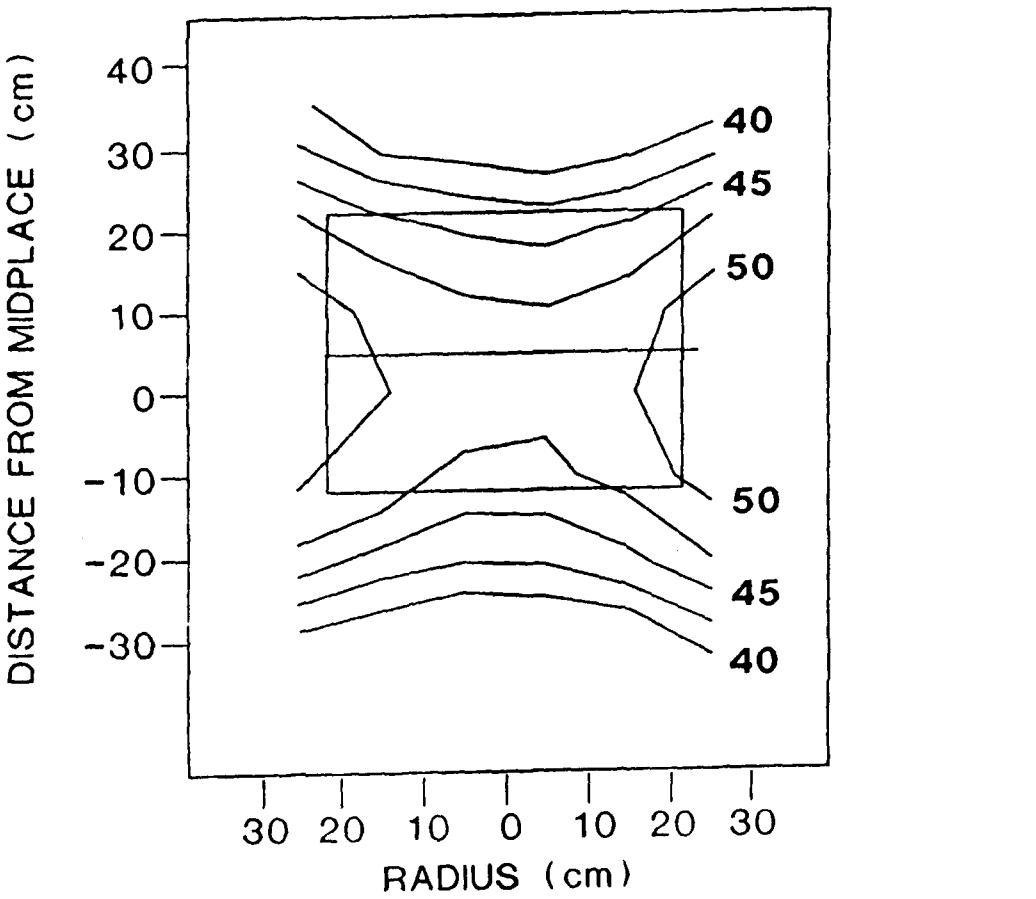
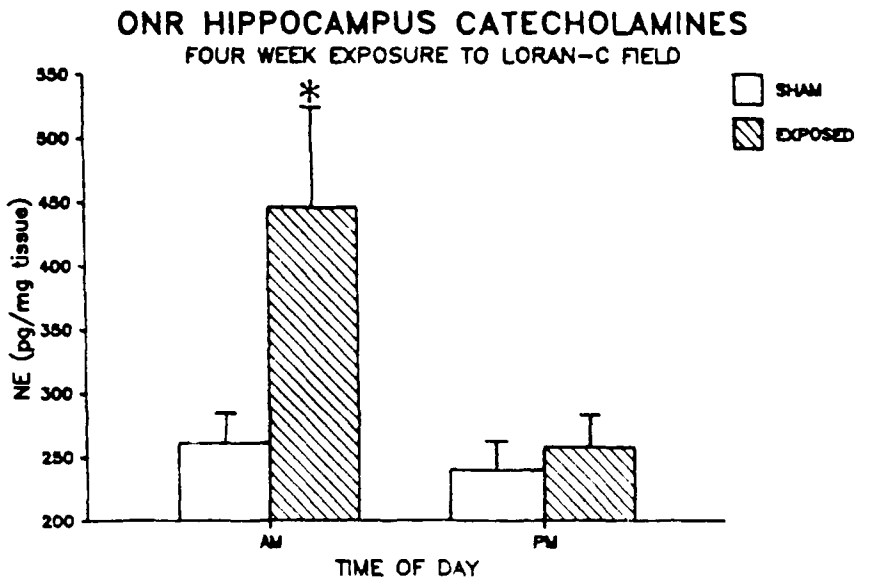
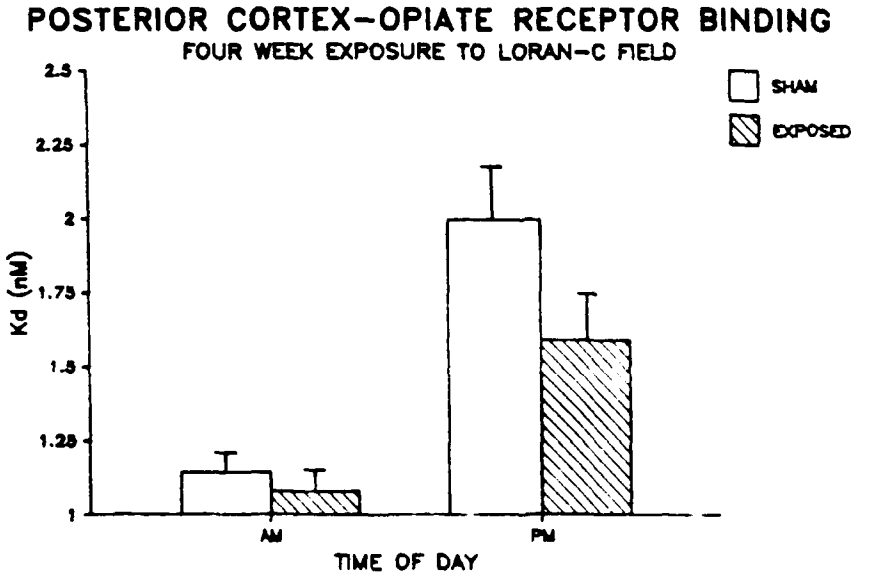

100 KHZ ELECTRIC FIELD (5% CONTOURS V/M RMS)
DISTANCE FROM MIDPLACE (cm)
40
30
20
10
0
-10
-20
-30
-40
2400
2700
3000
3300
3300
3000
2700
2400
2100
10 cm
5 cm
RESOLUTION
RESOLUTION
30 20 10 0 10 20 30
RADIUS (cm)


100 KHZ MAGNETIC FIELD (5% CONTOUR, μT RMS)
DISTANCE FROM MIDPLACE (cm)
40
30
20
10
0
-10
-20
-30
40
45
50
50
45
40
30 20 10 0 10 20 30
RADIUS (cm)

A

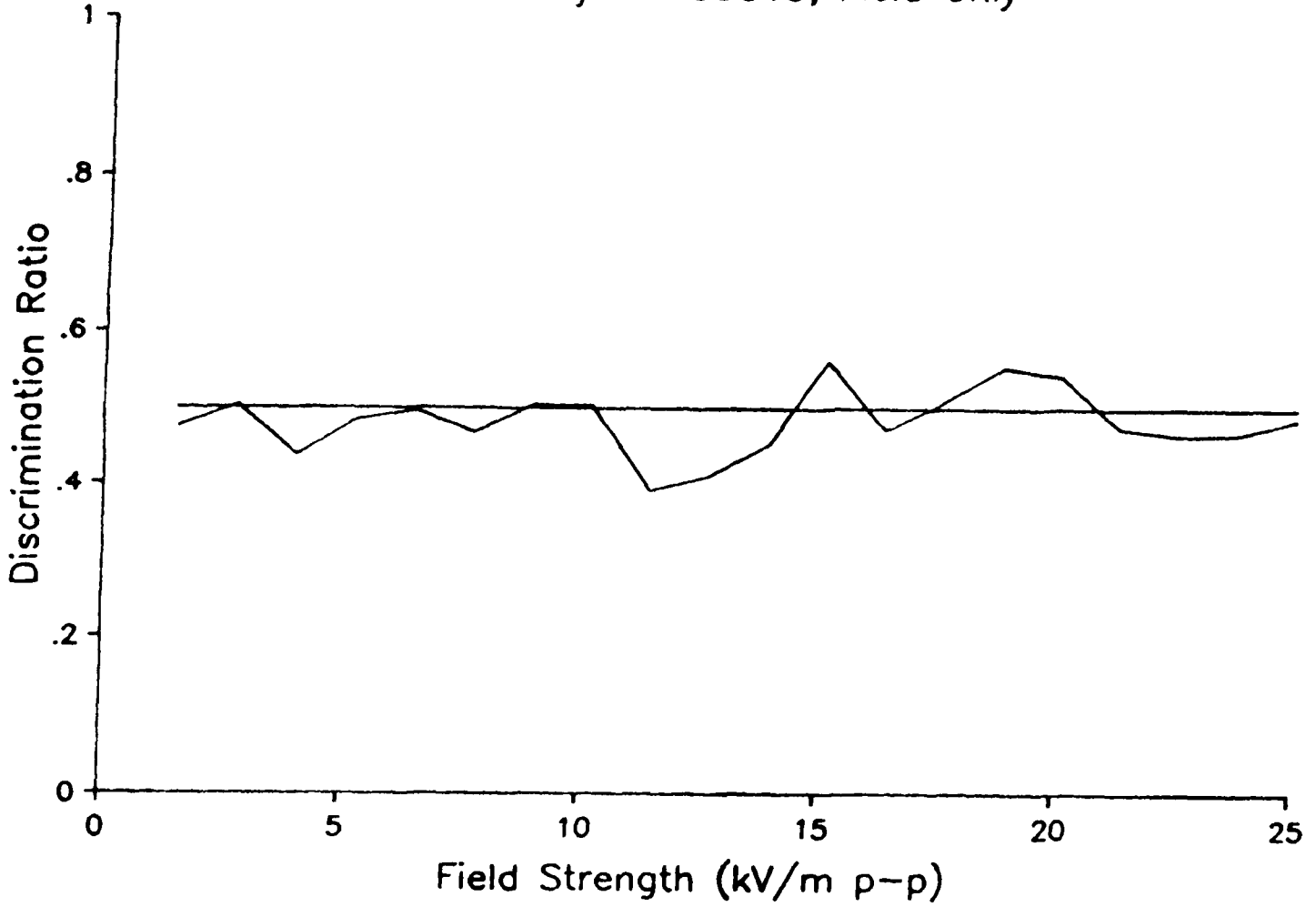

ONR HIPPOCAMPUS CATECHOLAMINES
FOUR WEEK EXPOSURE TO LORAN-C FIELD
SHAM
EXPOSED
NE (pg/mg tissue)
550
500
450
400
350
300
250
200
*
AM
PM
TIME OF DAY

B


POSTERIOR CORTEX-OPIATE RECEPTOR BINDING
FOUR WEEK EXPOSURE TO LORAN-C FIELD
SHAM
EXPOSED
Kd (nM)
2.5
2.25
2
1.75
1.5
1.25
1
AM
PM
TIME OF DAY

Psychometric Function

ONR Subject R85015, Field Only



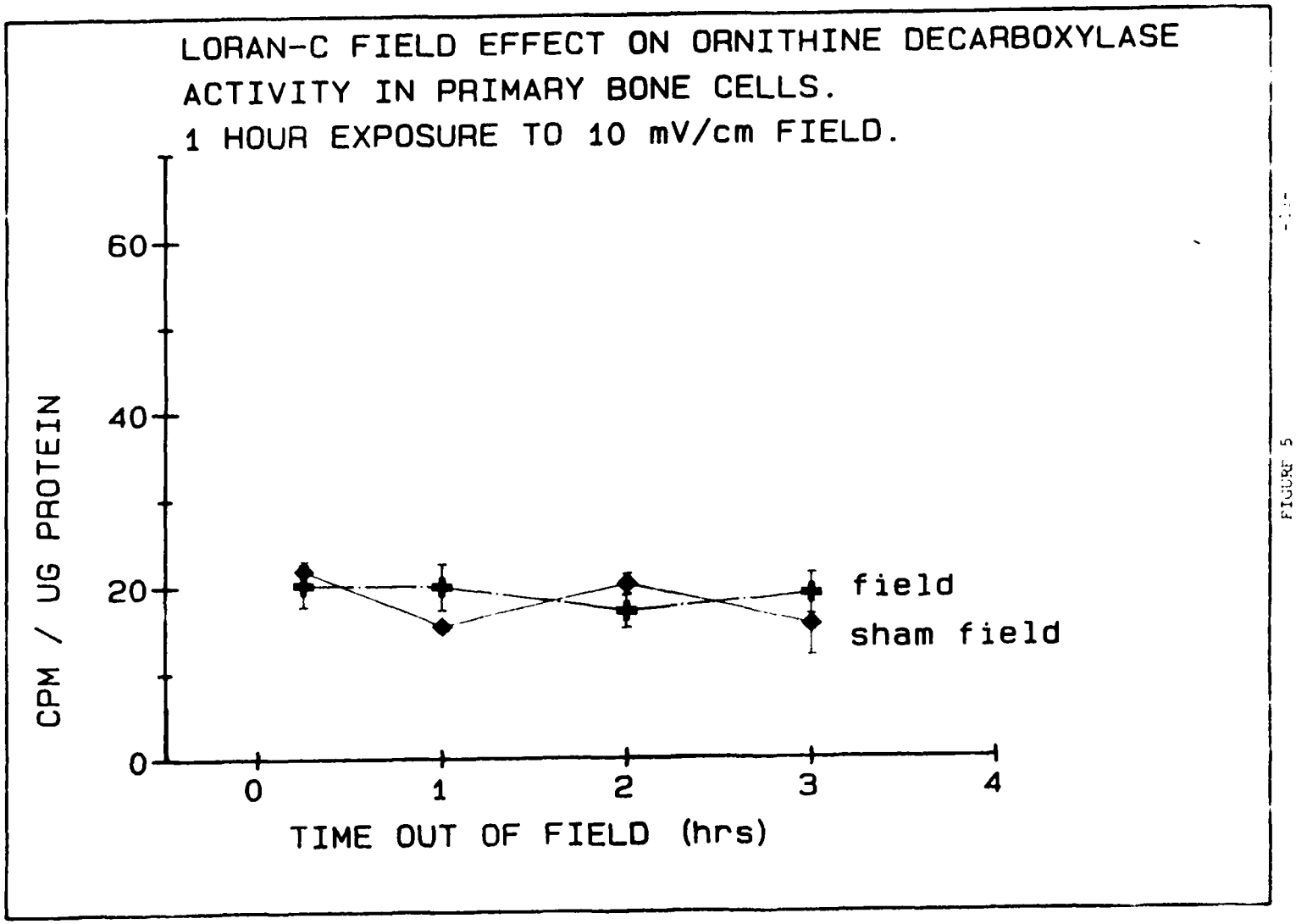

LORAN-C FIELD EFFECT ON ORNITHINE DECARBOXYLASE
ACTIVITY IN PRIMARY BONE CELLS.
1 HOUR EXPOSURE TO 10 mV/cm FIELD.
CPM / UG PROTEIN
60
40
20
0
0
1
2
3
4
TIME OUT OF FIELD (hrs)
field
sham field

FIGURE 5

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