

**STUDY OF FLUORESCENT EMISSION FROM
THE CAT CEREBRAL CORTEX**

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FOREWORD

This study was initiated by the Biophysics Laboratory of the 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(615)-1206, 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. Arthur C. Peters, D.V.M., as co-investigator, was responsible for animal procedures. Mr. Wayne Allton assisted in all phases of the experimental studies. Captain Lothar O. Hoeft, Biodynamics and Bionics Division, was the technical monitor for the Air Force. The research sponsored by this contract was started in January 1964 and was completed in August 1964.

Acknowledgment is made to Dr. Ernest Retzlaff and Mrs. Joan Fontaine of the Columbus Psychiatric Institute for helpful discussion and technical assistance during the course of the research.

This technical report was reviewed and is approved.

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ABSTRACT

Experiments were conducted to investigate the existence of changes in fluorescence properties of the cat cerebral cortex during cortical and peripheral electrical stimulation. In anesthetized cats, the exposed cortex was illuminated with the light output from a monochromator and fluorescence changes were detected by use of a photomultiplier tube. Appropriate filters were used to prevent response of the photomultiplier to light from the excitation source. When electrical pulse stimulus of electroconvulsive intensity was applied by means of a wick electrode to the brain surface, there were observed signals of several millivolts amplitude from the photomultiplier tube interpreted as fluorescence changes. An interpretation is tentatively proposed which is based upon changes in relative proportions of oxidized and reduced pyridine nucleotide in the cortical tissue.

TABLE OF CONTENTS

	<u>Page</u>
SECTION I: INTRODUCTION.	1
SECTION II: METHODS	1
GENERAL	1
ANIMAL PROCEDURES	1
Animal Selection	1
Anesthesia	1
Surgical Preparation	1
INSTRUMENTATION	2
Photomultiplier	2
Excitation Source.	3
Stimulation and Recording.	3
EXPERIMENTAL PROCEDURES	4
SECTION III: RESULTS	5
GENERAL OBSERVATIONS	5
DISCUSSION OF RESULTS	11
REFERENCES.	11

LIST OF ILLUSTRATIONS

FIGURE 1. TWO STAGES IN THE OPERATION EXPOSING THE CAT CEREBRAL CORTEX FOR FLUORESCENCE STUDIES	2
FIGURE 2. ARRANGEMENT OF EXPERIMENTAL APPARATUS FOR FLUORESCENCE STUDIES	3
FIGURE 3. SET-UP OF STIMULATION AND RECORDING APPARATUS	4
FIGURE 4. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 3	6
FIGURE 5. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 7	7
FIGURE 6. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 10	8
FIGURE 7. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 12	9

LIST OF TABLES

TABLE 1. SUMMARY OF RESULTS	10
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SECTION I

INTRODUCTION

The purpose of this research was to determine whether there occur in the mammalian cerebral cortex changes in fluorescence that reflect the state of activity of the tissue. The fluorescence of tissues in various states of excitation has previously been investigated and employed as a tool in studies of functional biochemistry. Fluorescence changes in muscle during stimulation have been reported by Chance and Jöbsis (ref 1). Fluorescence changes in invertebrate giant axons during stimulation have been reported in a recent paper by Shtrankfeld and Frank (ref 2). They employed a photographic densitometry technique for recording the emission of radiant energy. The excitation energy was in the wavelength range 240-400 millimicrons. Increased fluorescence was observed under conditions of stimulation. The authors attributed this to a change in the condition of axoplasmic protein, leading to increased fluorescence. The presence of various organic dyes in the tissues contributed to the observed changes. An earlier study at this laboratory (ref 3), concerned primarily with luminescence of the cerebral cortex during local and peripheral stimulation, suggested the possibility that at least part of the observed emission might be the result of fluorescence.

SECTION II

METHODS

General

The experimental animal was the cat. The cerebral cortex was surgically exposed and illuminated by means of a monochromator. A photomultiplier tube, having a photosensitive surface that was masked by a filter, which excluded light of the wavelength incident upon the cortex, but which passed light of longer wavelengths, was positioned so as to respond to light emitted from the cortex.

Provisions were made for applying electrical stimuli either to the cortex or to other selected regions of the animal. The photomultiplier signals and stimulus signals were displayed upon a two-beam oscilloscope and recorded photographically.

Animal Procedures

Animal Selection

Cats used in these experiments were young, mature animals selected for good general condition. They were maintained in quarantine for at least 3 weeks after purchase to insure freedom from disease. They were within the weight range from 4 to 5.5 pounds. At the time of receipt cats were administered distemper serum.

Anesthesia

Sodium pentobarbital anesthesia was used throughout these experiments. Slow injection of anesthetic into the cephalic vein was performed until the desired depth of anesthesia was attained. The vein was then cannulated with polyethylene tubing so that further injections of pentobarbital could be made as required during the experimental procedure. Initial dosage of anesthetic was 30 mg per kg. Over the course of the experiment of 5-8 hours duration, an additional 30 mg per kg was usually administered.

Surgical Preparation

A medial longitudinal scalp incision was made and by use of a Stryker saw, two rectangular blocks of cranial bone were removed in symmetrical fashion to expose both cerebral hemispheres. The fenestrations were placed so as to expose most of both lateral gyri and the suprasylvian gyri. 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, +20 to 0 mm, and laterally, as left 14 to right 14. In later experiments, a bone bridge covering the central sulcus was not removed because of difficulty in preventing hemorrhage in this region. The dura was removed from both hemispheres and rectangles of saline-soaked Gelfoam were used to protect the brain surface until beginning the experiment.

A typical surgical exposure is shown in figure 1.

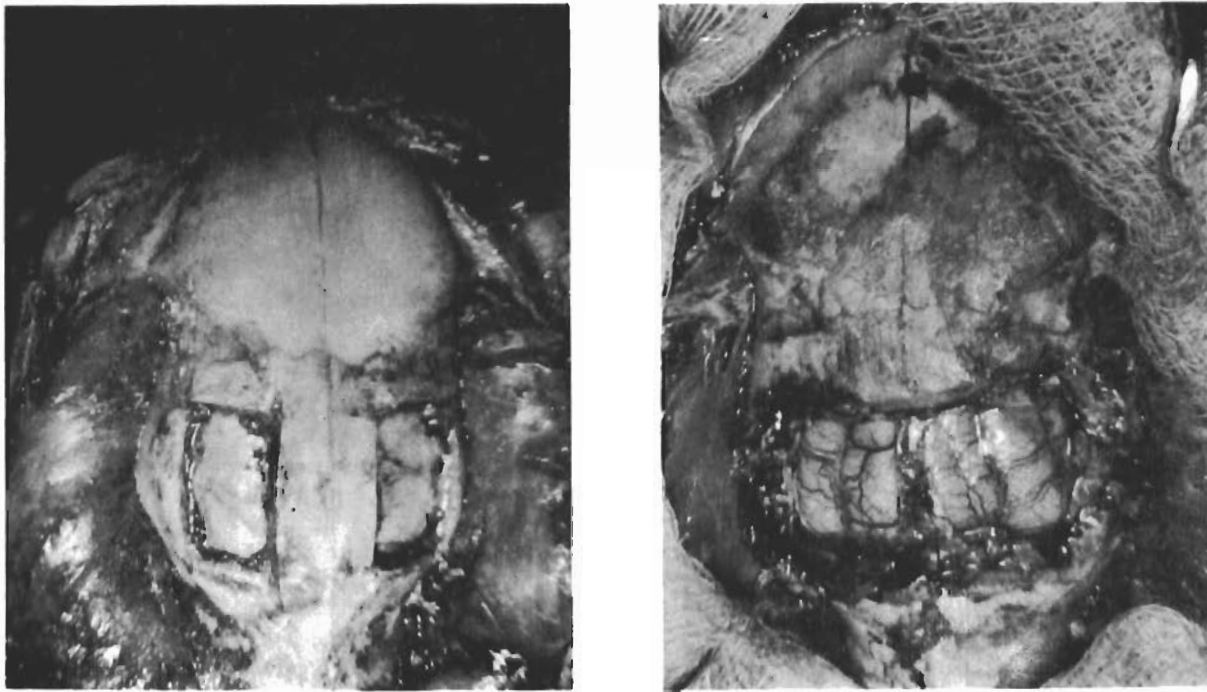


FIGURE 1. TWO STAGES IN THE OPERATION EXPOSING THE CAT CEREBRAL CORTEX FOR FLUORESCENCE STUDIES

The photograph at left shows the double fenestration completed. At right, the bone bridge and dura have been removed to expose the brain.

Instrumentation

Photomultiplier

A Dumont 6292 end-window 10-stage photomultiplier tube fluorescence sensing transducer was used. The tube was enclosed in a light tight m μ -metal shield provided with a holder for positioning light filters in front of the tube window. A Corning type 3-73 filter was employed to exclude from the phototube radiation of wavelength less than 400 m μ . The filter holder fixture was provided with a slide which could be used to cover the tube window to prevent the entrance of light. The entire phototube fixture had provision for universal mounting from a laboratory ring stand.

Excitation Source

The source of fluorescence-exciting illumination was a Bausch and Lomb high-intensity monochromator whose light source was a tungsten-iodide lamp. This instrument was equipped with a quartz-fluoride projector lens to permit focussing an image of the source at distances up to 10 inches. The grating employed permitted the selection of any illumination wavelength in the range of 350-800 millimicrons. To suppress higher order spectra present in the output of this instrument, a Kodak type 7-60 filter was positioned between the lamp and the monochromator entrance slit. The entrance and exit slits were adjusted to result in a nominal half intensity band width of ± 30 millimicrons from the center wavelength.

The intensity of the monochromator beam at a wavelength setting of 365 millimicrons was measured by use of a calibrated total radiation thermopile as 81 ergs per square centimeter per second at a distance of 20 cm. from the exit lens, the standard working distance employed throughout these experiments.

Figure 2 is a photograph showing the arrangement of apparatus in position for measurement upon an exposed cat cortex.



FIGURE 2. ARRANGEMENT OF EXPERIMENTAL APPARATUS FOR FLUORESCENCE OBSERVATIONS

At right is the projector lens of the monochromator light source. Positioned above the head of the cat is the photomultiplier radiation detector. The wick electrode is shown in position for cortical stimulation.

Stimulation and Recording

Electrical stimulation was furnished by a Grass Type S4G Stimulator. The use of a Grass Type SIU-4B Isolation Unit was found to decrease stimulus artifact in the photomultiplier signal. A Tektronix Type 502 Dual-Beam Oscilloscope was used for signal and stimulus display. A Grass C-4 Oscilloscope Camera recorded the traces. A manual switch was used to apply stimulus to the cat. A saline-wetted cotton wick electrode resting upon the brain surface was used to apply stimulus current to the animal. The polarity of the applied pulses was negative-going at the cortex. The return electrode was a member of the stereotactic animal holder in contact with the roof of the mouth.

Contrails

A stimulus frequency of 20 pps, with pulse duration of 10 ms, was used throughout these experiments except where otherwise noted. These values were selected for reasons detailed under "General Observations" below.

Figure 3 is a photograph of the arrangement of stimulation and recording instruments.

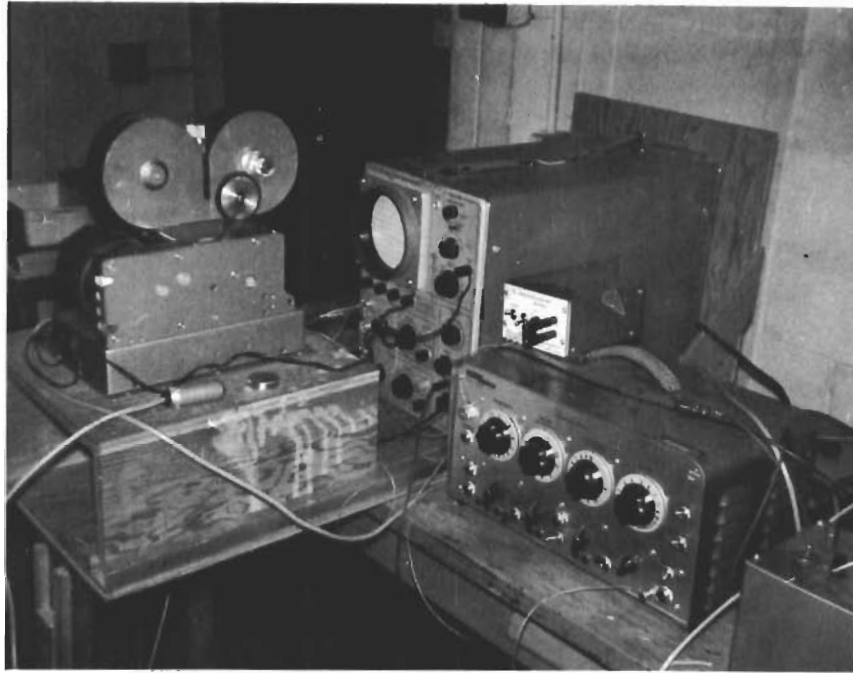


FIGURE 3. SET-UP OF STIMULATION AND RECORDING APPARATUS

Experimental Procedures

The surgically prepared animal, mounted in a stereotactic holder which maintained the head in a rigidly fixed position, was transferred to a steel-walled room from which all external light could be excluded. The beam of the monochromator was aimed at the exposed cortex by temporarily setting the wavelength within the visible range and adjusting the position of the monochromator so that the light spot approximately covered one of the skull fenestrations, uniformly illuminating the brain surface. Positioning was such that the angle of incidence of the light beam upon the cortex was about 30 degrees from the horizontal. The photomultiplier fixture was then adjusted in position so that the entrance window of the tube was as close as possible to the exposed cortex without interrupting the monochromator beam. This distance was approximately 4 cm. The stimulus electrode was positioned on the cortical surface at a relatively low angle to minimize occlusion of the incoming light beam and the fluorescence radiation.

By this stage of the experiment, the initial anesthetic dose was usually losing effect and a further injection of anesthetic was slowly administered to arrive at stage 3 anesthesia. The wavelength setting of the monochromator was adjusted to the excitation frequency of 365 millimicrons. All lights were extinguished in the experimental chamber and an observer remained in the dark room to report on cat responses while the experimenter outside the room applied stimulus pulses and observed fluorescence signals for a primary test of the set-up.

RESULTS

General Observations

In most experiments in which electrical stimulation was applied to the cerebral cortex, photomultiplier signals were observed that were interpreted as due to fluorescence changes. These were voltage changes of magnitude generally less than 10 mv in the direction of decreased light intensity.

To elicit a response, the stimulus voltage had to exceed a threshold value which varied from one animal to another. In all cases, the threshold value for the 20 pps, 10 ms pulse stimulus was 20 volts or greater, and the usual threshold was about 25 volts. At stimulus voltages less than the threshold value, a transient oscilloscope deflection was sometimes observed which is referred to here as an incomplete response. When the stimulus voltage exceeded the threshold, a sustained oscilloscope deflection was observed which was maintained until after the stimulus signal was removed. This is referred to here as a full response. The latency between application of a supra-threshold stimulus and appearance of the full response signal was usually of the order of 0.1-0.2 second, although occasionally a latency as great as 0.5 second was observed. Decline of the response signal coincided with the removal of the stimulus, as nearly as can be measured on the oscillographs.

The stimulus strength required to elicit a full response was usually only a few volts less than the threshold for electro-convulsive reaction on the part of the cat, and in the range in which strong, repeated movements of the contralateral limbs were observed. In earlier experiments, movement artifact was suspected as the cause of the observed photomultiplier signals. This possibility was explored by having the observer in the darkroom manipulate the cat in efforts to reproduce photomultiplier signals. Even vigorous manipulation of the animal failed to give rise to signals resembling those observed. Movement artifact could be at most a minor contribution to the observed signals.

The observed fluorescence signal appeared not to depend strongly upon any parameter of the electrical stimulus other than the voltage. Reduction of the pulse duration to less than 3 ms caused an increase in the threshold voltage for a full response. Increasing stimulus frequency above 20 pps had no effect upon threshold. Reducing the frequency to less than 10 pps resulted in an increase in observed threshold voltage.

In two experiments, Nos. 7 and 11 in the Summary of Results, Table I, the cats died shortly after the beginning of cortical stimulation, apparently from respiratory failure. In all other experiments, observations were maintained over a period of at least two hours. Responses over a duration of several hours were quite reproducible with respect to threshold and details of the response signals. Figure 6 shows series of responses to 1-second stimuli at periods separated by approximately 4 hours.

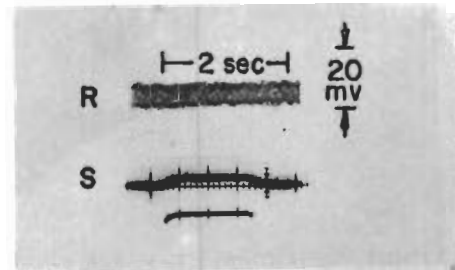
With regard to experiments in which fluorescence changes were not observed, Nos. 8 and 11 in Table I, no specific reason for the failure to observe such signals was apparent. Operational checks upon the measurement equipment in these cases indicated satisfactory function. The apparent depth of anesthesia was no greater in the case of these animals than for the others. Undetected variations in either the state of the animals or in details of the preparations afford the most likely explanation.

Details of the fluorescence responses may be seen in the oscillograms, figures 4-7. In figures 4 and 5, an oscillatory component is apparent in the case of the suprathreshold stimuli. This component, which has a frequency of about 12 cps, was occasionally observed in the responses of nearly all the experiments in which fluorescence changes were seen.

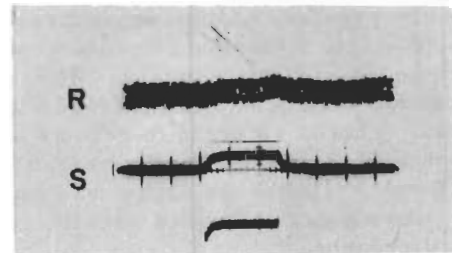
Fluorescence changes were occasionally observed in the absence of stimulus. Unlike the changes due to stimulus, these were in the direction of increased fluorescence, and were usually limited to less than 5 mv in amplitude and durations of less than 0.5 second. Figure 5, a portion of a record from a cat in which one of the cerebral hemispheres was damaged during surgery, exhibits this form of activity.

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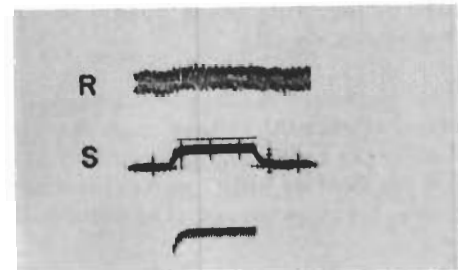
- A. 10-volt stimulus: no somatic response.



- B. 20-volt stimulus: no somatic response, slight fluorescence signal.



- C. 24-volt stimulus: contralateral leg flexure.



- D. 35-volt stimulus: generalized seizure activity, fluorescence signal ~5 mv.

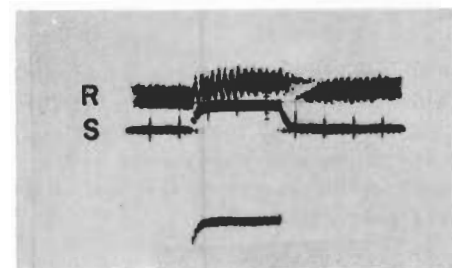
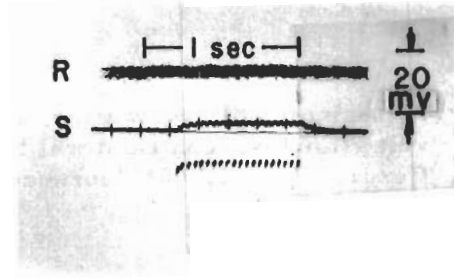


FIGURE 4. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 3

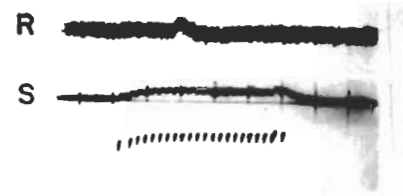
In this and succeeding figures, the trace S represents the stimulus and and trace R is the photomultiplier signal representing fluorescence change. Amplitude of the photomultiplier signal may be estimated by comparison with vertical scale line. An upward inflection of the trace S indicates decreased fluorescence.

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A. 12-volt stimulus: no response.



B. 15-volt stimulus: incomplete fluorescence response.



C. 25-volt stimulus: generalized convulsive activity of cat, 10 mv. fluorescence signal.

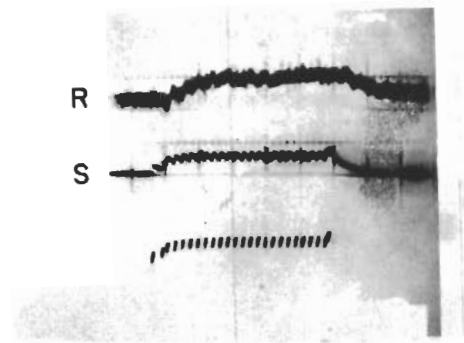
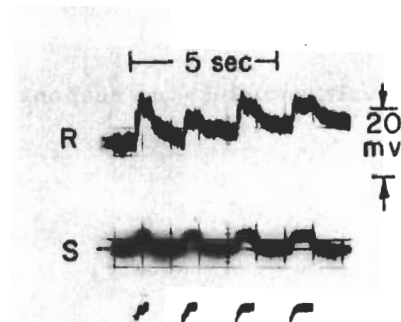


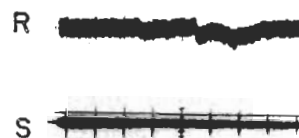
FIGURE 5. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 7

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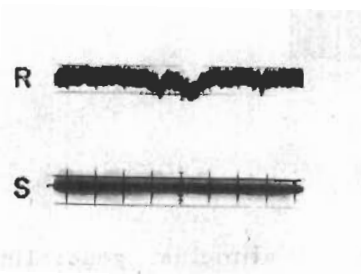
- A. At beginning of observation, 20-volt stimulus: contralateral leg flexure, 15 millivolt fluorescence signal.



- B. At beginning of observation, spontaneous fluorescence activity.



- C. Spontaneous activity at 3 hours after beginning of observation.



- D. Four hours after beginning of observation. Strong fluorescence signal is still present in response to 20-volt stimulus.

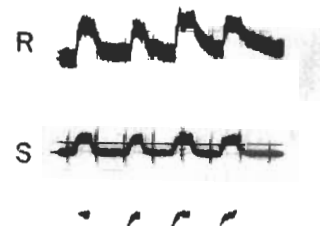
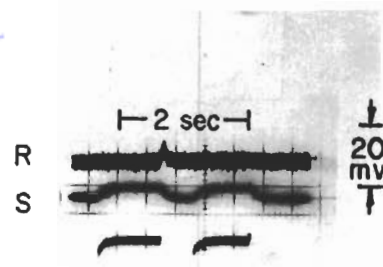


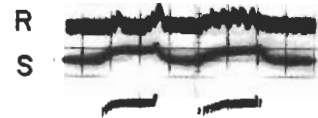
FIGURE 6. EXCERPTS FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 10

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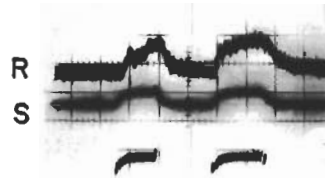
- A. 15-volt stimulus: incomplete fluorescence response, no somatic movement.



- B. 15-volt stimulus: incomplete fluorescence response, contra-lateral leg flexure.



- C. 20-volt stimulus: complete fluorescence response, strong repetitive bilateral leg movement.



- D. 25-volt stimulus: complete fluorescence response of 10 mv. signal strength; generalized convulsive activity.

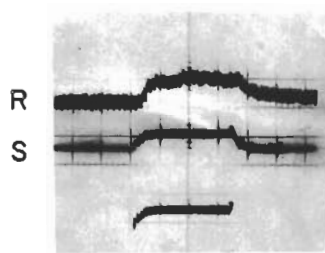


FIGURE 7. EXCERPT FROM OSCILLOGRAPHIC RECORD, EXPERIMENT NO. 12

TABLE I. SUMMARY OF RESULTS

The results of the twelve experiments are summarized in tabular form below.

Exp. No.	Sex	Procedures	Fluorescence Observed	Remarks
1	M	Peripheral mechanical and electrical stimulus	?	Signal obscured by ripple from A. C. exciter source
2	M	a) peripheral stimulus (elec.) b) cortical stimulus (elec.)	a) - b) +	a) stimulus artifact only b) threshold--30V
3	M	Cortical stimulus (elec.)	+	Threshold--20V
4	M	a) cortical stimulus (elec.) b) peripheral stimulus (elec. and mech.)	a) + b) -	a) threshold--25V b) --
5	M	a) cortical stimulus (elec.) b) cortical stim. (strychnine)	a) + b) -	a) threshold for fluor. signal--35V b) --
6	M	Cortical stimulus (elec.)	+	Threshold--20V
7	M	Ditto	+	Threshold--25V. Cat died shortly after starting observation
8	M	"	-	--
9	M	"	+	Observation continued over 6 hrs.
10	M	"	+	Cortex cut during surgery; high level of spontaneous activity
11	F	"	-	Cat died 30 min. after beginning observations
12	F	"	+	Threshold--<20V

In experiment 5, chemical stimulation was attempted by placing upon the cortical surface a small square of absorbent paper soaked in a solution of strychnine sulfate. In several other early experiments, peripheral mechanical stimulation was applied to determine whether the cortical fluorescence level would thereby be altered. Neither of these methods of stimulation resulted in changes in the observed fluorescence levels.

Discussion of Results

Published specifications of the type 6292 photomultiplier indicate a radiation sensitivity at 4500 Å of 10^{-6} ampere of anode current/ 2.5×10^{-11} incident watt of radiant energy. Across the 2-megohm load resistance of the signal circuit, a 10 mv signal would thus represent a change of approximately 1.2×10^{-13} watts incident at the tube face. Estimating the solid angle subtended by the tube face as $1/4$ steradian, the change in energy radiated by the brain surface per mv oscilloscope deflection is approximated as 10^{-12} watts. The exposed area of the cortex was nearly 5 cm^2 ; the radiance change represented by a typical stimulus response thus amounted to 0.2×10^{-12} watts/ cm^2 .

Detection of this minute quantity of energy was facilitated by the fact that it occurred at a frequency which nearly coincides with the sensitivity peak of the type S-11 photomultiplier surface.

The likely source of the fluorescence observed in these experiments was the reduced pyridine nucleotide of the tissue under observation (ref 4). The decrease in fluorescence would be due to oxidation of the pyridine nucleotide, possibly due to increased concentration of ADP as indicated by the studies of Chance and Williams (ref 5). No reference has been found to studies of this sequence of biochemical events during electrical activity of neural tissue. Further fluorescence studies upon isolated neural components such as large axons, fibre bundles, or isolated ganglia are suggested by the results of the present study.

The occurrence of fluorescence during stimulus-induced activity of the cerebral cortex suggests the possible application of this phenomena to the study of activity of the brain surface of an animal in functional condition. However, the observations reported here were carried out under conditions far removed from the normal functional state of the cerebral cortex, upon fully anesthetized animals using stimuli of electroconvulsive intensity. The possible relationship of the observed fluorescence signals to the normal functional activity of the cortex is suggested as an appropriate subject for future study. It would appear technically feasible to extend studies of the kind reported above to the observation of the fully functional cortex by the use of chronically prepared animals with cranial windows. Experimentation of this type would seem to be desirable from the standpoint of developing novel methods for studying cerebral function. Intensification of the fluorescence image of the cortex is judged to be technically feasible within the present state of development of electronic image-intensifying devices. An optical method for observing transient states of activity of the cerebral cortex might prove to be of high value in psychophysiological investigations.

REFERENCES

1. Chance, B., and Jöbsis, F., "Changes in Fluorescence in a Frog Sartorius Muscle Following a Twitch", Nature **184**, pp 195-196 (1959).
2. Shtrankfeld, I., and Frank, G., "O Lyuminestsentsii Gigantskix Nervnix Volokon pri Vozbyshdenii (On the Fluorescence of Giant Nerve Fibers During Stimulation)", Biofizika **9**, pp 321-326 (1964).
3. Roppel, R., "A Study of Near Infrared Emission From the Mammalian Cerebral Cortex", Rpt. Nr. AMRL-TDR-63-66 (AD 417125), Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio, June 1963.

Contrails

4. Chance, B., and Baltscheffsky, H., "Respiratory Enzymes in Oxidative Phosphorylation", J. Biol. Chem. 233, pp 736 (1958).
5. Chance, B., and Williams, G. R., "Respiratory Enzymes in Oxidative Phosphorylation", J. Biol. Chem. 217, pp 409 (1955).

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13. ABSTRACT Experiments were conducted to investigate the existence of changes in fluorescence properties of the cat cerebral cortex during cortical and peripheral electrical stimulation. In anesthetized cats, the exposed cortex was illuminated with the light output from a monochromator and fluorescence changes were detected by use of a photomultiplier tube. Appropriate filters were used to prevent response of the photomultiplier to light from the excitation source. When electrical pulse stimulus of electroconvulsive intensity was applied by means of a wick electrode to the brain surface, there were observed signals of several millivolts amplitude from the photomultiplier tube interpreted as fluorescence changes. An interpretation is tentatively proposed which is based upon changes in relative proportions of oxidized and reduced pyridine nucleotide in the cortical tissue.		

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Cortical fluorescence						
Brain fluorescence						
Tissue fluorescence						
Cerebral cortex						
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Cortical activity						
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