

PRIMARY AND SECONDARY FINDINGS IN A SERIES OF ATTEMPTS TO TRANSPLANT CEREBRAL CORTEX IN THE ALBINO RAT

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EIGHT FIGURES

INTRODUCTION

This paper is to report the findings of a somewhat extended attempt to transplant cerebral cortex. In two instances, at least, the attempt to keep alive the neurons within the tissue seemed to meet with success, although not in the manner desired, since the tissue transferred from one animal to a second animal became adherent in a position such that the extending axons could not grow into adjacent nervous tissue.

The secondary findings have some interest also, and the entire problem has a value because of its bearing on the question of the vitality of nervous tissues. Continuation of the life and growth of nervous tissue in vitro has been accomplished successfully, Harrison, '07, Burrows, '11, Lewis, '12, but the perpetuation of the vitality of nervous tissue transferred from one region of the nervous system to another region has met with greater difficulties.

In the earlier attempts at transplantation it was found that the transplanted mass did not disintegrate entirely and disappear, but that the neurons died, leaving the supporting structures to represent the original transplanted portion. Of the earlier attempts at transplantation those of W. Gilman Thompson, '90, of Saltykow, '05, and of Del Conte, '07, may be cited. In

1909, Ranson reported successful transplantation of the spinal ganglion into the brain. Marinesco and Bethe had earlier transplanted ganglia to a position adjacent to the sciatic nerve. In these cases of successful transplantation the neurons remained alive.

In 1904, while research assistant in the Neurological Laboratory of the University of Chicago in charge of Professor H. H. Donaldson, I began some experiments in the transplantation of cortical cerebral tissue in young albino rats.

MATERIAL SELECTED

The albino rat was selected because of its adaptability as a laboratory animal. In contrast to the adult animals which had earlier been selected for attempted transplantation, a more immature animal was utilized. At the ninth or tenth day from birth the cerebral tissue of the albino rat is not mature, the hairy coat is not sufficiently developed to interfere with operation, and although the young must be left in the mother's care after the operation, they are not given that assiduous care which renders operation on very young animals so difficult. It is possible to return the operated young to the nest without fear of attack upon them from the mother, if the precautions mentioned under the discussion of methods of operation are observed.

The records show that in all forty-six rats were operated. Two of these rats died soon after operation. Nine other brains showed nothing of interest on macroscopical examination. Thirty-five brains were sectioned and studied.

For purposes of tabulation as appears in table 1, the forty-six operations are divided into four series made up of an irregular number of groups. The term group had a definite connotation and marked the number of animals operated at one time. A group usually consisted of the young of one litter, the transplantation being made from one to another young rat of one litter. The brains were thus equally mature at time of operation and the rats of the closest consanguinity. It was hoped that these two points might have weight in the preservation of

TABLE 1

	SEX	BORN	OPERATED	KILLED	AGE IN DAYS	DAYS AFTER OPERATION
Series I						
Group I, Rat 1.....	?	12/16/1903	12/28/1903	4/ 5/1904	111	99
Group II, Rat 1.....	?	12/23/1903	1/ 4/1904	1/12/1904	20	8
Group III, Rat 1.....	♂	1/ 4/1904	1/14/1904	4/ 9/1904	96	86
Rat 2.....	♂	1/ 4/1904	1/14/1904	4/ 9/1904	96	86
Group IV, Rat 1.....	♀	1/12/1904	1/23/1904	4/ 7/1904	86	75
Rat 2.....	?	1/12/1904	1/23/1904	4/ 8/1904	87	76
Rat 3.....	♀	1/12/1904	1/23/1904	4/ 9/1904	88	77
Series II						
Group I, Rat 1.....	♀	5/30/1904	6/ 9/1904	7/12/1904	43	33
Rat 2.....	?	5/30/1904	6/ 9/1904	7/18/1904	49	39
Group II, Rat 1.....	♂	6/12/1904	6/22/1904	8/10/1904	57	49
Rat 2.....	♀	6/12/1904	6/22/1904	8/10/1904	57	49
Group III, Rat 1.....	♂	7/10/1904	7/20/1904	10/26/1904	108	98
Rat 2.....	♂	7/10/1904	7/20/1904	10/26/1904	108	98
Series III						
Group I, Rat 1.....	♂	4/ 7/1905	4/17/1905			
Rat 2.....	♀	4/ 7/1905	4/17/1905			
Rat 3.....	♀	4/ 7/1905	4/17/1905			
Group II, Rat 1.....	?	4/17/1905	4/27/1905	6/ 7/1905	51	41
Rat 2.....	♀	4/17/1905	4/27/1905	6/ 7/1905	51	41
Rat 3.....	♂	4/17/1905	4/27/1905	6/ 8/1905	52	42
Group III, Rat 1.....	♂	5/10/1905	5/20/1905	9/14/1905	127	117
Rat 2.....	♂	5/10/1905	5/20/1905	9/14/1905	127	117
Rat 3.....	♂	5/10/1905	5/20/1905	9/14/1905	127	117
Group IV, Rat 1.....	?	9/11/1905	9/21/1905	11/ 6/1905	54	44
Rat 2.....	?	9/11/1905	9/21/1905	11/11/1905	59	49
Rat 3.....	?	9/11/1905	9/21/1905	11/21/1905	69	59
Rat 4.....	?	9/11/1905	9/21/1905			
Series IV						
Group I, Rat 1.....	?	11/24/1906	12/ 4/1906	2/27/1907	95	85
Rat 2.....	?	11/24/1906	12/ 4/1906	3/20/1907	116	106
Group II, Rat 1.....	♀	2/24/1907	3/ 5/1907	5/ 3/1907	68	59
Rat 2.....	♂	2/24/1907	3/ 5/1907	5/ 9/1907	74	65
Group III, Rat 1.....	?	4/14/1907	4/22/1907	4/29/1907	15	7
Rat 2.....	♂	4/14/1907	4/22/1907	6/26/1907	73	65
Group IV, Rat 1.....	♀	4/17/1907	4/26/1907	7/ 1/1907	75	66
Rat 2.....	♀	4/17/1907	4/26/1907	7/ 1/1907	75	66
Rat 3.....	♂	4/17/1907	4/26/1907	7/ 1/1907	75	66

TABLE 1—Concluded.

	SEX	BORN	OPERATED	KILLED	AGE IN DAYS	DAYS AFTER OPERATION
Group V, Rat 1.....	♀	5/14/1907	5/23/1907	10/ 4/1907	143	134
Rat 2.....	♀	5/14/1907	5/23/1907	10/ 4/1907	143	134
Rat 3.....	♂	5/14/1907	5/23/1907	10/ 4/1907	143	134
Rat 4.....	♂	5/14/1907	5/23/1907	10/ 4/1907	143	134
Group VI, Rat 1.....	♂	5/23/1907	6/ 1/1907	9/24/1907	125	116
Rat 2.....	♂	5/23/1907	6/ 1/1907	11/23/1907	185	176
Rat 3.....	♂	5/23/1907	6/ 1/1907	12/28/1907	220	211
Rat 4.....	♂	5/23/1907	6/ 1/1907	12/28/1907	220	211
Group VII, Rat 1.....	♂	6/16/1907	6/27/1907	11/30/1907	167	156
Rat 2.....	♂	6/16/1907	6/27/1907	12/28/1907	195	184
Rat 3.....	♂	6/16/1907	6/27/1907	12/28/1907	195	184

vitality in the transplanted material. The consanguinity may have particular value if closely related individuals have a similar metabolism and hence a like chemical constitution of the body tissues.

METHOD OF OPERATING

For the convenience of the operator, the left hemisphere of the brain was chosen for operation in each case. A portion of the cerebral cortex was selected for removal, the loss of which would least interfere with the nutrition of the operated animal. The skin was first opened near the median line of the head by an incision carried from the region of the eyes to the nape of the neck, an incision of not excessive length in the young albino rat. The flap on the left side was retracted by pulling on the skin at some little distance from the line of incision. Then with fresh sterile scissors a cartilaginous flap was made in the parietal region of the skull. This was accomplished by a crescent-shaped incision with the attached base just above the ear. This procedure was repeated on a second rat. Then with a thin knife a triangular portion of the thin cortex was removed from the first rat and replaced by a similar portion from the second rat. The incision was usually made in such a way that the

apex of the triangle extended downward. The time consumed in the transfer of the cortex was made as short as possible. In the earlier operations the lateral ventricle was often accidentally opened, although the intention had been to remove and replace a thin superficial portion of cortex only. The apparently successful cases were found to be those in which the ventricle had been opened and a bit of cortex had become adherent to the choroid plexus. During and after the operation the dura was preserved if possible but in rats of ten days the cerebral membranes are very delicate and difficult to differentiate from one another. Sometimes the dura remained conveniently attached to the cartilaginous flap. After transference of the cortical mass the cartilaginous flap was freed, the skin drawn over it and the edges of the skin retained in proximity by a collodion dressing.

Attention to a number of details was found to be advantageous. Complete anaesthesia before and during the operation was found necessary, otherwise the struggles of the animal caused protrusion of the cerebral substance through the incision. Asepsis was secured by using successive sets of sterile instruments. No antiseptic was used other than the ether of the collodion dressing. Maintenance of the body warmth both during and after the operation was essential. This care for the maintenance of the body heat and that for the exclusion of antiseptics were the very helpful suggestions of Prof. C. S. Sherrington, who was a visitor to the laboratory during the early experimental period. Care was exercised not to injure adjacent cerebral structures. Transplanted material was handled rapidly and with a warm knife. An almost insuperable difficulty appeared to be that of retaining the transferred material in the desired place. At ten days of age the cerebral cortex of the albino rat is soft and plastic. The removal of tissue leaves an irregular and almost imperceptible cavity, so that the transferred tissue is displaced almost immediately from the convexity of the cerebral hemisphere. In the later series an attempt was made to permit the formation of a thin blood clot lying over the transferred bit of tissue and extending to the

adjacent parts of the hemisphere. This splint would be, I believe, an important factor in ultimate success.

The operation was done under ether anaesthesia and the animal kept warm, both during the operation and for two or three hours after the operation, until it had thoroughly recovered from the anaesthetic, and the odor of the ether from the anaesthetic and from the collodion dressing had disappeared. Then the young rats were returned to the nest and met with no interference from the mother other than futile attempts to remove the collodion dressing. The quiet and seclusion of the nest during the few days after operation aided convalescence. Young rats are not inclined to stray from the nest until the eyes open, about the fourteenth day of life.

EXAMINATION OF THE MATERIAL

No microscopical studies of the early conditions of the transplanted material were attempted, as the attention was centered on an effort to ascertain whether such transplanted material would later contain mature neurons with medullated axons. The brains of a few rats which died soon after operation gave no suggestion of the survival of the transplanted tissue.

A few of the operated brains especially from the rats of the earlier operations, showed some inflammatory changes, with disintegration of the cerebral substance, about the region of the incision.

The rats upon whom these experiments were carried out gave the appearance of normal rats. No convulsions or paralyses were noted in the operated animals. Control rats were studied during the course of the first operations but, when no sequellæ of the operative procedure were noted, the observation of control rats was abandoned.

The examination of the material removed post-mortem was of two kinds. The first of these was the gross examination at the time of autopsy, when notes were made regarding the condition of the skull, of the meninges, and of the cerebral substance as to the superficial extent of the wound, location of the cicatrix, et cetera. The brains were then removed and fixed in

ten per cent formalin. Some weeks before the brains were to be embedded for cutting, they were mordanted in toto in Müller's Fluid. The blocked material was cut serially either in thirty or forty-five micra sections and stained by the Weigert-Pal method. Alternate sections were counter-stained with Upson's Carmine.

The sections were studied individually under low and high power.

I am indebted for the drawings to Miss Katherine Hill and Mr. A. B. Stredain.

The completion of this study was made possible through the courtesy of Dr. R. R. Bensley and Dr. C. J. Herrick, who granted me an additional amount of free time for the microscopical study of the sections.

The rats used were bred in the laboratory, with the exception of Series IV, Group I, for which I am indebted to Dr. J. B. Watson.

THE FINDINGS FOR CEREBRAL TRANSPLANTATION

The investigation now reported was undertaken for the purpose of determining the possibility of maintaining the life of nerve cells in bits of transplanted cerebral cortex. This continuity of vitality has been found possible and growth has gone on in the neurons transplanted. The neurons which have survived have assumed their morphological relations to other neurons within the transplanted bit. The growth changes within the transplants are very similar to those of normal material of about the same age. Medullation is fully accomplished. The number of medullated fibers is however relatively smaller than in normal material but this is probably due to the absence of such fibers as grow into any cerebral region from other parts of the brain. The growth of the individual neurons has been very considerable. Watson, '03, found that the cortex of the brain of the albino rat is but slightly developed at the tenth day of life, the age at which transplantation was attempted, and that medullation appears much later. The transplanted neurons must therefore have been very immature.

The possible relations of these transplanted neurons with neurons outside of the transplanted portions have not been determined by the results of these experiments. In no brain of the four, with successful transplants, did the transplanted bit so attach itself that fibers could cross the line of attachment to unite functionally with adjacent neuron masses.

The two points of chief importance in successful cerebral transplantation are first, the retention in place of the material transferred, and second, the furnishing to it of an adequate blood supply. Apparently the death of neurons in blocks of transplanted cerebral cortex has been due to some factor which has not affected the vitality of other tissues. The supporting tissues of the cortex have lived and retained the mass form of the transplanted bit. This may suggest the lack of sufficient nourishment for the nervous elements. In my own successful operations, the transplanted portions have remained adherent to the denuded portions of the cortex but have taken some position near the choroid plexus of the lateral ventricle and have apparently received their blood supply from that source. Dr. Ranson permits me to mention that in carrying on some further (unreported) studies in the transplantation of nerve ganglia into the brain he found the most nearly normal conditions in those ganglia which were within or adjacent to the choroid plexus. This may have been due to the more complete anchorage of the material or to a more adequate nourishment, and my own experience would put emphasis on the latter reason. It would seem then that after the mechanical difficulties of securing juxtaposition have been solved, the viability of the transplanted tissues will be secured by guaranteeing sufficient nourishment.

My chief reason for believing these four to be true transplantations of cerebral cortex is the finding in each instance a line of cicatricial tissue about the mass of cortex in question. To follow the enclosing cicatrix it was necessary to study serial sections, and to assure oneself that the tissue mass in question was not partly separated from the remainder of the brain, or a bit which had been twisted out of its original position in the

course of the operation and had retained its vitality because of its ability to draw nourishment from its original blood supply.

To illustrate the conditions found in successful transplants, drawings have been made from sections of the brain of Rat 2 of Series IV, Group IV. Figures 1 and 2 are drawn from sections 119-120 of this brain. Figure 2 is a detail from the region

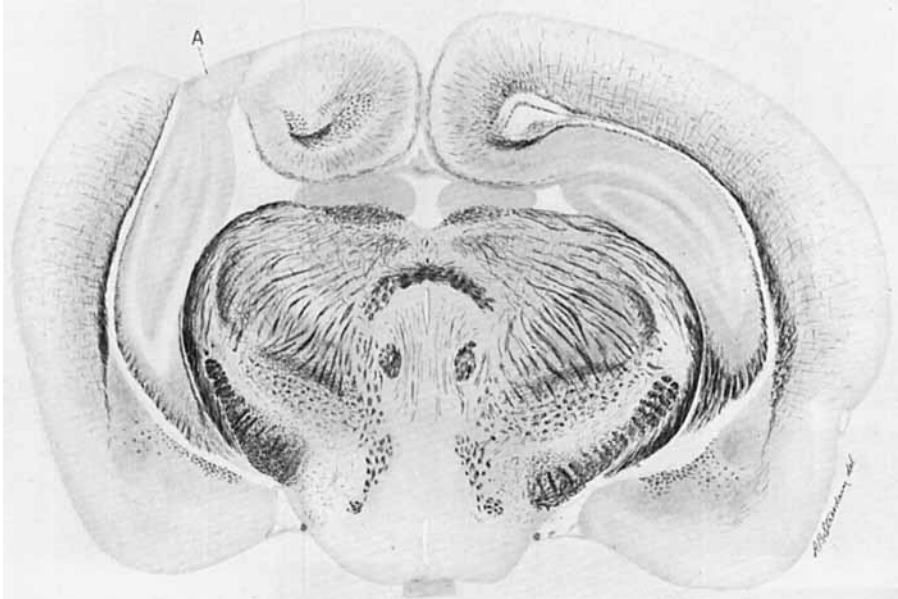


Fig. 1 Showing at *A* a bit of transplanted cerebral cortex in the albino rat. From sections 119-120, Series IV, Group IV, Rat 2. $\times 7.5$.

marked *A* in figure 1. This is by chance the first true transplantation to be noticed as all the material was carried through before detailed studies were made upon the completed slides. Later in the course of reëxamination, other true transplants were observed.

On the discovery of the transplant, figures 1 and 2, it was thought possible that it might be a portion of tissue pinched off from the hippocampus, to which it lies adjacent. However a rather wide band of cicatricial tissue could be seen in the double stained sections, separating the mass from the adjoining

structures. The position of the perikarya, also, and the relations of the medullated nerve fibers are those of a bit of inverted cerebral cortex. The capillary blood supply appears to be derived through the cicatricial adhesion to the plexus choroideus of the lateral ventricle.

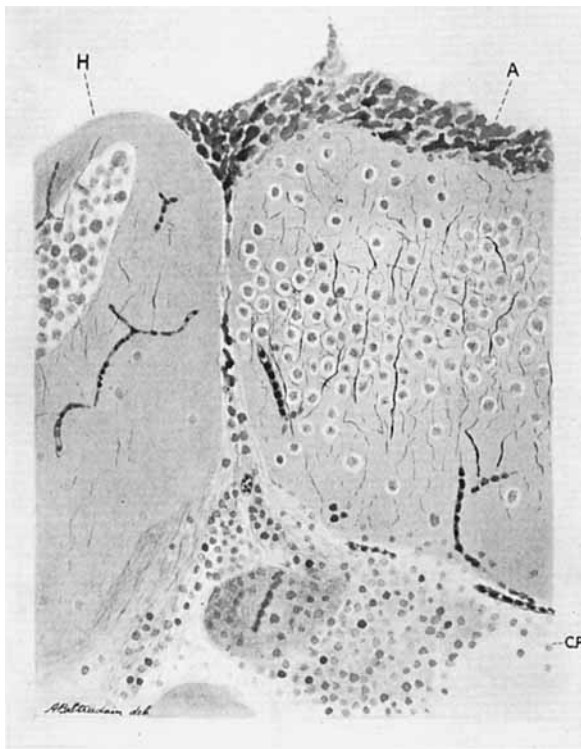


Fig. 2 Detail of figure 1. Showing attachment of transplanted portion (A) to hippocampus (H) at the left. The cortex is inverted and adherent to the choroid plexus (C. P.) from which it seems to derive its blood supply.

While the general type of cerebral cortex prevails in the portions transplanted, certain differences from normal cortex can be detected. Such areas have a slightly different reaction to staining agents than have surrounding areas. The colors vary slightly in intensity and in shade from those of the rest of the section. After fixation and staining the neurons appear some-

what fragmented, especially the free endings of the medullated nerve fibers which easily fray. The blood supply is less ample in such regions, the capillaries being more slender and less well filled.

THE MASSING OF CORTICAL FIBERS

In addition to what we may regard as true transplantation of cerebral cortex, other interesting results of the operations were noted. One of these was reported at a joint meeting of the Chicago Neurological Society and the Biological Club of the University of Chicago, March 30, 1909, under the title "On the course of cortical tangential fibers developing after ablation of encephalic cortical substance." Perhaps the use of the term 'tangential' in this connection is misleading. The fibers to which the report refers were parallel to the surface of the brain and located at various depths throughout the cortex. They were not tangential in the narrower sense of the term as it is applied to the fibers lying near the surface of the cortex. At a later time it was noted in other brains that vertical fibers were also apparently increased in number. The materials in which these conditions were noted were produced in the following way. When the operator accidentally opened into the lateral ventricle in the course of operation, there was a tendency for the sub-ventricular substance to protrude through and to widen the original opening. It was while studying serial sections of a brain in which this had occurred that the apparent increase of fibers about the open space was noted. In the normal cerebral cortex of the albino rat many scattered medullated nerve fibers may be found at various depths running parallel to the surface of the cortex. In those brains in which considerable openings occurred, there appeared, in transverse serial sections, to be a massing of fibers parallel to the cortex. These bands of fibers could be traced from outlying cortex and were found to merge into cerebral tissue which had about the normal number of fibers which were parallel to the surface. It seemed at the time that neuron processes which in their growth were not able to follow the path usual to them had been deflected by the wall of the

open space and formed a band along the margin. It appeared that the massing was more noticeable when the margin of the opening was near the center of the antero-posterior diameter of the hemisphere than when it was near the frontal or occipital



Fig. 3. Showing margin of wound with a massing (*M*) of cortical fibers. These fibers can be traced in successive sections, to the surrounding margin of the open space produced by the ablation. Zeiss microscope, camera lucida. Outline on table level with Ocular 2, Objective 16.0 mm.

pole of the hemisphere and that this was correlated with the marked increase in the number of such fibers in the corresponding region. It seemed possible then to interpret these fibers as association fibers because they could be found at various

levels in the cortex, could not be traced to projection fibers, and extended some distance through the cortex. Fibers of this kind can be found at *M* in figures 3 and 4 and in figures 5 and 6.

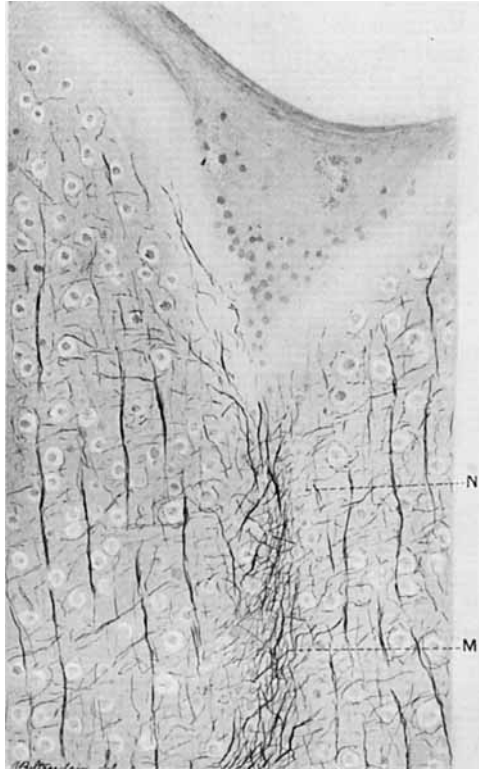


Fig. 4 Detail of figure 3. Showing the upper part of figure 3. Some small neurons (*N*) may be seen among the massed fibers. Zeiss microscope, camera lucida, outline on table level with Ocular 2, Objective 8.0 mm.

The more recent work of Greenman, '16, suggests a satisfactory interpretation of the number of medullated nerve fibers found circling the gap in the cortex. Dr. Greenman found in studies on the regeneration of peripheral nerves that the number of nerve fibers on the proximal side of the lesion in the regenerating nerve was enormously increased over the normal.

This increase apparently depended on the amount of obstruction offered by the connective tissue through which the nerve fibers must grow to reach their peripheral terminations. This interpretation may well account for the large number of fibers found in such sections as those shown in figures 3 and 4 and figures 5 and 6. The growth of tissue around a gap in central nerve sub-

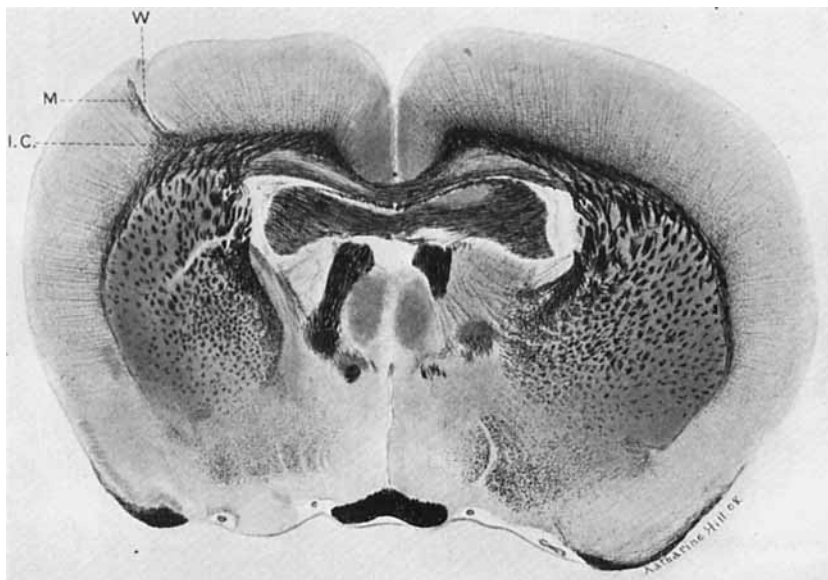


Fig. 5 Showing massing of cortical fibers along the open wound (*W*). Fibers apparently extend into the internal capsule (*I. C.*). Freehand drawing from Section 121, Series I, Group IV, Rat 3.

stance is comparable to their growth through connective tissue in peripheral nerves and may be governed by the same laws.

THE GROWTH OF MEDULLATED NERVE FIBERS ACROSS CICATRICAL TISSUE

Ranson, '03, demonstrated that processes of neurons could grow across cicatricial tissue and develop their medullary sheaths. These findings were on stab wounds of the corpus callosum in the albino rat. The cerebral cortex of operated

rats, in my experiments, yielded similar nerve fibers in such instances as the incision had extended for some distance into otherwise normal tissue and the coapted edges had united by the formation of cicatricial tissue. The fibers traversing the cicatricial tissue are no more numerous in my material than they were in that of Dr. Ranson, but are distinctly to be seen and, in favorable sections, may be traced for some distance.

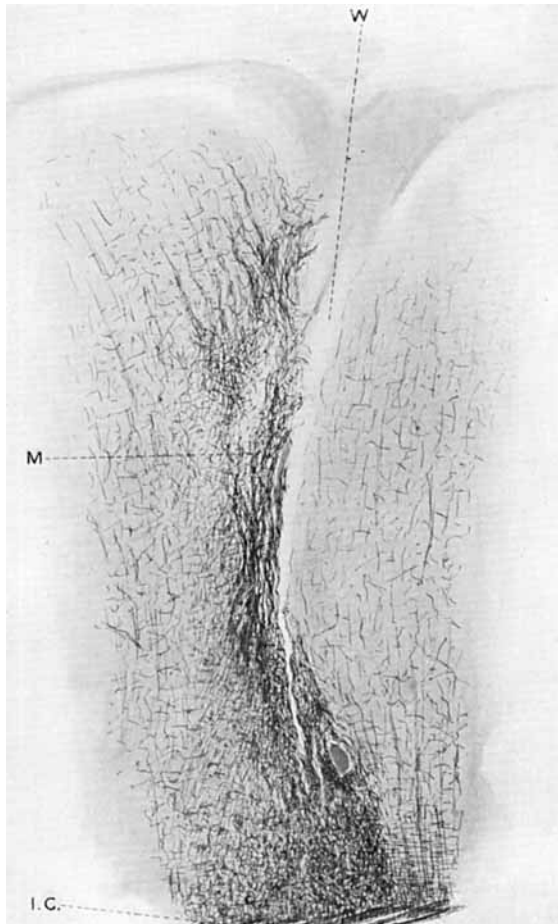


Fig. 6 Detail from figure 5. Freehand drawing. Magnification unknown.

Ranson interpreted these fibers as processes of neurons which were immature at the time of operation. Their perikarya may be located at some distance from the cicatrix. At *F*, in figures 7 and 8, may be noted such medullated nerve fibers crossing cicatricial tissue in the cerebral cortex of an albino rat.

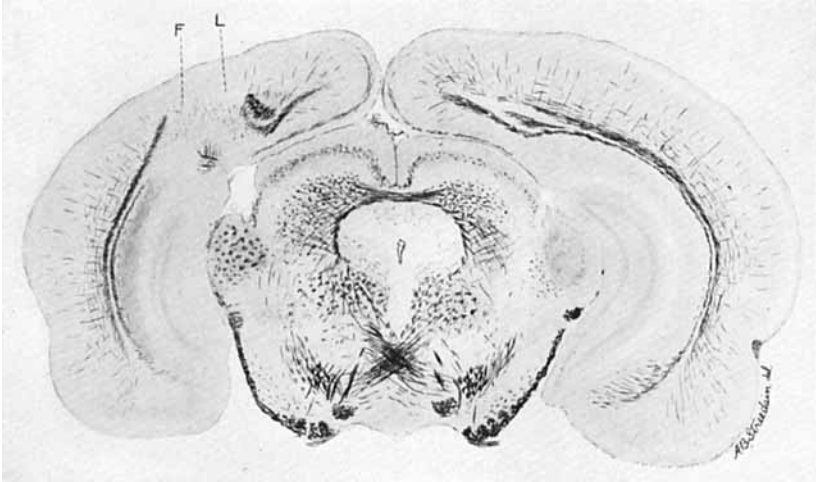


Fig. 7 Showing a section of the brain of the albino rat from a region near the posterior pole. In the dorsal part of the left hemisphere is a light line (*L*) marking the line of incision, with a few medullated nerve fibers crossing it (*F*). From sections 81-82, Series IV, Group IV, Rat 3. $\times 6$.

SUMMARY

By the use of immature nervous tissue from the brain of the albino rat the life of the constituent neurons in the cerebral cortex has been maintained after transplantation.

After many unsuccessful attempts this result was obtained by utilizing a thin covering blood clot to retain the graft in position.

The best nourished grafts were those which lay near the plexus choroideus of the lateral ventricle.

In the neurons of the transplanted cortex certain differences from those of normal tissue were detected. These differences were in the staining intensity and morphology of the perikarya and medullated fibers. The blood supply was less ample.

A massing of tangentially placed medullated nerve fibers was found about the open spaces produced by accidental ablations of cortex. These tangential fibers probably connect different parts of the cerebral cortex. This aberrance of nerve fibers shows that a new path may be routed when the usual path has been permanently blocked.

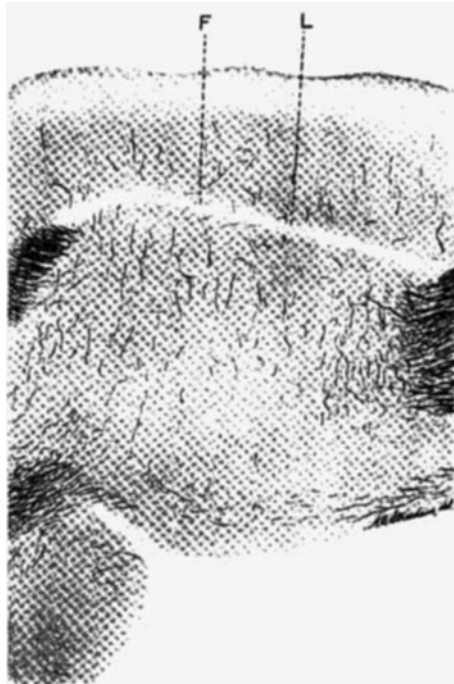


Fig. 8 Detail from figure 7, showing the line (*L*) of cicatricial tissue with several medullated nerve fibers (*F*) crossing it. $\times 28$.

Similar bands of projection fibers were noted along the margins of incised wounds.

Corroborative evidence was noted for Ranson's finding regarding the growth of medullated nerve fibers across cicatricial tissue in the nervous system.

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