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A STUDY OF NEAR INFRARED EMISSION FROM THE MAMMALIAN CEREBRAL CORTEX

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AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Contract Monitors: Dr. Joseph Mundie and Lothar O. Hoeft, Capt., USAF
Project No. 7232, Task No. 723204

(Prepared under Contract No. AF 33(657)-8056 by
Richard M. Roppel of Battelle Memorial Institute, Columbus, Ohio)

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FOREWORD

This study was initiated by the Biophysics Laboratory of the 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was conducted by Battelle Memorial Institute of Columbus, Ohio, under Contract No. AF 33(657)-8056, Project No. 7232, "Research on Logical Structure and Function of the Nervous System", and Task No. 723204, "Bionic Neurophysiology". Dr. Richard Roppel, Biophysicist, was the principal investigator for Battelle Memorial Institute. Dr. Joseph Mundie and Captain Lothar O. Hoeft, Biodynamics and Bionics Division, were the contract monitors for Biophysics Laboratory. The research sponsored by this contract was started in April 1962 and was completed in March 1963.

Acknowledgment is made to the following persons for helpful discussion and technical assistance during the course of the research:

Dr. Ernest Retzlaff and Mrs. Joan Fontaine of the Psychiatric Institute and Hospital at Ohio State University; Dr. Barnes and Mr. Douglas Worth, Barnes Engineering Company, Stamford, Connecticut; Dr. Ronald Melvack, Department of Psychology, Massachusetts Institute of Technology; Juan Negrin, M.D., New York City; Mr. Charles Reeder of the Reeder Instrument Company, Detroit, Michigan.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

ABSTRACT

The following experiments were conducted to investigate the existence and quantitative characteristics of photon radiation from the mammalian cerebral cortex, in excess of that due to thermal radiation.

Surgical exposure of the cerebral cortex of several mammalian species was performed and various forms of radiant energy transducers were employed to detect and/or to measure the intensity of radiation of cortical origin. In some experiments, observations were carried out during electrical and chemical stimulation of the cortex. Measurements of emitted radiation were also made during termination of the animals by injection of cytotoxic materials into the brain via the carotid artery to achieve a rapid cessation of vital activity of the cortical tissue.

During the termination experiments, small changes in radiancy of the cortical tissue were observed; the magnitudes of observed changes were not consistent. During the experiments in which electrical stimulation was applied, radiation changes were occasionally observed which were so related in time to the stimulus as to suggest causation by the stimulus. Transducers used included a commercial infrared radiometer with a thermistor detector, a thermocouple radiometer, and an infrared sensitive photomultiplier tube.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

Jos M Quashnock

JOS. M. QUASHNOCK
Colonel, USAF, MC
Chief, Biophysics Laboratory

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INTRODUCTION

This research originated as an attempt to answer the question as to whether vital neural tissue emits photon radiation in excess of thermal radiation. The author had previously observed that the exposed cortical surface of mammals appears bright relative to surrounding tissue when viewed through an infrared image converter device. Other living organs and tissues exhibit relatively low apparent brightness. Intense electrical simulation of the palmar surface of the forepaw of a cortex-exposed cat resulted in the appearance of a gradient of cortical surface brightness in which apparent brightness was maximal at the somatic projection area. The original observations were made using an optical system and image converter tube of small resolving power. The observations were interpreted as being due either to a change in surface reflectivity, surface emissivity, or cortical radiancy, correlated with the functional state of the cortex.

The purpose of this investigation was to determine by instrumental measurement whether such emissive activity exists, and if so, to determine approximately the intensity of emitted radiation and whether it could be correlated with functional state of the tissue.

Although the original observations were made by use of an image converter tube limited in wavelength response to approximately 1.4 microns, there was no a priori reason for restricting the wavelength range of the investigation to this region. It was decided initially to use wavelength-independent detectors to determine whether emissive activity was present in any spectral region. Devices of this kind, being responsive to the wavelengths involved in thermal radiation at near-ambient temperatures, could be used with appropriate filters to discriminate between thermal infrared emission and that due to other causes. It was planned also to make use of a photomultiplier detector having a photoemissive surface of approximately the same spectral response as the image converter tube with which the original observations were made.

A literature search has failed to disclose any published report of photon emission, or luminescence, of mammalian tissues in vivo. The bioluminescence phenomena associated with microorganisms, insects, and fish, which generally arise from specialized organs or chemical systems, have been extensively studied in the past. The fluorescence of vertebrate tissue components, which requires that the light-emitting state be excited by light of shorter wavelength, has been studied and employed as an effective tool in recent biochemical studies. B. Chance and F. Jöbsis at the Johnson Research Foundation, Philadelphia, Pa., have studied the fluorescence changes of frog muscle under stimulation (ref. 1). Chance, Conrad, and Legallais employed spectrophotometric methods to analyse similar fluorescence changes of isolated tissue components in terms of specific metabolic events (ref. 2).

During the early phases of the present study, an inquiry was made to the Barnes Engineering Co., Stamford, Conn., manufacturers of IR radiation-measuring instruments to learn whether Barnes instruments had been employed in studies of tissue radiancy, and also to learn whether specialized research devices manufactured by Barnes would be adaptable to this use.

Pursuant to this inquiry, technical personnel of Barnes, assisted by Dr. Ronald Melvack of the Dept. of Psychology, Mass. Institute of Technology, conducted an experiment in which the exposed cortex of a cat was surveyed with a thermistor radiometer. The instrument used was an infrared camera with the scan disabled. Stimulation of the retina by light consistently resulted in decreased radiancy of the visual projection area of the cortex. This was interpreted by the investigators as due to decrease of cortical surface temperature following light stimulus (ref. 3).

METHODS

Instrumentation

Thermocouple Radiometer

Figure 1 is a photograph of the Thermocouple Radiometer employed in the preliminary phases of this study. It was manufactured by the Reeder Instrument Company, 173 Victor Avenue, Detroit 3, Michigan.

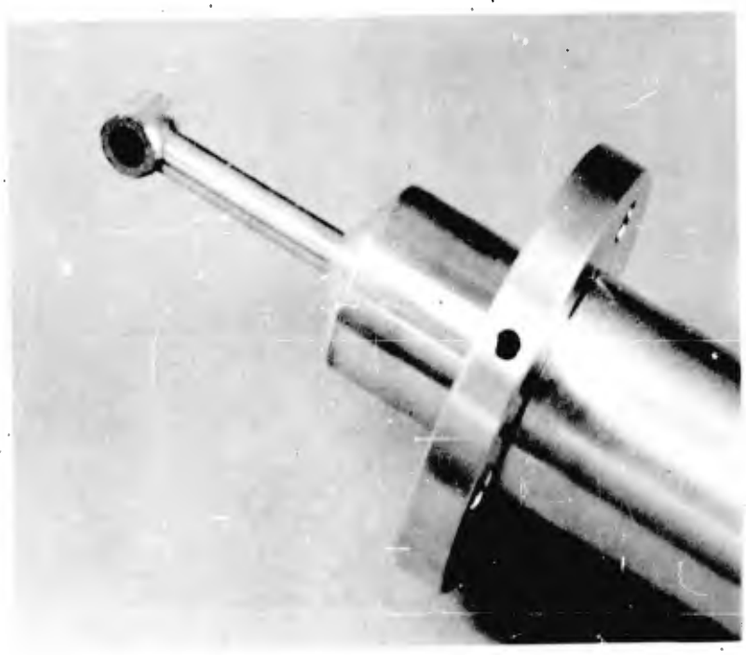

FIGURE 1. Thermocouple Radiometer

This instrument contains a pair of matched thermocouples connected in opposition. One of the couples is shielded from incident radiation, and the other is connected to a totally absorptive receiver area. The couples are enclosed in a vacuum chamber to which radiation is admitted by a barium fluoride window of diameter 5 mm. The receiver area is approximately 0.3 x 3.0 mm. The instrument produces 15 microvolts of output per microwatt incident on the receiver area. The internal resistance of the radiometer is approximately 10 ohms. The $BaFl_2$ window is essentially transparent from the visible to beyond 10 μ.

This radiometer was employed for measurement in conjunction with a spherical front-surface mirror of 7.5-cm focal length. The mirror was mounted above the specimen at a distance of approximately 20 cm. The radiometer was positioned at about 10 cm below the mirror at the plane where the cortical image was brought to focus. Mechanical arrangements were made to position a miniature incandescent lamp at the point occupied by the radiometer sensitive area so that the area of the cortex which was imaged at the detector could be accurately located. Figure 2 is a photograph showing this measurement system in use for measurement of cortical radiation.

FIGURE 2. Measurement Set-Up Using Thermocouple Radiometer

The thermocouple radiometer was used in conjunction with two different amplifiers: a Tektronix Type 122 a-c coupled preamplifier with gain of 1000 and bandpass of .8 - 80 cps; and a Kintel Model 121-A/A d-c coupled preamplifier of gain 1000 and bandpass 0-100 KC. The design of both amplifiers incorporates circuit features for maintenance of precise amplification ratios and minimal d-c drift.

Thermistor Radiometer

This instrument, designated as the R4D1 Radiometer, was manufactured by the Barnes Instrument Co., Stamford, Conn. It is designed primarily for the sensitive measurement of surface temperature, or temperature variations, of small or large magnitude. It incorporates a 4-inch-diameter parabolic mirror which images the area to be measured upon a thermistor receiver. The focal distance is variable between 24 inches and infinity. The point of aim of the telescope system may be indicated by a built-in light spot projector. The instrument system includes the sensing head which is adaptable to tripod mounting, and a separate chopper amplifier unit. A filter incorporated in the sensing head effectively cuts off radiation in the visible portion of the spectrum so that the instrument is responsive only in the infrared. This makes possible its use in a fully illuminated environment.

As employed in these experiments, the output of this device was connected to the input of a Dumont-Type 502 oscilloscope, usually operated at the maximum deflection sensitivity of 200 μv/cm. With the low-pass filter of the Barnes instrument switched on, the peak-to-peak noise level exclusive of transients due to line variations was about 15 μv.

Calibration of Radiometers

A fixture was devised for calibration of the radiometers in terms of output microvolts/degree C change in emittor temperature. This consisted essentially of a strip of 1-mil blackened brass to the surface of which was spot-welded a thermojunction of 36 ga. chromel and alumel wires. Provision was made for applying to the strip a brief pulse of electric current to raise its temperature by a small increment. The strip was positioned so that the thermojunction was at the image point of the radiometer thermocouple. The thermojunction output was displayed on one trace of a two-beam oscilloscope, and the amplified radiometer output was displayed on the second trace. The scope was triggered by the heating current pulse and the simultaneous deflections of the two traces were photographed. The temperature change of the emittor was calculated from the deflection of the first trace, and radiometer output was measured from the second trace.

A series of measurements upon the Reeder radiometer in combination with the 7.5 cm mirror resulted in the value of 118 μv at the amplifier input/degree C as the constant for this measurement system. The peak-to-peak noise of the system, referred to the amplifier input, was measured as 7 μv.

The Stefan-Boltzman relationship between surface temperature and radiancy was used to compute the change in radiance corresponding to an observed signal from the radiometer. The radiance at the surface of the brass strip was calculated to be 405 $\mu w/cm^2$/deg C at 300 K, assuming a (handbook) value of .62 for the emissivity of the brass strip. The sensitivity of the Reeder radiometer, in energy units, was computed as approximately .29 μv per microwatt per cm^2 of radiance change.

The fixture was also used to calibrate the thermistor radiometer. The constant relating surface temperature to oscilloscope deflection was measured as 25 μv/deg C = .12 cm/deg C at maximum scope gain, for the blackened brass surface of emissivity .62. In radiant energy units, this is equivalent to about 1 $\mu v/16 \mu w/cm^2$ (at 300°K). The temperature sensitivity of this measuring system is proportional to surface emissivity.

These sensitivity values derived for the radiometers are estimates based upon assumed values for emissivity and upon geometrical approximations. However, it was desired only to arrive at reliable order-of-magnitude estimates of intensity changes in cortical radiation levels; greater precision than that employed herein was believed to be unwarranted in the course of this preliminary investigation.

Photomultiplier Detector

The Dumont Type 6911 ten-stage photomultiplier tube was employed as a radiation detector. The spectral response curve of the photocathode is reproduced in Figure 3, page 5.

This tube was used in a conventional cathode-grounded configuration with the Dumont 502 oscilloscope input as the signal anode load. With 90-volts per stage and a 2.5-v shield potential, the dark current was measured as approximately 2×10^{-8} amperes. A .02 μfd capacitor was connected across the oscilloscope input to eliminate high-frequency noise from the oscilloscope image. The effective time-constant of the circuit, as measured by applying a light pulse to the tube, was approximately 20 milliseconds. Thus, the detection system was able to follow variations in radiant intensity of frequencies up to about 50/sec. The random noise on the trace was about 50 μv peak-to-peak. The high voltage power supply was a 900-v battery.

The Type 6911 photomultiplier was employed in two different experimental arrangements. In one arrangement, a concave front surface mirror of 7.5 centimeter focal length was mounted above the cat preparation and a plane mirror of 1.5 centimeter diameter was mounted at an angle of 45 degrees to the light path to provide a 90 degree deflection of radiation to the photomultiplier tube, as in a Newtonian telescope. Figure 4 shows this experimental arrangement.

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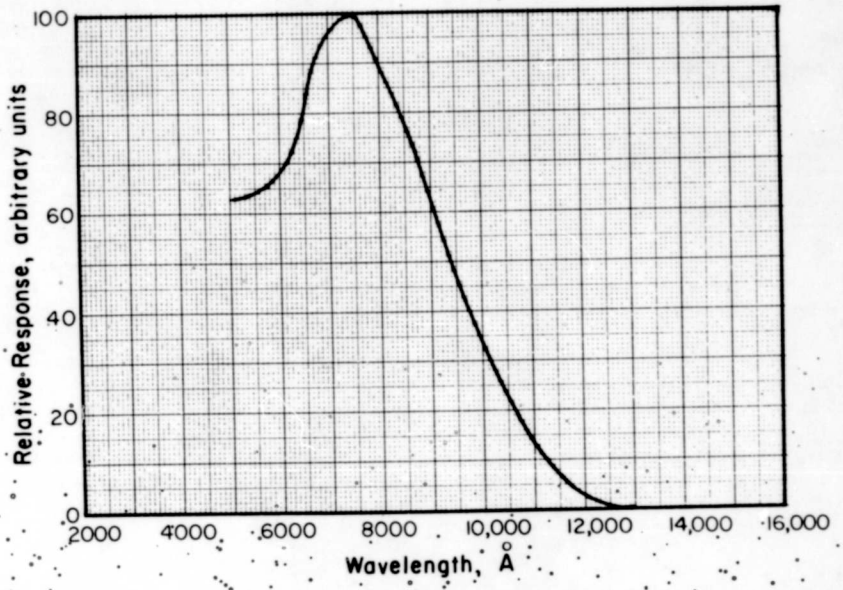

FIGURE 3. Typical Spectral Response of Type 6911 Multiplier Phototube

FIGURE 4. Experimental Set-Up Using Photomultiplier Detector

In an effort to obtain increased optical efficiency, an in-line arrangement was tested consisting of a converging lens between the specimen and the photomultiplier. The lens employed had a focal length of 10 centimeters and a diameter of 7-1/2 centimeters. Because the infrared transmissivity of the glass employed in this lens was unknown, an approximate determination of its transmissivity at 1.3 microns was made. By use of a monochrometer illumination source and a calibrated bolometer detector, the transmissivity of the lens was estimated as 90 percent at 500 millimicrons, 87 percent at 1 micron, and 60 percent at 1.2 microns. Because the relative sensitivity of the Type-S1 multiplier surface is essentially zero at 1.2 microns, only a relatively small proportion of the energy within the spectral range of the multiplier surface was absorbed by the lens.

Photomultiplier Sensitivity

A method for calculating the photomultiplier sensitivity, based upon the tube manufacturer's published specifications, was employed for the purpose of estimating the value of radiant energies involved in cortical emission.

By interpolation from published specifications, the sensitivity of the tube to radiation of 7500 Å at 90 volts per stage was estimated as 500 microamperes of anode current per microwatt incident upon the photocathode. Across the 2-megohm load, the tube would develop a signal of 1 volt per 10^{-9} incident watts. Assuming an optical efficiency of 5 percent in the coupling of emitted energy from the cortex to the tube face, a 100-microvolt oscilloscope deflection would represent a change in cortical radiance of 2×10^{-12} watts over the exposed cortical area. This estimate is believed to be probably accurate within one order of magnitude.

Signal Recording

During the earlier experiments reported below, recording of observed signals was carried out by photography of scope traces with standard oscilloscope-mounted cameras, either on 35-mm roll film or with Polaroid film. Later in the experimental work a strip-film camera became available, Model C-4, manufactured by the Grass Instrument Co., Quincy, Massachusetts. This camera incorporates a continuous film transport of variable speed which may be used to furnish an accurate time base upon which vertical oscilloscope deflections may be displayed. In this recording method the oscilloscope sweep circuit is not used. The Grass camera was used with 35-mm film having a special base and emulsion as specified by the camera manufacturer.

Both traces of the Tektronix Type-502 oscilloscope were used for recording. Ordinarily the radiation signal was applied to one beam and the other trace was used as reference line and event marker.

Animal Procedures

Animal Selection and Care

The hamsters and rabbits used for preliminary experiments were mature animals chosen for relatively large size and good general condition.

Cats were procured from an animal supplier and held under observation for a sufficient length of time to insure freedom from disease. On the basis of weight and apparent age, they were classified as immature (young, growing) specimens, generally in the weight range of 2.5 - 3.5 lb, or mature specimens of 3.5 - 5 lb. Some cats showed signs of respiratory embarrassment and even failure due to obstruction of the airway in early stages of anesthesia. This was possibly due to unrecognized respiratory tract infection.

Anesthesia

Several anesthetic agents were used during surgical preparation and observation.

For most of the preliminary work, a urethane-chlorolose mixture was selected which produced excellent anesthesia for at least 4 hours without objectionable physiological effect. This was formulated as follows:

5 g Urethane (Ethyl Carbamate) - Eastman
.5 g Alpha Chlorolose - Matheson, Coleman & Bell
10 cc 9N Saline + 5% Dextrose
Saline is mixed with Urethane warmed to 37 C, added to chlorolose and mixed at 37 C. Dosage: 1100 mg/kg.

At a later stage in the experimental work when variable responses were observed, the anesthetic agent was suspected as a possible cause of nonreproducibility. Other agents were therefore tested, including sodium pentobarbital and sodium pentothal. The usual doses of pentobarbital were in the range of 20 to 30 mg/kg, and of pentothal, 20 to 30 mg/kg. In the tabulation of results, the anesthetic agent used in each experiment is stated together with a judgment of the degree of anesthesia at the time of the experiment.

Surgical Preparation

The cat was anesthetized, a medial longitudinal scalp incision was made, and a rectangular block of cranial bone was cut out with a dental burr to expose a brain area 20-25 mm in width and 25-30 mm in length. The placement of the opening was such as to expose most of both lateral gyri and portions of the suprasylvian gyri, the anterior limit generally including portions of the cruciate sulci. In terms of the Jasper and Ajmone-Marsan stereotactic system (which takes the interaural line as frontal reference zero), the exposed area may be identified as, frontally, +25 to -5 mm, and laterally, as left 12 to right 12. This area includes some of both primary and secondary visual projection areas and portions of the somatic projection area. Further anterior exposures were sometimes attempted but resultant intrusion into the frontal sinuses led to excessive blood seepage and difficulty in maintaining an optically clear field.

The dura was carefully cut off the hemispheres. The exposed brain surface was covered with saline-soaked gauze which was taped in place without brain compression until beginning the experiment.

Some experiments required the abrupt termination of the animal. For this purpose, a polyethylene cannula (PE 20) was tied into one carotid artery directed brainward. The artery was clamped off below the cannula. A 10 ml syringe filled with a cytotoxic agent, usually 10 per cent potassium cyanide, was connected to the cannula with a suitable clamp to prevent premature injection of the poison into the artery. In one experiment as noted below, 10 per cent formalin was used instead of cyanide solution.

Experimental Procedures

Animals were anesthetized by slow injection of anesthetic into the cephalic vein until the desired depth was obtained. While surgery was performed, adjustments in depth of anesthesia were made as required. About 1-1/2 hours were required for the operation. The animal was then transferred to a standard stereotactic instrument equipped with clamps to hold the head in a fixed position. The mounted animal was placed in position under the radiation transducer and the necessary adjustments made to the optical system for proper focus of the image of the cortex upon the transducer. The alignment was checked by placing a point light source (miniature mercury arc or "grain-of-wheat" lamp) at the transducer position and noting the image point on the cortex.

In experiments requiring electrical simulation of the cortex, electrodes were placed prior to the beginning of experimental observation. A stainless steel screw, fixed in the skull during preparatory surgery, was used as return electrode. For the earlier experiments reported below, the cortical electrode was a blunt-tipped 20-ga. steel wire. During later experiments, this was replaced by a saline-saturated wick electrode to avoid mechanical and chemical damage to the cortex, and also to achieve stimulus over a larger area. The stimulus applied was a biphasic pulse, initially negative-going, at the cortex. The pulse duration was 1 ms and pulse frequency was 30 cps unless otherwise noted in the Summary of Results below. The usual placement of the cortical electrode was forward on the left lateral gyrus.

Experimental manipulations, such as applying stimulating voltage, were carried out by an assistant while an observer watched the oscilloscope signal and placed event markers as required on the auxiliary trace of the oscilloscope.

The usual experimental protocol consisted of a strip camera recording of at least 30-seconds duration without a stimulus to establish base level activity, followed by a series of 3-second stimuli of 3V amplitude. If no response or change in radiation signal was noted, after 30 seconds a series of 3-second stimuli were applied at 30V amplitude. If the observer had noted any trace deflection suggestive of a change in radiation with applied stimulus, this sequence was repeated.

In experiments in which termination by injection of toxic agents was included, this was carried out after the stimulus experiments. The observer marked the time of starting the injection and its conclusion.

Experiments in which the radiometers were employed as detectors were carried out in a laboratory with reduced illumination from fluorescent lamp sources. Photomultiplier experiments were carried out in a room from which all illumination, including that due to the electronic equipment, was excluded.

RESULTS

Summary of Experimental Results

The results of 31 experiments in which attempts were made to observe and measure the intensity of photon radiation from the exposed cerebral cortex of animals are summarized in tabular form below:

Expt. No.	Expt'l Animal	Anesthetic	Instrumentation	Experimental Procedures	Measured Values	Photo Record	Comments
1	Rabbit, mature female	U-C*	TC** radiometer, AC amp.	Cortical stim., 1 ms pulses, 30 pps, 0-25 v	No response	No	Difficulty in keeping field clear of blood
2	Cat, mature male	U-C	Peripheral and cortical elec. stim.	Peripheral and cortical elec. stimulation	No response	35 mm	
3	Cat, mature male	U-C (light)	Ditto	Ditto	Ditto	35 mm	Severe electrical noise present
4	Ditto	Ditto	"	"	"	No	
5	"	"	Thermistor radiometer***	Cortical stim. at point of focus of radiometer, 0-15 v, 1 ms, 30 pps	No response above noise	35 mm	Excessive noise traced to instrumentation malfunction

*Urethane-Chlorolose formulation.
**Reeder Thermocouple.
***Barnes R4D1.

Summary of Experimental Results (Continued)

Expt. No.	Expt'l Animal	Anesthetic	Instrumentation	Experimental Procedures	Measured Values	Photo Record	Comments
6	Cat, immature female	U-C (light)	TC radiometer + DC amp.	(a) Simultaneous recording of ECG and radiation. (b) Peripheral and cortical elec. stim. (c) Termination by massive pentothal injection	(a) No correspondence by visual obs. (b) No resp. (c) 18 μv change in 40 min after inj. ΔT of cortex was 7.5 deg in same period	35 mm	Observed change due to temp. decrease after death
7	Cat, mature male	U-C (light)	TC radiometer + AC amp.	(a) Simultaneous ECG and radiation measure. (b) Peripheral and cortical elec. stimulation	(a) No resp. (b) No resp.	No	
8	Cat, mature female	Ditto	Ditto	Ditto	Ditto	No	
9	Cat, mature male	"	"	"	"	No	
10	Cat, mature male	Pento-barbital (light)	1P21 photo-multiplier	(a) Peripheral and cortical elec. stimulation	"	35 mm	1P21 was used as temporary substitute for 6911 pm, not yet available
11	Cat, mature male	U-C	Ditto	Ditto	"	No	
12	Cat, mature male	Ditto	6911 photo-multiplier	(a) Simultaneous ECG and radiation obs. (b) Cortical and peripheral elec. stimulation	"	Polaroid	
13	Cat, immature female	"		(a) Simultaneous ECG and rad. obs. (b) Cortical and peripheral stim. (c) Obs. of rad. emission during death from anoxia	(a) ΔV at photoanode 1 mv p-p*** (b) ΔV = -1 mv during cortical stim. (c) ΔV = -500 μv during anoxic death (5 min)	No	Animal became anoxic and died during preparation for photo recording
14	Cat, mature female	"	6911 pm	(a) Cortical stimulation (b) Simultaneous ECG and radiation measurement	No resp.	No	Long-term visual observation - 2 hr
15	Cat, mature female	Pento-barbital 69	6911 pm + strip camera	(a) Cortical stimulation (b) Electrical and mechanical peripheral stimulus	Ditto	Strip film	

***Negative sign indicates signal change in direction of diminished radiation.

Summary of Experimental Results (Continued)

Expt. No.	Expt'l Animal	Anesthetic	Instrumentation	Experimental Procedures	Measured Values	Photo Record	Comments
16	Cat, immature male	Pentothal	6911 pm + strip camera	(a) Cortical stimulation (b) Electrical and mechanical peripheral stimulus	No resp.	Strip film	
17	Cat, immature male	U-C	Ditto	(a) Peripheral and cortical elec. stimulation (b) Rad. meas. during anoxic death	(a) No resp. (b) ΔV = -100 μv at termination		ΔV was too small for certainty of effect. Might have been due to scope drift.
18	Cat, immature female	U-C	"	(a) Peripheral and cortical elec. stimulation (b) Radiation meas. during termination by cyanide injection via carotid	(a) No resp. (b) ΔV = -2 mv within 45 sec after start of injection	Strip film (Fig. 5)	First recording of definite radiation response
19	Cat, mature female	U-C	"	Ditto	No resp.	Strip film	
20	Cat, mature female	U-C	"	(a) Peripheral and cortical elec. stimulation (b) Cyanide inj. via carotid	(a) No resp. (b) ΔV = 2.5 mv within 25 sec. after start of injection	Strip film (Fig. 6)	
21	Cat, mature male	U-C	6911pm and strip camera	(a) Peripheral and cortical stim. (b) Cyanide inj.	(a) No resp. (b) Ditto	Strip film	
22	Cat, mature male	U-C (light)	Ditto	(a) Peripheral and cortical stim. (b) Cyanide inj.	(a) No resp. (b) ΔV = -2.7 mv/40 sec after start of inj.	Strip film (Fig. 7)	
23	Cat, mature female	U-C (light)	"	(a) Cortical stimulus (b) Cyanide injection	No resp.	Strip film	
24	Cat, mature female	U-C	"	(a) Cortical stim (b) Cyanide inj.	(a) No resp. (b) Very small $\Delta v \simeq .5$ mv	Strip film	
25	Cat, immature female	U-C	"	Ditto	No resp.	Strip film	
26	Cat, immature female	Pento-barbital & pentothal	6911 pm + strip camera	(a) Cortical electrical stimulation (b) Cyanide injection	(a) ΔV = +1 mv with stimulus of 3 v, 1 ms, 10 pps (b) No resp.	Strip film (Fig. 8)	(a) Repeated 2x in succession; response failed thereafter. Apparent response might be due to movement by cat.

Summary of Experimental Results (Continued)

Expt. No.	Expt'l Animal	Anesthetic	Instrumentation	Experimental Procedures	Measured Values	Photo Record	Comments
27	Cat, immature male	U-C	6911 pm + strip camera	(a) Cortical stim. (b) Cyanide inj.	(a) No resp. (b) $\Delta V = -1$ mv within 50 sec. after start of injection	Strip film (Fig. 9)	
28	Cat, mature male	U-C	6911 pm with interposed lens	Ditto	No resp.	Strip film	
29	Cat, immature male	U-C	Ditto	"	Ditto	Ditto	
30	Cat, mature female	Pentothal	"	"	"	"	
31	Cat, mature female	"	"	"	"	"	

Discussion of Results

In experiments with radiation measuring devices other than the infra-red sensitive photomultiplier tube, no variation in radiant emission from the cortex was measured except that due to temperature change of the cortex after death of the animal (experiment No. 6).

Of 20 experiments in which the infrared sensitive photomultiplier was employed as detector of cortical radiation, in five there was observed a significant change in radiation emission within a short time following injection of potassium cyanide solution into the brain via the carotid artery. The photomultiplier signal voltage changes in these instances were within the range of .5 - 2.7 millivolts, representing radiancy changes of the order of $1\text{-}4 \times 10^{-11}$ watts taking place over the 4-6 cm^2 exposed cortical area.

It may be estimated that the total population of cortical neurons exposed to view through the skull aperture was of the order of 10^7. Using these values, the poison-induced change in the time rate of individual radiative events per cell may be calculated.

The energy value of a photon at the 8000 A sensitivity peak of the photomultiplier is 25×10^{-12} ergs. Thus a radiancy change of 10^{-11} watts represents a change of 4×10^6 emitted photons per second. If it is assumed that the 10^7 exposed neuronal bodies are the sources of this number of photons, the observed radiancy change corresponds to a decrement of about .4 radiative events per cell per second.

No reason can be advanced at present for the irregularity of occurrence of changes in photon emission activity at the time of death. The conditions under which the experiments were conducted are believed to preclude the possibility that undetected artefact is responsible for the observed signal changes. The possibility is not excluded that varying conditions obtaining during each experiment might be responsible for the irregular occurrence of the emission phenomenon.

As a hypothetical source of these variations, the possibility exists that the observed emission phenomenon is, in fact, a long-term fluorescence due to a normal tissue component and excited by exposure to light prior to measurement of emission. Different experimental conditions existing during specimen preparation, such as varying degrees of illumination of the cortex, or variable duration of time between preparation and use, might account for the variable observations. The experimental records are not sufficiently detailed to permit even tentative conclusions concerning this point. There appears to be no correlation between either sex or age of the experimental animal and occurrence of the phenomenon.

In only one case, experiment number 26, was there evidence of a photomultiplier signal voltage change in response to electrical stimulation of the cortex. This response, repeated twice in trials separated by 30 seconds, consisted of a signal change in the direction of increase in radiation intensity of amplitude about 1 millivolt (Fig. 9). The handling of the animal in this

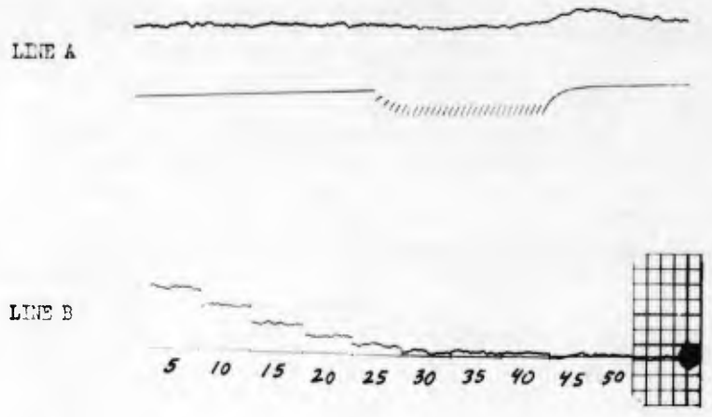

FIGURE 5. Excerpts From Film Strip Record, Experiment No. 18

In both lines A and B, the upper trace represents the photomultiplier signal and the lower trace, the reference including marker signals. In line A, the marker indicates the beginning of cyanide injection via the carotid artery. Note brief positive deflection (radiation increase) immediately following the start of injection.

Line B shows 1 sec. excerpts at 5 sec. intervals after the beginning of injection. Each vertical scale unit equals 500 microvolts. The total deflection shown is approximately -2 mv within 45 seconds after start of injection. The film transport speed was 2.5 horizontal scale units per second.

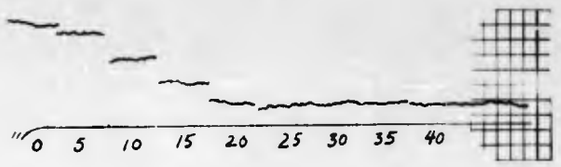

FIGURE 6. Excerpts From Film Strip Record, Experiment No. 20

The upper trace represents the photomultiplier signal; the lower trace is a reference line. Numerals indicate time in seconds after cyanide injection. Each excerpt represents 1 second of record length. Film transport speed was 4 horizontal scale units per second. Each vertical scale unit is equal to 500 microvolts. The total deflection was approximately 2.5 millivolts within 25 seconds after start of injection.

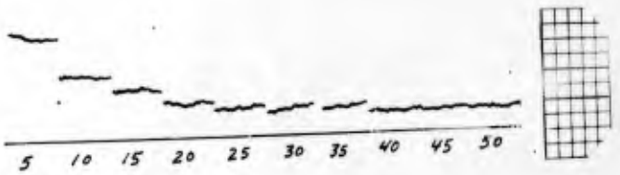

FIGURE 7. Excerpts From Film Strip Record, Experiment No. 22

Each excerpt represents 1 second of record length. Numerals indicate time in seconds after cyanide injection. Film transport speed was 4 horizontal scale units per second. Each vertical scale unit represents 500 microvolts of photomultiplier signal change. The total deflection occurring within 40 seconds after cyanide injection is equal to approximately 2.7 mv in the direction of decrease of radiation intensity.

FIGURE 8. Excerpts From Film Strip Record, Experiment No. 26

In both lines A and B, the upper trace represents the photomultiplier output and the lower trace is the reference line including event markers. Line A shows the signal change resulting from the application of pulse stimulation to the cortex. The marker indicates the duration of the stimulus. Line B shows a repetition of this experiment after a 30-second interval. The film transport speed was 4 horizontal scale units per second. Vertical deflection sensitivity was 500 microvolts per vertical scale unit.

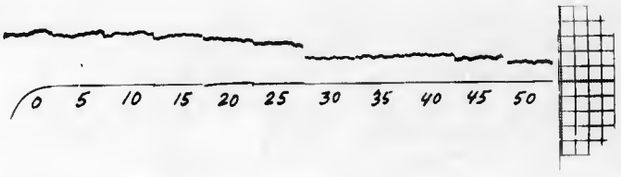

FIGURE 9. Excerpts From Film Strip Record, Experiment No. 27

Each segment represents a 1-sec. length of record; segments are taken at 5 second intervals. Numerals indicate time after beginning of cyanide injection. Film transport speed was 4 horizontal scale units per second. Each vertical scale unit equals 500 microvolts of photomultiplier signal. Total deflection within 50 seconds after start of injection was approximately 1 mv.

experiment differed in details from other experiments. The cat was anesthetized to stage 1 level with sodium pentobarbital. A relatively short-acting agent, sodium pentothal, was used thereafter in sufficient doses to permit surgery and placement of the animal. At the time of the measurement slight reflex reactions to painful peripheral stimuli were present.

The observed radiation response may have been due to the relatively light anesthesia in the case of this animal; alternatively, it may have been the result of slight movement on the part of the animal at the time of application of the stimulus. No repetition of this observation was achieved in similar experiments.

Another singular observation was recorded in experiment No. 13 in which ECG and radiation signal were observed simultaneously by use of the two oscilloscope channels, the Type 6911 photomultiplier being used as radiation detector. For a period of about 2 minutes during which the signals were monitored visually, ECG and radiation waveforms were strongly similar. The ECG consisted of slow negative-going deflections of low frequency and small amplitude (1-5 mv). The radiation signal exhibited changes in the direction of increased radiation intensity coincident with the onset of the negative-going cortical potential.

The cat used in this experiment had been anesthetized with the urethane-chlorolose mixture to the level at which reflex response to a toe-pinch was absent. It was probably anoxic during the time of the observation, for it was found to be in respiratory arrest during the observation and died shortly thereafter while preparations were being made to photograph the related waveforms. A small change (-500 microvolts) in the photomultiplier signal was observed during the period of respiratory arrest and before cardiac function totally disappeared.

CONCLUSIONS

Under certain conditions which have not been defined by experimental studies to date, photon radiation of small intensity is emitted from the cerebral cortex of the cat. This radiation declines rapidly in intensity following poisoning of the cortical tissues by a cytotoxic agent. The foregoing observations are unlikely to be due to changes in thermal radiation alone because they are based upon use of a photomultiplier detector whose emissive surface is responsive only within the visible and near infrared spectral regions. However, for complete assurance on this point, further studies upon the temperature sensitivity of the photomultiplier would be required.

Of 20 cats whose exposed cerebral cortex was observed by means of the photomultiplier radiation detector, only one gave evidence of a cortical radiation emission whose waveform was distinctly similar to the simultaneously observed electrocorticogram. In six of the 20 animals, a decrease in emission was observed following injection of a sufficient quantity of cyanide solution to cause rapid death of the cortical tissues. For the case of the single experiment in which there was an apparent increase in radiation signal level during cortical stimulation, the most probable explanation is that the signal change was caused by movement of the animal.

The magnitude of the decrease in cortical radiancy at death was of the order of 10^{-11} watts/cm^2, which would imply a decrement of less than one-radiative event per second per cell of the exposed cortex.

RECOMMENDATIONS

The methods of measurement thus far applied to this problem made use of readily available radiation detection and measurement devices and optical components. We recommend that in any future study of this problem, optical means specifically designed for optimal efficiency of radiation collection be employed. We further recommend that regions other than the frontal cortex be examined by radical brain exposure.

The observation that at least a portion of the radiation change takes place in a spectral region within the wavelength sensitivity range of the Type S-1 photoemissive surface makes feasible the use of a conventional type of image converter for study of vital function of the cortex. However, the minute energy available for observation indicates the need for energy-integrative methods of study.

An approach that would seem to offer advantages is that of using an off-axis parabolic mirror to image the cortical surface upon a photographic plate of appropriate spectral sensitivity. Alternatively, the image might be projected upon a "light-amplifier" tube of appropriate spectral response (S-1) whose output might be photographically recorded. Although these techniques would not be applicable to the study of short-term variations in intensity of emitted radiation, they would permit the study of long-term variations in response to various forms of stimulation.

An experimental program of broader scope might be designed, in which the study of cortical luminescence would constitute one of two phases. The other phase would consist of observation and measurement of localized fluorescence, based upon a refinement of the technique which Chance and Jobsis (ref. 1) applied to muscle studies. These investigators illuminated muscle with light of wavelength .366 microns and observed the changes in fluorescence at .450 microns by means of a photomultiplier tube.

The fluorescence changes were related to specific metabolic events within the tissue. The addition of an optical system for image formation and the provision of a light-amplifier type of image converter tube would make possible the viewing of localized patterns of activity, assuming the photon flux changes would be large enough for image formation.

The first step in this experimental program might be the study of fluorescence changes of cerebral cortex in various functional states by the use of non-image forming devices. The experimental arrangement employed in the present study, with the addition of a monochromator light source and appropriate filters, could be adaptable to this use. Further studies of cortical luminescence could be carried out employing essentially the same experimental arrangement. Because of the greater possibility of yield of useful information, it is recommended that future study of the phenomenon of photon emission from the cortex include study of both luminescence and fluorescence.

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4. Rabbit
5. Bionics
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