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Electrical Desiccation as a Method for Marking Functional Cortical Areas

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ELECTRICAL DESICCATION - EARP

ELECTRICAL DESICCATION AS A METHOD FOR MARKING FUNCTIONAL CORTICAL AREAS

By

Kenneth R. Earp

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of Master of Science

in Psychology

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This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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The writer wishes to express appreciation to Dr. George M. Peterson for the assistance and advice given while this study was in progress.

I am also indebted to Mr. Donald K. Gucker for his advice and information on the equipment and on the histological procedures used in this investigation.

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Investigators in the fields of psychology and physiology have postulated and searched for changes in the nervous system due to practice. However, there has been no direct evidence concerning the nature of this change. Hilgard (1956, p. 481) states that "one of the most crying needs is for a crucial experiment identifying specifically a change occuring in neural tissues (or in bio-electrical fields related to such tissue) as learning takes place."

Recent progress in the development of highly refined apparatus for electrical stimulation and for the recording of neural activity has produced some evidence and theory concerning possible neural changes in the spinal cord (Eccles 1953). There has been no direct evidence, however, concerning cortical changes which presumably take place when learning occurs. (Hebb 1949).

An approach to the problem has been made possible by Peterson's (1934) work on handedness in rats. Peterson and Gucker state that, "Preferential handedness in the rat offers a means of attacking the issue without too many taxing assumptions and with certain advantages over other simple behavioral phenomena. It is highly localized in the cerebral cortex (Peterson and Fracorol, 1938) and can be influenced by practice (Peterson, 1951). A histological approach to the study of

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structural changes is feasible only when precise localization is possible. Such an investigation requires comparison of tissue before practice with tissue after practice, and this ordinarily calls for two animals" (1959, p. 2). Handedness offers an advantage because comparison of the cortical tissues in the areas controlling the preferred and non-preferred limbs can be made within a single animal.

Attempts to find a common cortical locus for handedness in all rats, however, has failed (Peterson and Fracorel, 1938), and therefore electrical stimulation has been used as a means for locating the area involved (Peterson and Gucker, 1959). Peterson and Gucker suggest that a means should be found for marking the point where electrical stimulation locates the area for handedness, so that histological examination of the tissue in this area can be made.

However, when tissue is marked or treated in any manner, there is risk of producing a functional change. If the cortical elements operative in handedness are destroyed or changed in an attempt to mark them for identification, then the mark has made further histologic investigation useless. One way to determine whether such a change has occurred is to test for handedness transfer after the rat has been marked.

The present study is concerned specifically with this problem, i.e., whether a means can be found to make a mark at

structural changes in specific cells where precise localization is possible. Such an investigation requires examination of tissue before [illegible] with the [illegible] [illegible], and this ordinarily calls for two animals [illegible]. [illegible] offers an advantage because comparison of the [illegible] in the areas controlling the preferred and non-preferred limbs can be made within a single animal.

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the point where handedness has been localized by electrical stimulation without producing changes in that area sufficient to alter its functioning.

It has been suggested that the cells influencing handedness behavior are located in layer V or below in the cortex (Peterson, 1934). As a working assumption this implies that marks should not be made which penetrate the cortex beyond layer IV.

In order to make destructions of this magnitude, it is necessary to utilize an instrument which will give us the advantage of minute destructive effects. Tissue destruction utilizing high-frequency currents appears to give us this advantage. William L. Clark (Kelly and Ward, 1932) was the first man in this country to describe the destructive effects of high-frequency currents on tissue. He noted that the cells became dehydrated and consequently terms this kind of destruction desiccation.

Desiccation of cortical tissue was produced in this this experiment with the use of a Hyfrecator[1]. The Hyfrecator

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[1]. A Hyfrecator is an electrosurgical device which produces radio frequency currents of 2.5 magacycles at a voltage of approximately 1800 volts maximum and at low amperage. It is used primarily for the removal of tumorous growths, and is manufactured by the Birtcher Corporation in Los Angeles, California.

was chosen for this work because it offered the opportunity to combine the stimulating electrode and the desiccating needle into one, thus making it possible to locate and mark the handedness area in a single procedure. Other advantages of this instrument are that the amount of current and the length of time this current is supplied to the desiccating needle can be controlled precisely, and that the tissue is sterilized in the desiccated area. Since capillaries are sealed using this technique, damage due to bleeding is minimized.

Cortical tissue desiccated in this experiment had the appearance of being more lightly stained than surrounding tissue with an absence of neurons in a conically shaped area, with the apex of the cone directed toward the center of the tissue. These differences were used in determining the boundaries of the destruction for the measurement of the desiccated tissue.

II. Method

Animals:

Male albino rats, 3-4 months old, were used in this study. Sixteen rats were selected at random for the preliminary experiment. For the main experiment ten rats which met a criterion for 95% single-handedness were selected.

Apparatus:

The Peterson (1931) food-reaching situation was used to determine handedness of the rats.

A modified stereotaxic instrument was used to hold the rat in position during stimulation and marking. The base of the instrument consisted of two brass plates with smooth faces which allowed them to slide with moderate friction over each other. The top plate held a V block for the rat's jaws and a wire clamp for the teeth. To the lower plate was attached a steel arm which held the needle used for stimulation and/or desiccation. The needle could be raised or lowered vertically and was rigid horizontally. In order to stimulate various points on the cortex, the plate holding the rat was moved.

Electrical stimulation was performed with interrupted direct current at 200 pulses per second and ranging from 5 to 10 volts. This was supplied to the stimulating electrode by a Tektronix Type 160 A power supply, a Type 162 Waveform Generator, and a Type 161 Pulse Generator.

General Procedure:

Operations were performed using ether anaesthesia. A 3/16 inch trephine was used to expose the contralateral frontal cerebral cortex. Care was taken not to injure the pia mater during this process.

The tip of the stimulating electrode was placed on various points of the exposed cortex until the rat responded with the appropriate arm movement which consisted of an adduction of the preferred limb. The arm was then moved back and the movement confirmed by someone other than the operator. Stimulation ceased at this point and a double switch was thrown which activated the equipment used for producing the mark. The mark was then made. The needle used for the stimulating electrode was also used for the desiccation, and the relative position of the needle to the cortex was maintained with the stereotaxic instrument. After the rat had been stimulated and marked, the incision was closed and sutured. Post-operative observations were then made within 24 hours.

After reobservation for handedness, the rat was sacrificed, the brain removed and placed in 10% neutral formal solution and allowed to harden for a minimum of 24 hours. The quarter of the brain containing the destruction was frozen and sectioned at 50 microns. The sections were placed in 20% alcohol and serially affixed to albuminized

General Procedure

slides and allowed to dry in a 40°C. oven for 24 hours. The sections were then stained with thionin. The staining procedure will be found in Appendix B. All sections containing destructions were examined microscopically and the amount and locus of the destruction determined.

The system used for marking the cortex consisted of a Birtcher Hyfrecator, a Hunter Decade Timer which was monitored by a Standard timer, and a Triplit A.C. Voltmeter which monitored the amount of volts coming from the power line through a variac to the primary coil of the Hyfrecator.

A block diagram of the instrumentation for electrical stimulation and desiccation is shown in Figure 1:

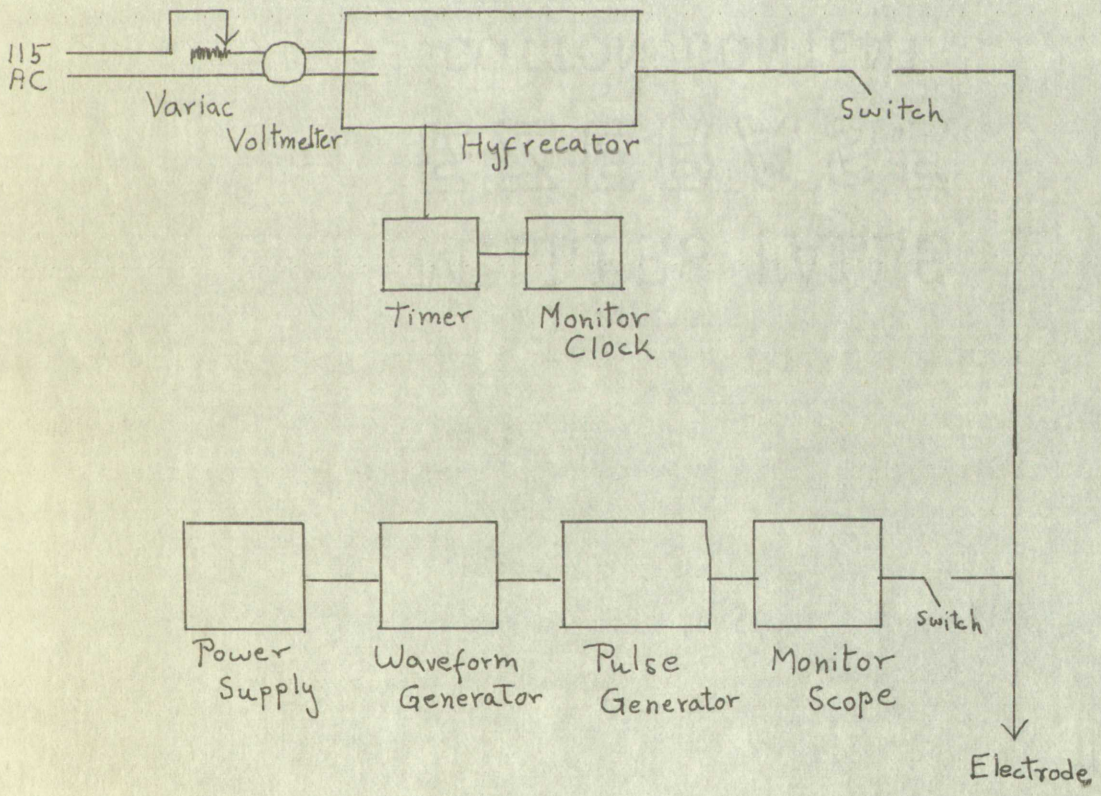
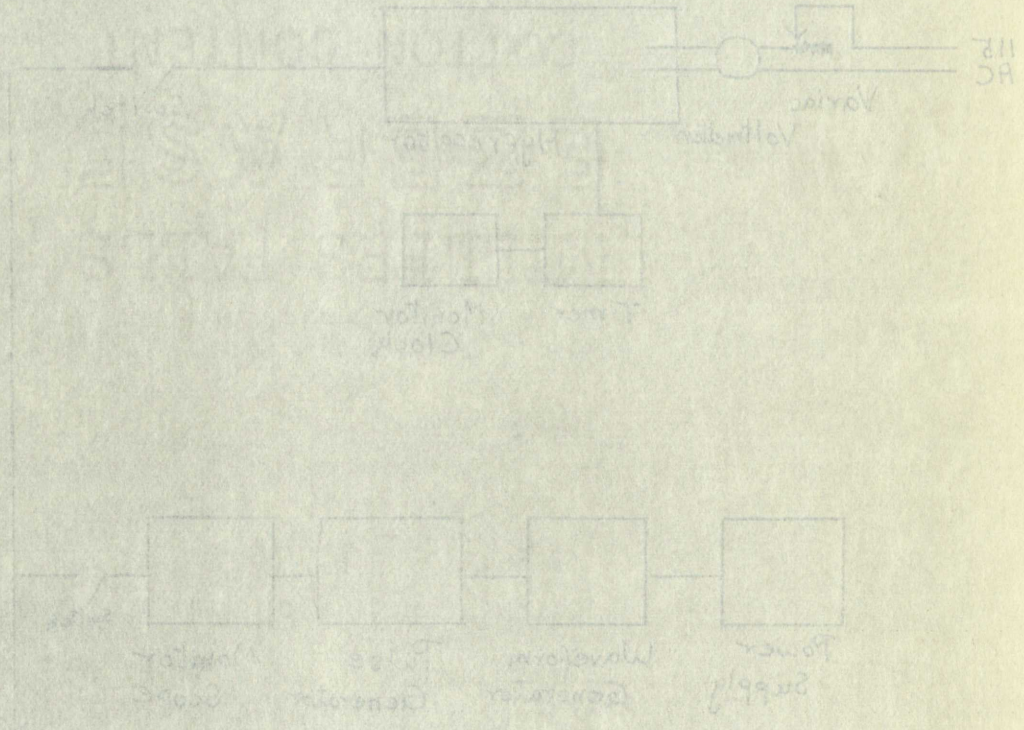


Figure 1

Block Diagram of Electrical Equipment



III. The Preliminary Investigation

Method:

A preliminary experiment was performed in order to determine the utility of the Hyfrecator for marking. Since minute destructions were desired, the Hyfrecator was set at 25 (the lowest possible) and the low power output utilized. Time, voltage to the primary coil of the Hyfrecator, and the diameter of the desiccating needles were varied in order to determine the relative influence of these factors on the size of the destruction.

The time series was .2, .4, .6, and .8 seconds; and the voltages fed to the primary coil of the Hyfrecator were 25, 50, 75, and 100 volts. The diameter of the desiccating needles were .005 and .025 inches. This gives 4 x 4 x 2 or 32 combinations of factors.

The frontal cortex of each of the 16 rats was exposed and a mark involving one of the possible 32 combinations of factors placed on the left hemisphere, and another one of the possible combinations applied to the right hemisphere. After marking, the rats were allowed 24 hours to recover from the operation and the brains were then removed and sectioned at 50 microns. All sections which included the destruction were mounted and stained.

Method:

Results:

In general, measurement with an ocular micrometer revealed that the narrow needle resulted in destructions which were better defined than the needle with the large diameter. The amount of voltage on the primary coil tended to be positively related to the depth of the destruction. This experiment was not carried to completion, because as the data were being collected, it was apparent that results would be so variable as regards the time and voltage factors that it would not be beneficial to spend any more time preparing the slides in order to make the measurements of all the hemispheres marked. It was noted, however, that relatively small well-defined marks could be made using 25 volts on the primary coil, the narrow needle, and the Hyfrecator set at 25 power units for 0.4 seconds.

Results:

IV. The Main Investigation

Method:

Ten single-handed rats were divided into two groups of five in order to observe for immediate and delayed post-operational effects on handedness. The two groups were first observed for three days in order to determine handedness. Individual results are given in Appendix A.

They were then operated upon and marks placed on the hemisphere contralateral to the preferred hand. The mark was placed at the point where electrical stimulation had produced the arm movements. Group I rats were then observed within 24 hours post-operationally to check for any noticeable effects upon handedness. The brains were then removed and sectioned at 50 microns. All sections which included the destruction were mounted and stained. The locus of the destruction was then plotted and the extent of the destruction measured.

Post-operational observations in Group II were continued for four weeks, until a total of 500 reaches were taken. Slides of the destructions were prepared, although no measurements of the destructions were made because of healing and consequent influx of intact tissue into the desiccated area.

Results:

None of the rats in either Group I or Group II transferred nor were they noticeably affected in their reaching. There was a well-defined, and very small mark which located the area where stimulation produced arm movements. The destruction penetrated layer I and in most cases only superficially involved layer III of the cortex. (Layer II is not present in the frontal cortex of the rat.)

Group I

The location of the marks are plotted on the Lashley chart illustrated below. All the marks from each case are superimposed on one chart, and placed on the left hemisphere for comparison. The point indicates the locus of deepest penetration. The red circle indicates the surface area of the destruction.

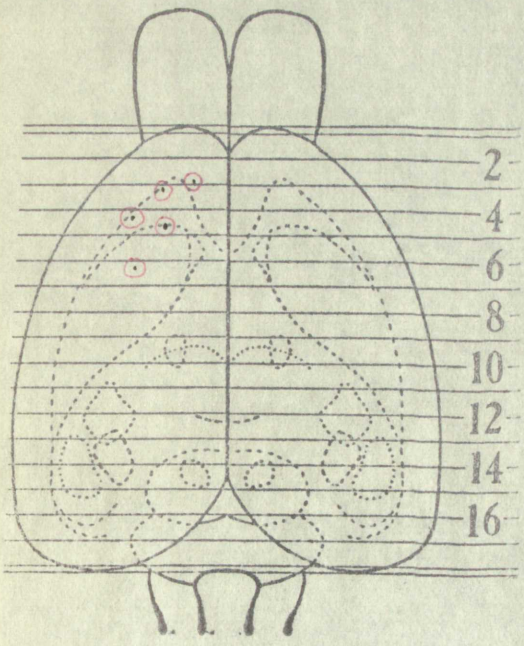
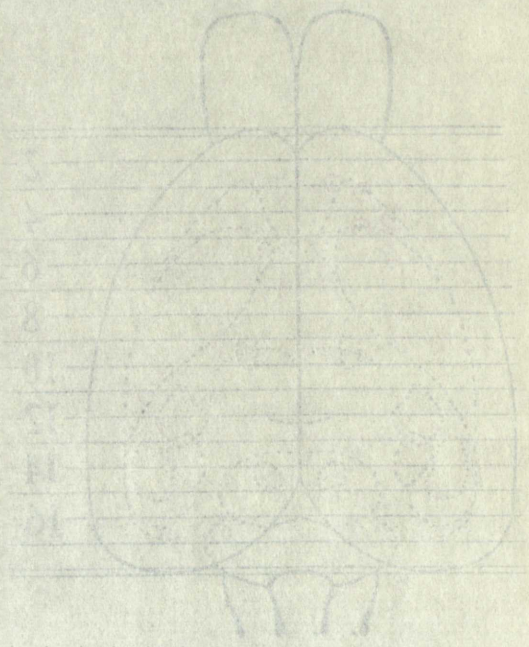


Figure 2

The Locus of the Destructions for Group I

Results:



The volume of the destructions were computed by plotting all sections which showed signs of desiccation (especially absence of neurons) on graph paper. These figures can be found in Appendix C.

The thickness of each section was known to be 50 microns, and the side of a square of the ocular micrometer on the microscope was known to be 0.21 millimeters. Therefore, each square which included destruction contained .050 mm. x .210 mm. x .210 mm. = .0022mm^3. All squares invaded for all sections within one brain were summed and multiplied by .0022 mm^3. and this result was the figure used as the volume of tissue destroyed.

The size of the destructions ranged from .092 mm^3. to .9062 mm^3.

Table I below summarizes the destruction for this group:

Table I

Volume of destruction for rats of Group I

Rat Number	Amount of destruction
3	.1452 mm^3
4	.0920 mm^3
11	.9062 mm^3
21	.1018 mm^3
31	.1232 mm^3

Photomicrographs of the most and lease extensive of these destructions can be seen in Appendix D.

Group II

The location of the marks for this group are plotted on the Lashley chart illustrated below. Marks were drawn in the same manner as was done with Group I.

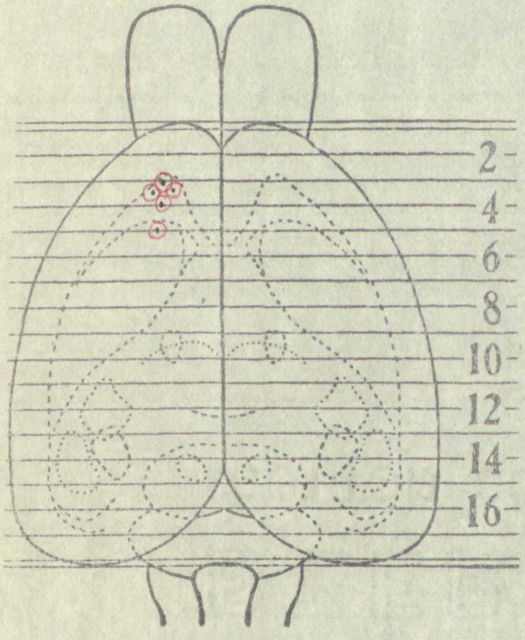
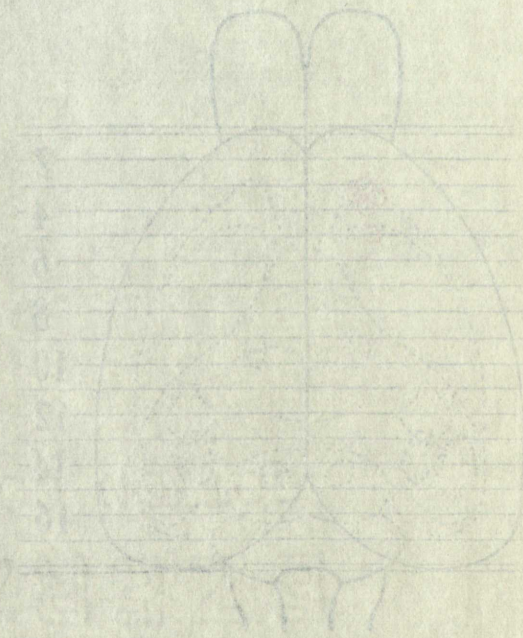


Figure 3

The Locus of the Destructions for Group II

Although the volume of destruction was not computed for this group because of healing, in all cases except case 51 the damage was less than the smallest destruction in Group I. Case 51 had approximately the same amount of destruction as case 21 of Group I (.1018 mm^3.).



V. Summary and Conclusions

Summary:

The possibility of making a mark at the point where handedness has been localized by electrical stimulation without producing changes in that area sufficient to alter its functioning was investigated. Two groups of single-handed rats were used, each group consisting of 5 rats. All rats were marked with a modified Hyfrecator which delivered high-frequency currents to a needle point of .005 inches in diameter for a period of 0.2 seconds. The voltage on the primary coil of the Hyfrecator was 25 volts, and the setting on the Hyfrecator was 25 units. The low power outlet on the Hyfrecator was used. The point where the mark was to be placed on the cortex was determined by electrical stimulation, and correct marking was insured with the aid of a stereotaxic instrument and the use of the same needle for both stimulation and desiccation.

Five of the rats were observed within 24 hours post-operationally and five were observed at weekly intervals for four weeks post-operationally. The brains were sectioned, stained, and the marks located and measured; also, the loci of these marks were plotted on a Lashley brain chart.

Summary:

Conclusions:

Rats can be marked at the most stimulable area of the cortex for handedness, and not be affected in their hand preference. This mark does not influence handedness over a period of four weeks.

It is thus possible to study the intact region immediately below this marking, except for the portion confined largely to layer I and to a slight extent the superficial portions of layer III of the cortex.

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Peterson, Geo. M., & [illegible], Donald [illegible]. [illegible] identification of the handedness area in the [illegible] cortex of the rat. [illegible]

APPENDIX

Appendix A

The Preoperational and Postoperational Reaching Records for Group I

OBSERVATIONAL PERIOD	Rat 3		Rat 4		Rat 11		Rat 21		Rat 31	
	R	L	R	L	R	L	R	L	R	L
Preoperation:										
1	46	4	50	0	48	2	50	0	0	50
2	100	0	100	0	99	1	100	0	2	98
3	50	0	50	0	50	0	50	0	0	50
24 Hour Postoperation:										
1	50	0	50	0	50	0	50	0	0	50

Appendix A

The Psychophysical and Psychological Scaling Procedure for Chapter I

The Preoperational and Postoperational Reaching Records for Group II

OBSERVATIONAL PERIOD	Rat 41		Rat 51		Rat 32		Rat 62		Rat 72	
	R	L	R	L	R	L	R	L	R	L
Preoperation:										
1	50	0	47	3	30	0	50	0	0	50
2	100	0	100	0	120	0	100	0	0	100
3	50	0	50	0	50	0	50	0	0	50
24 Hour Postoperation:	100	0	100	0	100	0	100	0	0	100
Weekly Postoperation:										
1	100	0	100	0	100	0	100	0	0	100
2	100	0	100	0	100	0	100	0	0	100
3	100	0	100	0	100	0	100	0	0	100
4	100	0	100	0	100	0	100	0	0	100

Die Verbreitung von Lophodermium nevadense auf Pinus spec. in Europa

Appendix B

Staining Procedure

Slides with tissue affixed are placed in the following solutions for the stated length of time.

	Solution	Time
1.	Toluene	5 min.
2.	Toluene	5 min.
3.	95% Alcohol	5 min.
4.	70% Alcohol	5 min.
5.	40% Alcohol	5 min.
6.	20% Alcohol	5 min.
7.	Distilled Water	10 min.
8.	Thionin (1.668 gr/200ml. Distilled Water)	5 min.
*9.	95% Alcohol	5 min.
*10.	100% Alcohol	5 min.
11.	Toluene	5 min.
12.	Toluene	5 min.

*Steps 9 and 10 may have to be shortened if the solutions are clear and too much bleeding of the dye from the tissue results.

Appendix C

Reproductions of Destructions

Explanatory note:

Small grid dividions are equal to 0.21 mm.

Each section including destruction is numbered serially from anterior to posterior position in the brain.

Dotted line in case 11 indicates the surface of the tissue.

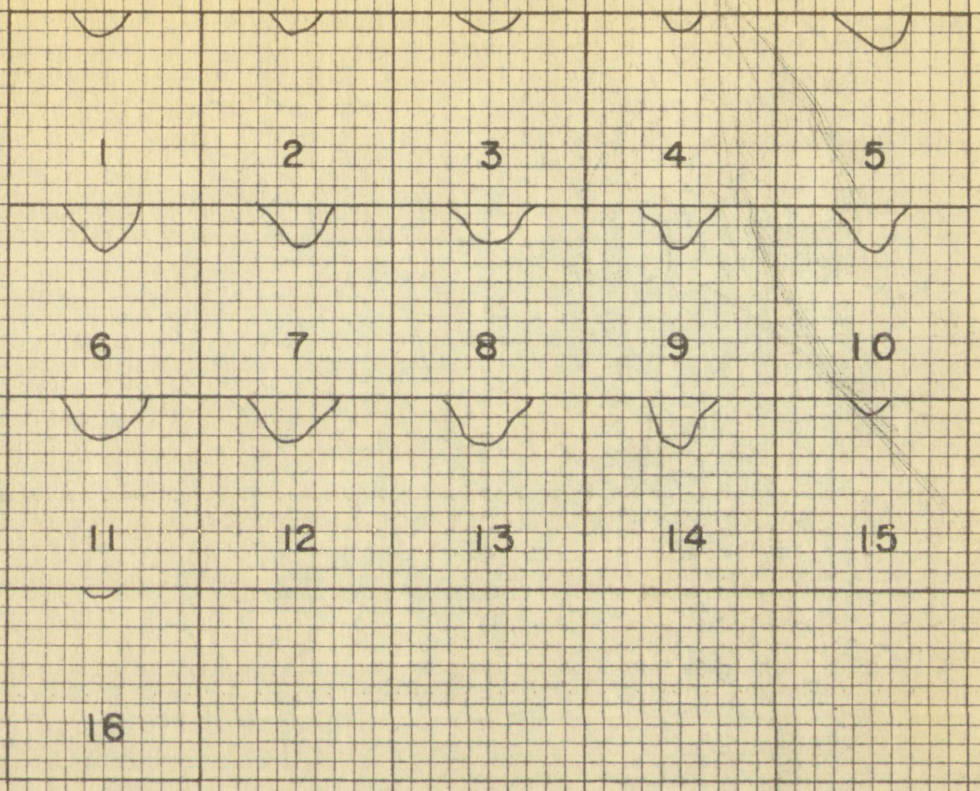


FIGURE 4

DESTRUCTIONS FOR CASE 3

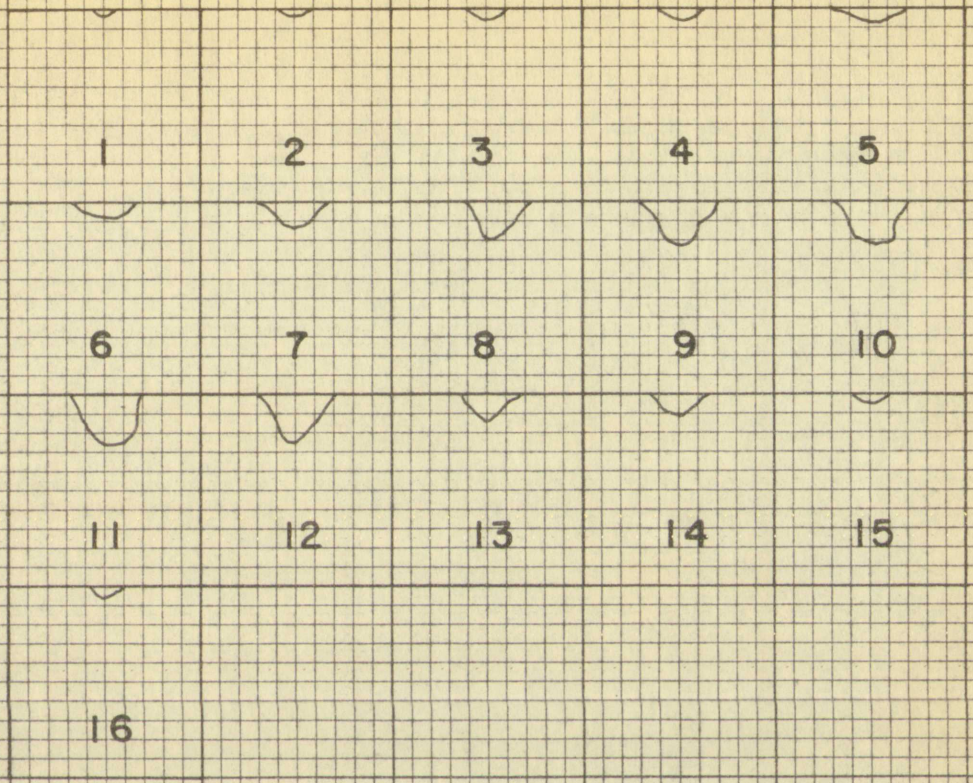


FIGURE 5

DESTRUCTIONS FOR CASE 4

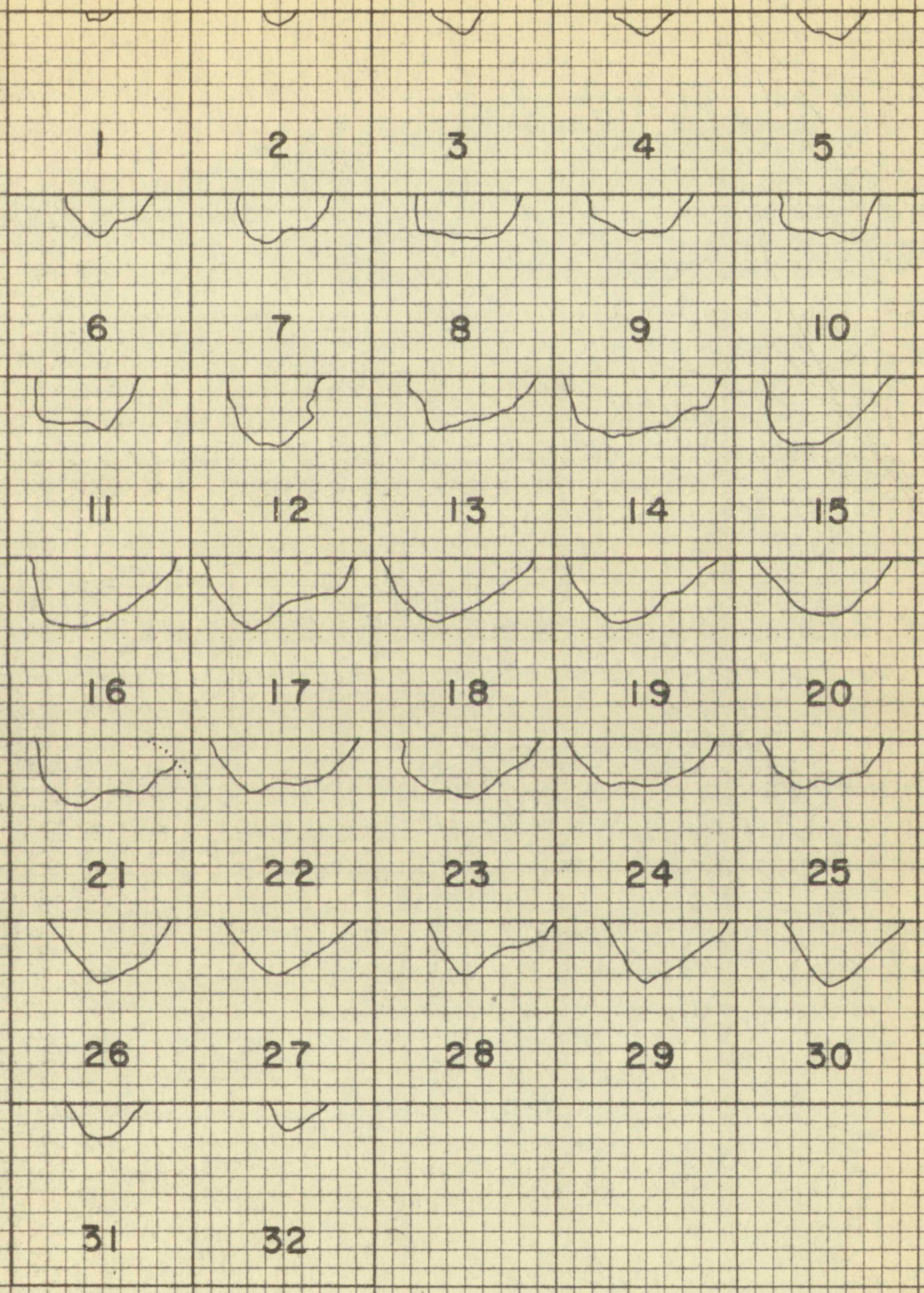


FIGURE 6

DESTRUCTIONS FOR CASE II

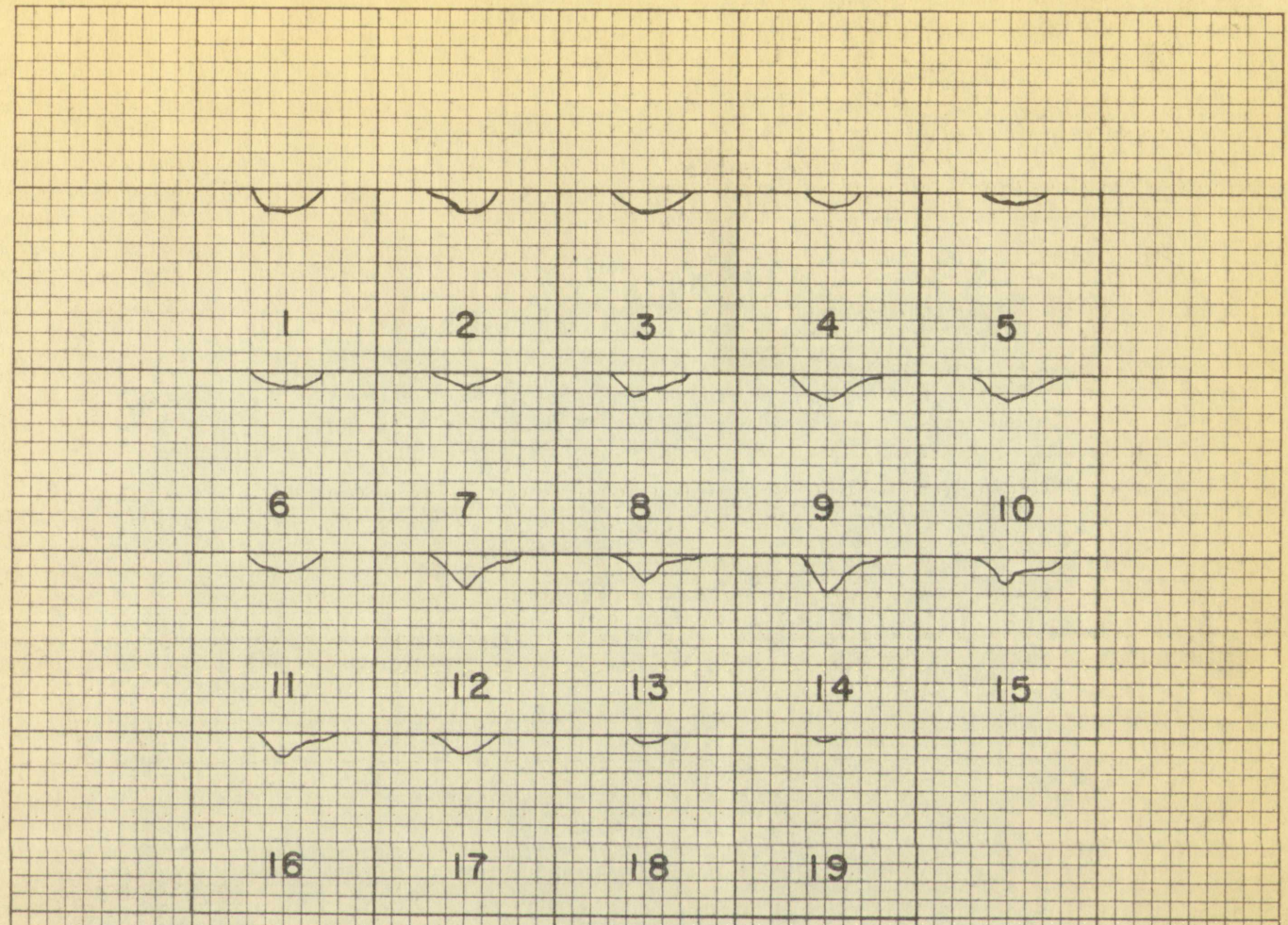


FIGURE 7

DESTRUCTIONS FOR CASE 21

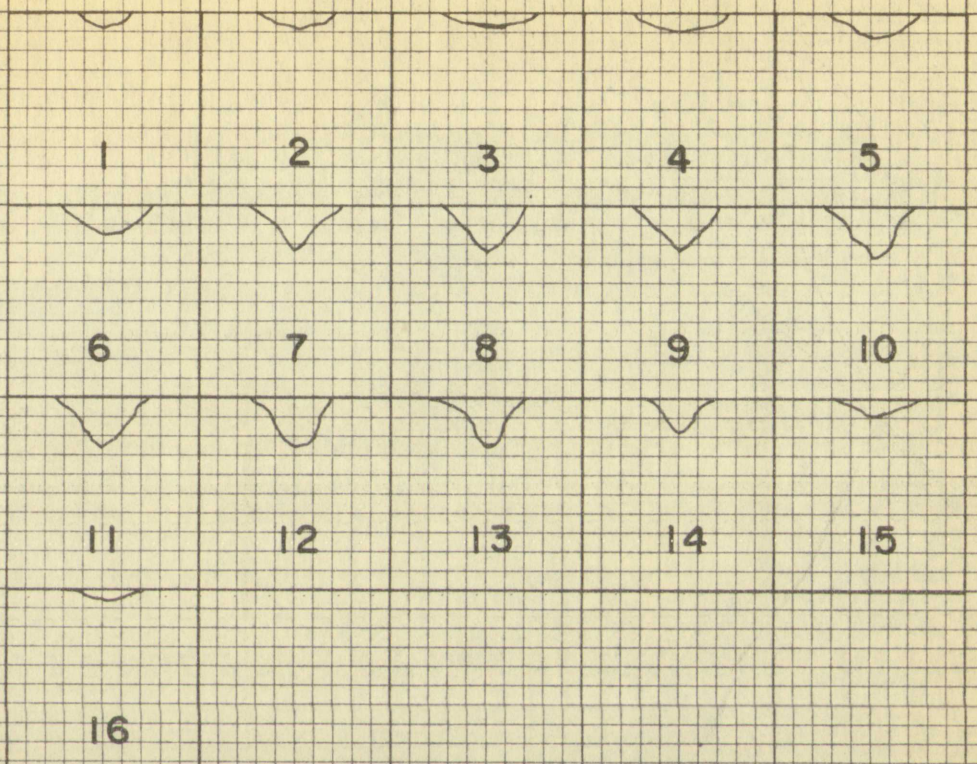


FIGURE 8

DESTRUCTIONS FOR CASE 31

Appendix D

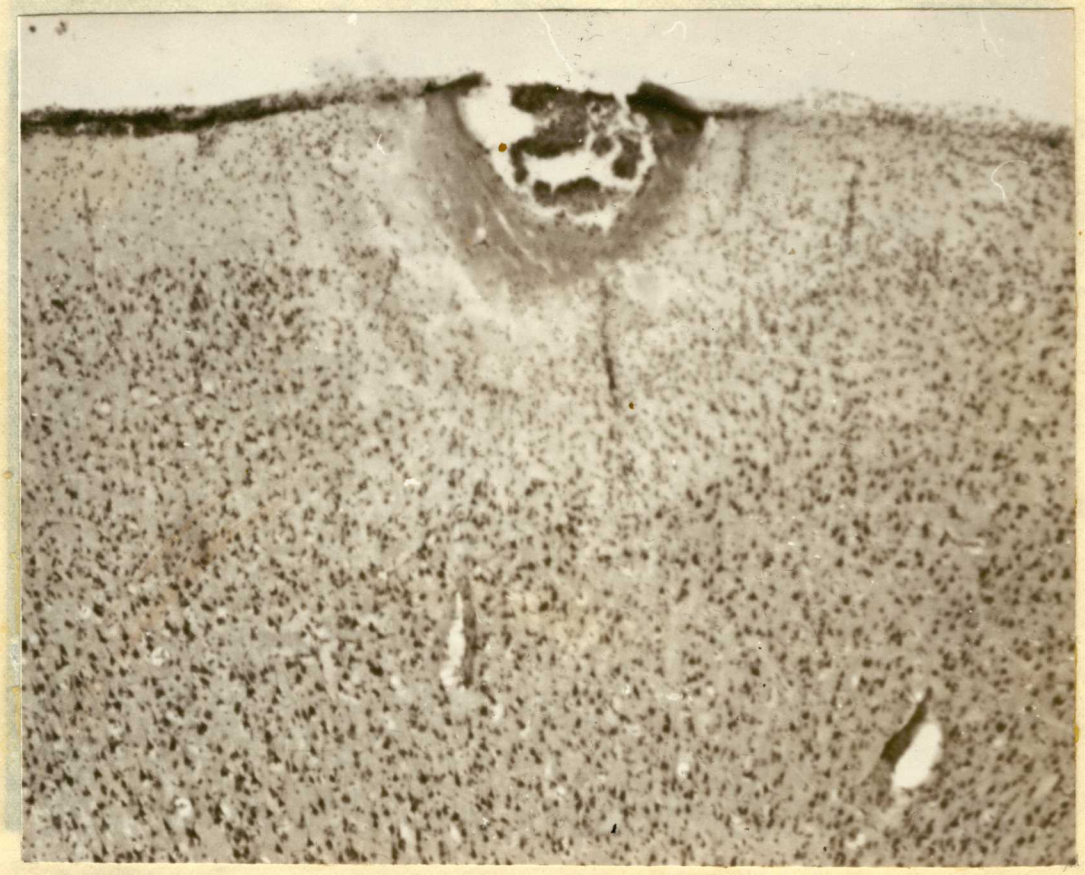
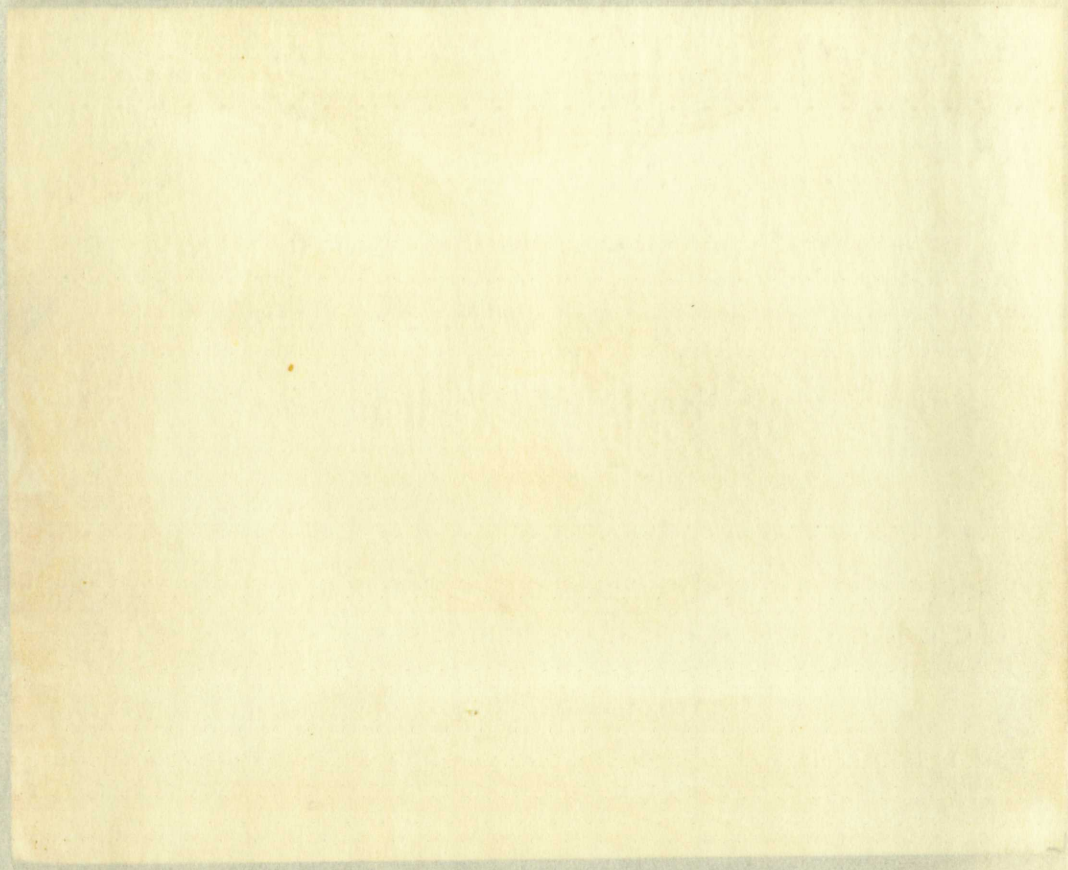


Figure 9

Photomicrograph of Most Extensive Destruction for Group I

(Case 11 Scale: 1.5 cm. = 210 microns)



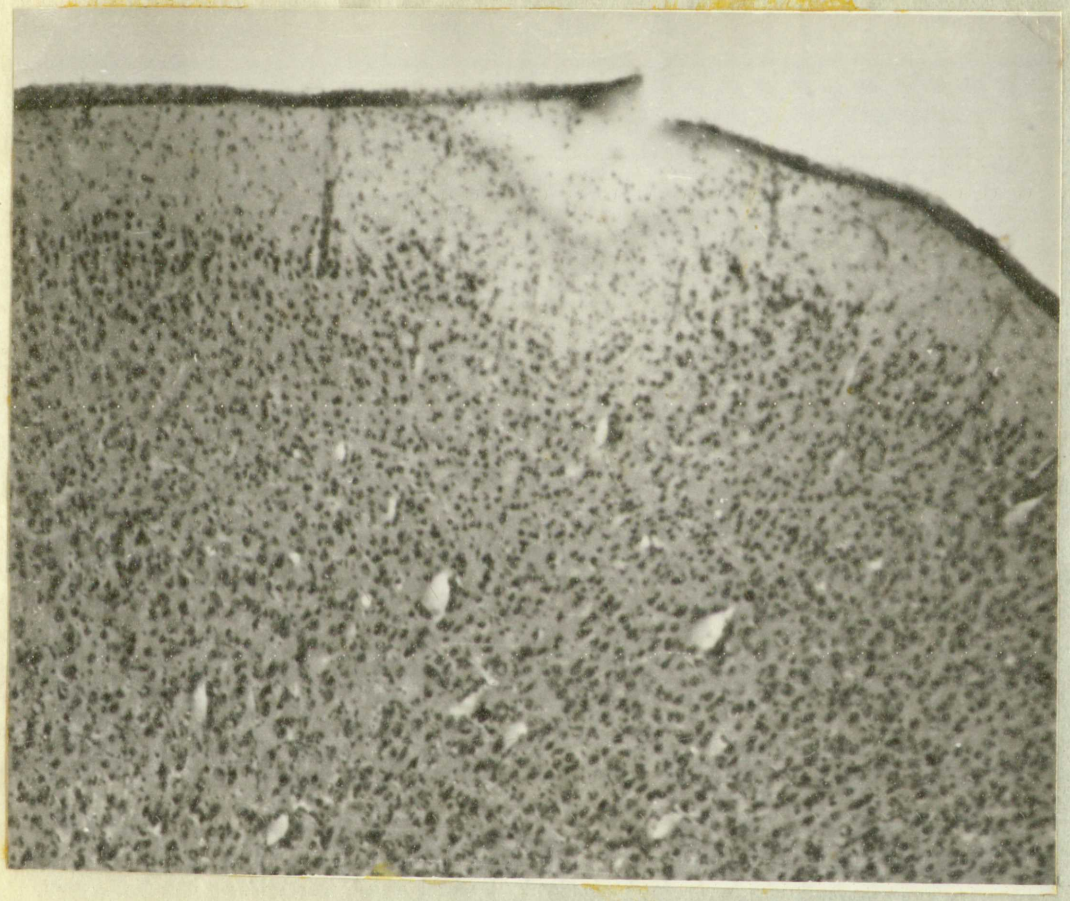


Figure 10

Photomicrograph of Least Extensive Destruction for Group I

(Case 4 Scale: 1.5 cm. = 210 microns)

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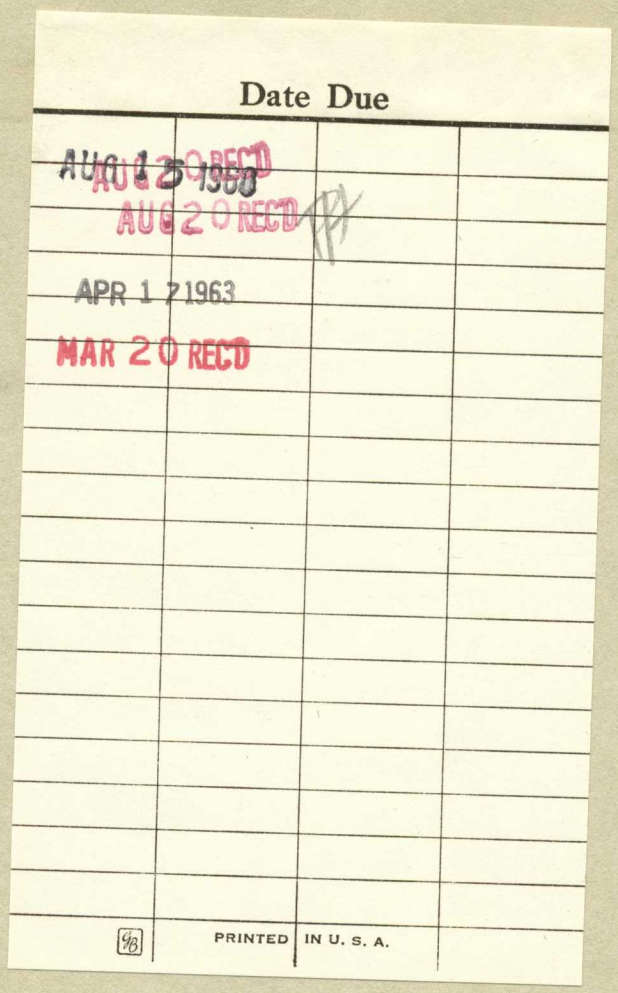

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