

Removal and Reimplantation of the Parietal Cortex of Mice During the First Nine Days of Life: Consequences for the Barreffield

Filipe Andrés

*Institute of Anatomy,
Rue du Bugnon 9,
1005 Lausanne, Switzerland*

ABSTRACT

Vibrissal follicles on one side of the mouse whiskerpad are topologically connected to barrels in the contralateral somatosensory cortex. Barrels develop from postnatal day 3 to 6. Recently, I have observed that the barreffields still develop in pieces of parietal cortex that were removed and reimplanted, in the same place and with the original orientation, on the day of birth, or on postnatal days 1 or 3. Now, two questions were asked: (i) Can the barreffield form and/or remain in place after interrupting thalamocortical connections at different ages (from birth to postnatal day 9)? (ii) How does the cortex behave, in terms of cellular layers, after the interruption of thalamocortical connections?

To answer these questions the parietal cortex was removed and reimplanted in the same place with the original orientation, in 79 mice from a C3H strain. Fifty-one mice survived and were processed for histology. Their brains were cut coronally to facilitate the identification of the limits of the reimplanted cortex and of its cellular layering. In 29 cases the reimplanted cortex could be identified, and in 17 cases barrel-like structures had developed. The "barreffields" were obtained from coronal sections of each piece of reimplanted cortex, by means of a computer program which permitted reconstructing these pieces of cortex and rotating them in

space. In this way, barrel-like structures and "barreffields" could be visualized as if obtained from sections made tangential to the parietal cortex. "Barreffields" were found in pieces of cortex reimplanted at different ages up to postnatal day 9. Cortical layers appeared to be more close to normal in cases operated after postnatal day 5.

INTRODUCTION

The barreffield is a cortical map that can be visualized in Nissl stained sections of the somatosensory neocortex of mice. It is formed by barrels. Each barrel is a multineuronal unit that represents topologically each vibrissal follicle of the animal's contralateral muzzle /13/. Each vibrissal follicle is a highly specialized sensory organ.

In a previous paper /1/ I described the consequences for the barreffield of removing and reimplanting relatively large pieces of the presumptive barreffield cortex of mice, on the day of birth (P0), on postnatal day 1 (P1), and at P3. It was then found that in a majority of animals a barreffield still forms. In those experiments the brains were cut tangentially to the surface of the parietal cortex, and the barreffields were reconstructed by drawing, from consecutive stained sections, the profiles of the barrels. However, with that approach it was not possible to study the lamination of the manipulated pieces of cortex.

The experiments described here were made to answer two questions: (i) Can the barreffield form and/or remain in place after interrupting the thalamocortical connections at different ages (from birth to P9)? (ii) How does the cortex behave, in terms of cellular layers, after the interruption of the thalamocortical connections?

Reprint address:
Dr. Filipe Andrés
Rua Tristao Vaz nr. 22, 3^o Esq.
1400, Lisboa, Portugal

MATERIAL AND METHODS

Animals

In this study 79 mice from a C3HeB/Fej stock were used. The number of mice operated for each age was: 3 at P0; 11 at P1; 14 at P2; 6 at P3; 13 at P5; 13 at P6; 7 at P7; 9 at P8 and 3 at P9.

Techniques of Operation

All mice were anesthetized before the operations began: up to P3 by cooling; after that age with Penthrane (Abbott) vapor. During the operations the mice were fixed onto a small platform and kept anesthetized.

The skin of the head was disinfected with 70% ethanol. A large C-shaped skin incision was made over the dorsolateral aspect of the left hemicranium (all operations were made on this side), and the resulting flap was reclined towards the midline. The skull was then opened with the sharp tip of a number 11 blade (Swann-Morton) and reclined, also towards the midline. The dura-mater was either torn while reclining the skull flap, or cut immediately afterwards, and also reclined.

A piece of cortex from the presumptive barrelfield region (roughly placed in the middle third of the fronto-occipital axis of the cerebral hemisphere, above the rhinal sulcus), was then removed and reimplanted in the same place, with the original orientation.

Three different types of instruments were used as "knives": (i) the cutting edge of a razor blade; (ii) the tips of two different hypodermic needles; and (iii) a special "window" knife made of a tungsten filament.

The razor blade was used in three cases. With this knife, the sides of a small block of neocortex (of about 2 x 2 mm as measured on the pial surface) were cut by inserting the cutting edge perpendicularly to the surface of the brain; next, its deep side was also cut, but the depth of this undercut could not be precisely controlled. Care was taken not to damage subcortical structures. Subsequently, the piece of isolated neocortex (covered by pia-mater) was put aside in a drop of sterile saline, and bleeding in the brain stopped with small fragments of Spongostan Special (M.E.D.U. D-25, Ferrosan). Afterwards, this piece of cortex was put back into the brain, preserving the original orientation.

Two different hypodermic needles were used to remove and reimplant a piece of cortex, in 65 cases. The outer and inner diameters of each needle were: 0.8mm and 0.5 mm, and 1.8 mm and 1.3 mm, respectively. The sharp point of the needle was inserted perpendicularly to the pial surface (with the hollow turned upwards) to a depth estimated to correspond to that of the thickness of the cortex (Figs. 1a, 2 and Table 1). Afterwards the needle was slanted laterally and pushed into the cortex, until 1 or 2 mm of cortical tissue were cut. Next, the needle and the piece of cortex were removed, leaving the peeled off pia-matter in place. Care was taken to avoid damage to subcortical structures. After the deposition of

TABLE 1

Age at operation	Type of knife used		Surviving mice	Mice in which manipulated cortex was identified		Cases with "barrelfield"	
	N	WK		N	WK	N	WK
P0	3		3	3		1*, 2	
P1	8		6	5		3*, 4	
P2	14		8	2(a)		5*(a,R)	
P3	6		5	3(a,a,a)		6*(a), 7(a)	
P5	9	4	7	2(a)	1	8*(a)	
P6	10	3	13	8(a,a,a,a,a)	1(a)	9(a), 10*(a)	13(R)
						11(a), 12(a,R)	
P7	7		1	0		0	
P8	5	4	5		2(a)		14*(a,R), 15(a,R)
P9	3		3	2(a)		16(a), 17*(a)	
TOTAL	65	11	51	25	4	14	3

a = cases showing cortical layering

N = cases in which parietal cortex was cut with injection needle

R = cases which received Rhodamine B isothiocyanate

WK = cases in which parietal cortex was cut with window knife

* = cases shown in Fig. 2

a drop of sterile saline the explant was put back into the brain with the original orientation, below the pia.

The "window" knife was made of a tungsten filament, with a diameter of 0.1 mm, which was folded to form a rectangle of 1 x 2 mm; the longer side, used to cut (Figs. 1b, c), was made thinner by means of electrolysis. The depth of 1mm was about that of the parietal neocortex. This knife was used to operate on 11 cases (Table 1). Three small incisions of about 2 mm each were made in the pia with the tip of a number 11 blade. The first incision was made laterally (above the rhinal sulcus), parallel to the midline, and the other two were made perpendicular to the first (from its cranial and caudal sides), towards the midline. The pia was cut in this manner to prevent it from peeling off. The cutting edge of this knife (C in Fig. 1b) was put against the surface of the cortex (through the lateral incision previously made in the pia), and pushed through it, until the upper side of the knife touched the pia-mater (Fig. 1b). Then, the cranial, the caudal and the deep sides of the piece of cortex were cut simultaneously, by moving the "window" knife towards the midline of the brain (Fig. 1b). When the required amount of cortex was cut, the knife was slowly pulled out until its cutting side came to touch the underface of the pia (Fig. 1b). Subsequently, the piece of cortex (covered by the pia) was lifted out and reclined towards the midline along the pial hinge, the knife removed (by doing the reverse of the movements described) and haemorrhages stopped (Figs. 1b, c).

In 51 cases microspheres (Sephadex G-25, Pharmacia Biotechnology International AB, Uppsala) were deposited on the cut surfaces of the explant, in an attempt to obtain a clear definition of its borders.

In 28 other cases crystals of rhodamine B isothiocyanate (British Drug Houses), were deposited inside the cavity created in the brain to mark the thalamic region projecting to the manipulated cortex.

Afterwards, the cut cortex was replaced to its original position and orientation. The cranium was closed by simply fitting the skull flap back in place. In some mice, a drop of Healon (sodium hyaluronate, Pharmacia) was deposited over the skull, before closing the skin. The skin was sutured with Ethilon monophyl 9/0 (Ethicon, Atralog) in younger animals; silk 5/0 was used in the others. After the operation each mouse was warmed up under a light bulb before being returned to the mother.

Survival Times and Preparation of the Brains for Histology

Operated mice survived up to ages ranging from P7 to

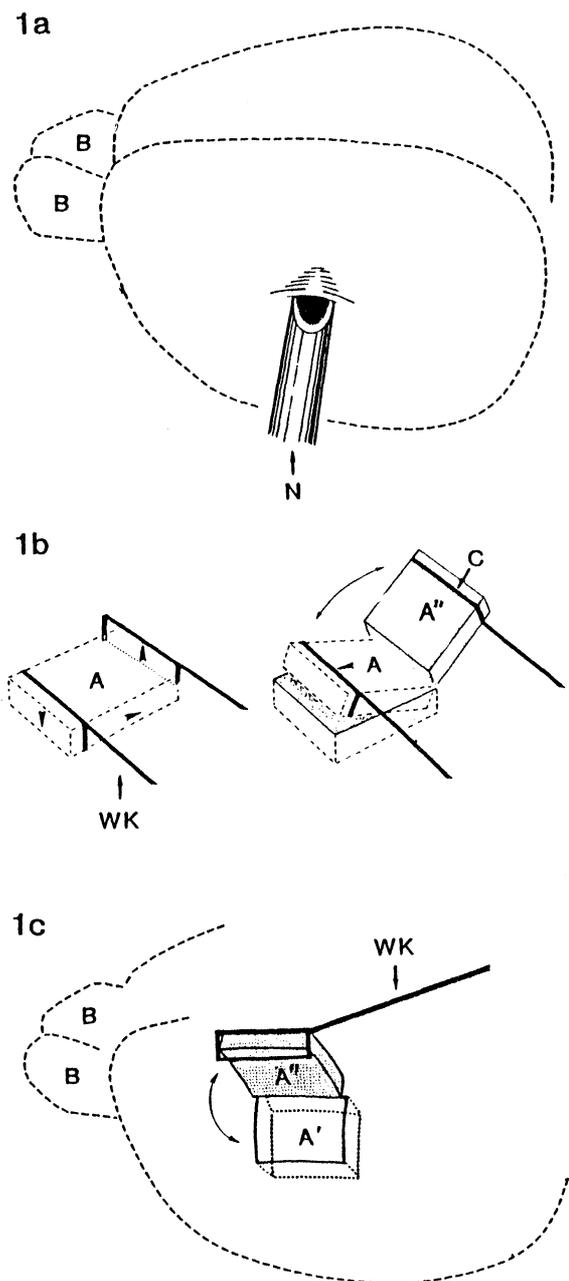


Fig. 1: Drawings showing some of the phases of the process of removing and reimplanting a piece of parietal neocortex: a) using an injection needle (this drawing shows a needle that has been slanted laterally, just before being pushed into the cortex to remove a piece of it); b) and c) using the "window" knife (profile indicated by the thicker line). Small arrow heads in b) indicate directions of movements to be made. Double arrows in b) and c) indicate movement of flap of neocortex when reclined towards the midline, along the pial hinge. A: dorsal surface of piece of parietal neocortex; A': cavity open in the brain after removal of A; A'': under surface of the reclined neocortex; B: olfactory bulb; C: cutting edge of window knife; N: injection needle; WK: window knife.

P75. Mice were anesthetized with pentobarbital (60 mg/kg body weight, i.p.) and perfused through the heart with 10% neutralized formalin in 0.9% NaCl. They were decapitated immediately afterwards, and the heads were left in the same fixative overnight. The brains were removed the next day and kept in the same fixative for 4 or 5 days. They were then transferred to a 20% solution of sucrose in phosphate buffer (0.1 M).

The brains were cut coronally from their frontal to

occipital pole (i 40 μ m thick sections) in a cryostat, at about -16°C . The serial sections were mounted on chrome-alum treated slides and stained with cresyl violet.

All sections were analyzed with the light microscope. The sections from the brains in which rhodamine had been deposited were examined with the fluorescence microscope.

CASE	AGE AT OPERATION	TYPE OF KNIFE USED	SURVIVAL TIME (DAYS)	LAMINATION	"BARRELFIELD"
1	P0	N	69		
3	P1	N	55		
5	P2	N	12		
6	P3	N	69		
8	P5	N	69		
10	P6	N	44		
14	P8	WK	10		
17	P9	N	75		
NON-OPERATED BRAIN					

Fig. 2: Presentation of barrelfields formed in reimplanted pieces of cortex, and aspects of cortical layers, in 8 cases. Left most column indicates case numbers. Right most column shows "barrelfields" obtained after computer processing. Cases 5 and 8 have two drawings because the middle regions of the reimplanted piece of cortex degenerated and the surviving fragments are separated by a gap. In the "barrelfields", as well as in the drawing of a normal barrelfield (non-operated brain): anterior is to the left; lateral is to the bottom of the picture. In the central columns are shown, from left to right: ages at which mice were operated, type of knife used to cut the piece of cortex (N for hypodermic needle; WK for "window" knife), survival time, and aspects of cortical layering for each age. The cortical layering is shown in inserts of photomicrographs of coronal sections, in which the borders of the reimplanted pieces of cortex are indicated by arrowheads.

Processing for 3-Dimensional Reconstructions

The computer processing consisted of three phases: (i) the borders of the piece of removed and reimplanted cortex, and the profiles of the barrel-like structures (from each histological section), were digitized by means of an interactive microscope and a graphics tablet /2,6/; (ii) the piece of removed and reimplanted cortex was reconstructed in 3-dimensions (Fig. 3a), by superposition of the corresponding (digitized) serial sections; (iii) the reconstructed piece of cortex was then rotated in space until the observer looked down on its pial surface, with an angle slightly smaller than 90° (Fig. 3b). This last image was printed on paper by a plotter and the profiles of its barrel-like structures were compared with those printed at the time of data acquisition (to be sure that none had been lost during the processing). Afterwards,

the domain of each barrel-like structure (as seen in tangential sections) was drawn on this printed paper (with the help of the corresponding coronal sections), by connecting (by hand) the profiles appearing in one section with those seen in adjacent sections (Fig. 3c).

Only when these drawings were completed, i.e., when all profiles found in neighboring sections had been connected, was it known whether they belonged to a barrel-like structure or not. This blind approach was useful because it reduced the impact of subjective factors during the phase of data acquisition, in the cases in which the profiles of the barrel-like structures were fragmentary and difficult to identify.

A barrelfield has also been reconstructed with the same technique, from coronal sections of an intact cerebral hemisphere, to be compared with those obtained from the operated cortices (Fig. 3d).

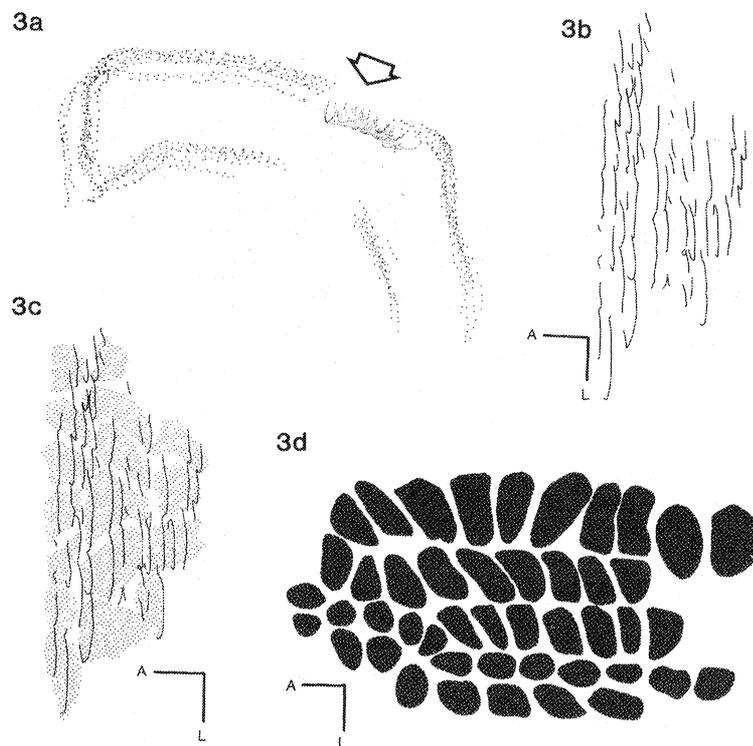


Fig. 3: a) Computer-generated image of 14 superimposed serial frontal sections corresponding to the region of the reimplanted piece of cortex of case 17 (shown in Fig. 2). Midline is to the left; anterior is towards the reader. Arrowhead indicates superimposed profiles of barrel-like structures. Dots indicate limits of the cortex in the corresponding frontal sections of the brain.
 b) Computer-generated image of the piled sections shown in a) after rotation in space. Lines represent profiles of barrel-like structures as seen from the side of the arrowhead in a), i.e., as if the brains had been cut tangentially. Anterior is to the left; lateral is to the bottom of the picture.
 c) Hand-generated image of the "barrelfield" of case 17. Barrel-like structures correspond to dotted spaces and are obtained by connecting corresponding profiles, sometimes incomplete, in adjacent sections. Anterior is to the left; lateral is to the bottom of the picture.
 d) Image of normal barrelfield reconstructed by the same method.

RESULTS

Cases Analyzed

The brains of 51 surviving mice, out of the 79 used in these experiments, were studied (Table 1). In the three cases operated at P1, with the razor blade, the manipulated cortex was not found. In 25 cases, out of the 65 operated at different ages with injection needles, the reimplanted cortex has been identified. In 4 out of the 11 cases operated with the window knife at P5, P6, and P8, the reimplanted pieces of cortex were found.

Rarely, two small fragments of the reimplanted cortex were found fixed in different places of the edge of the cortical wound. This explains why in some cases the reconstructed "barrelfields" (see cases 5 and 8 in Fig. 2) are divided.

In most cases the microspheres that had been deposited between the reimplanted cortex and the remainder of the brain were lost during the preparation of the brains for histology.

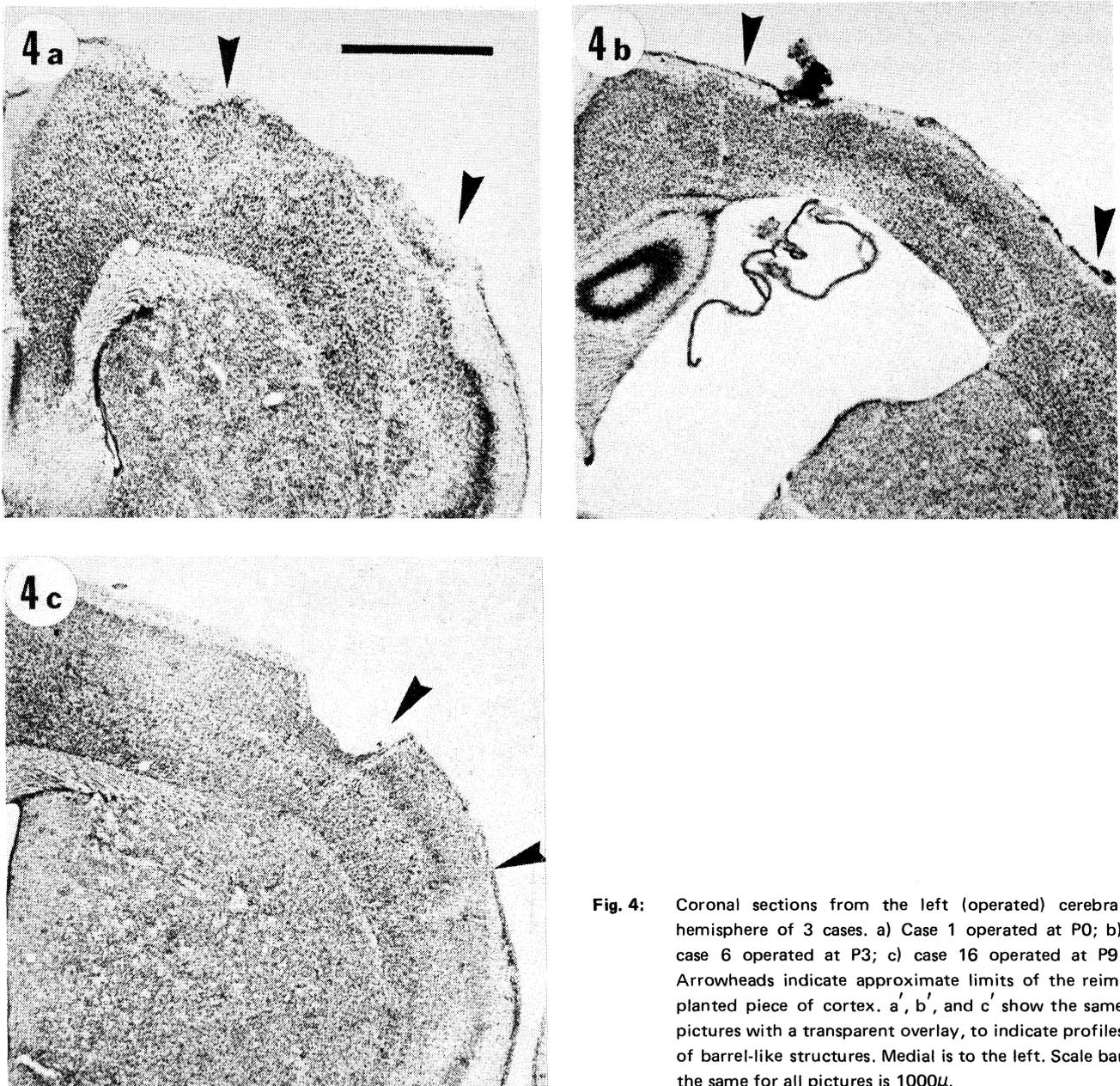
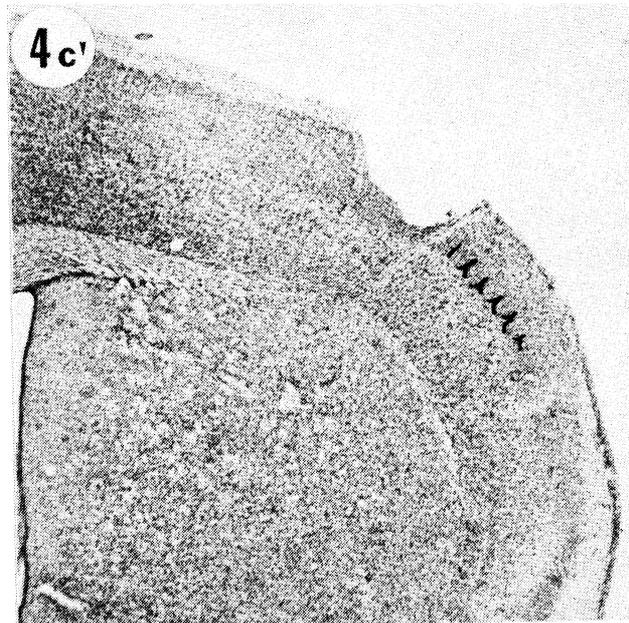
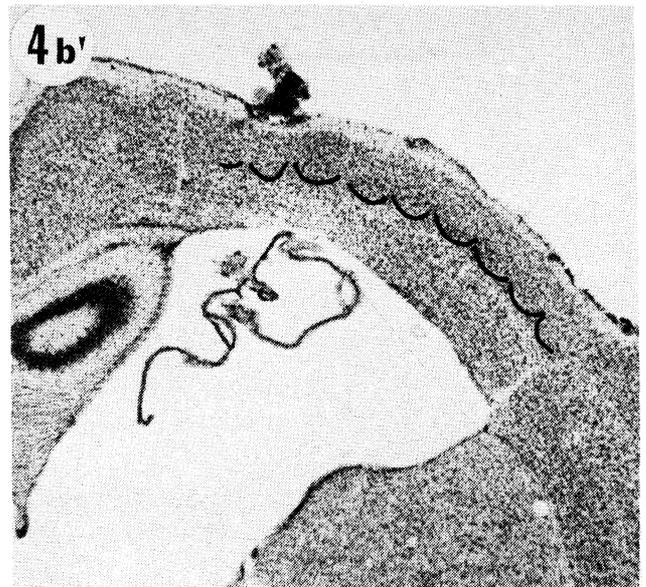
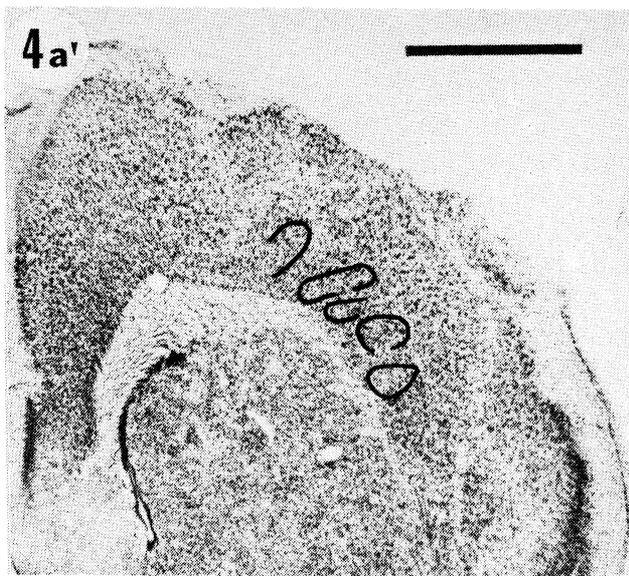


Fig. 4: Coronal sections from the left (operated) cerebral hemisphere of 3 cases. a) Case 1 operated at P0; b) case 6 operated at P3; c) case 16 operated at P9. Arrowheads indicate approximate limits of the reimplanted piece of cortex. a', b', and c' show the same pictures with a transparent overlay, to indicate profiles of barrel-like structures. Medial is to the left. Scale bar the same for all pictures is 1000 μ .



Cortical Thickness

In the cases in which the manipulated cortex was found, its thickness, as well as that of the adjoining cortex, was less than that of the corresponding parts in the intact cerebral hemisphere (compare Fig. 5a with 5c and 5d). But the overall thickness of the cortex away from the manipulated region was close to normal.

Lamination

The reimplanted pieces of cortex of cases operated at P0 and P1 showed no cellular layers (Fig. 4a). In case 5

(Table 1 and Fig. 2), operated at P2, a rudimentary layering is visible. In two cases operated at P3, the layers show a profile that is irregular and thinner than that of the non-operated side (Figs. 4b and 5c). In one case operated at P5 the infragranular layers are missing. After P5, cellular lamination is nearly normal (Fig. 4c), with the exception of case 13, operated at P6, which does not show any layering.

Lamination becomes more evident in cases operated at more advanced ages; however, in some of these cases the molecular layer is missing and layer VI is difficult to identify.

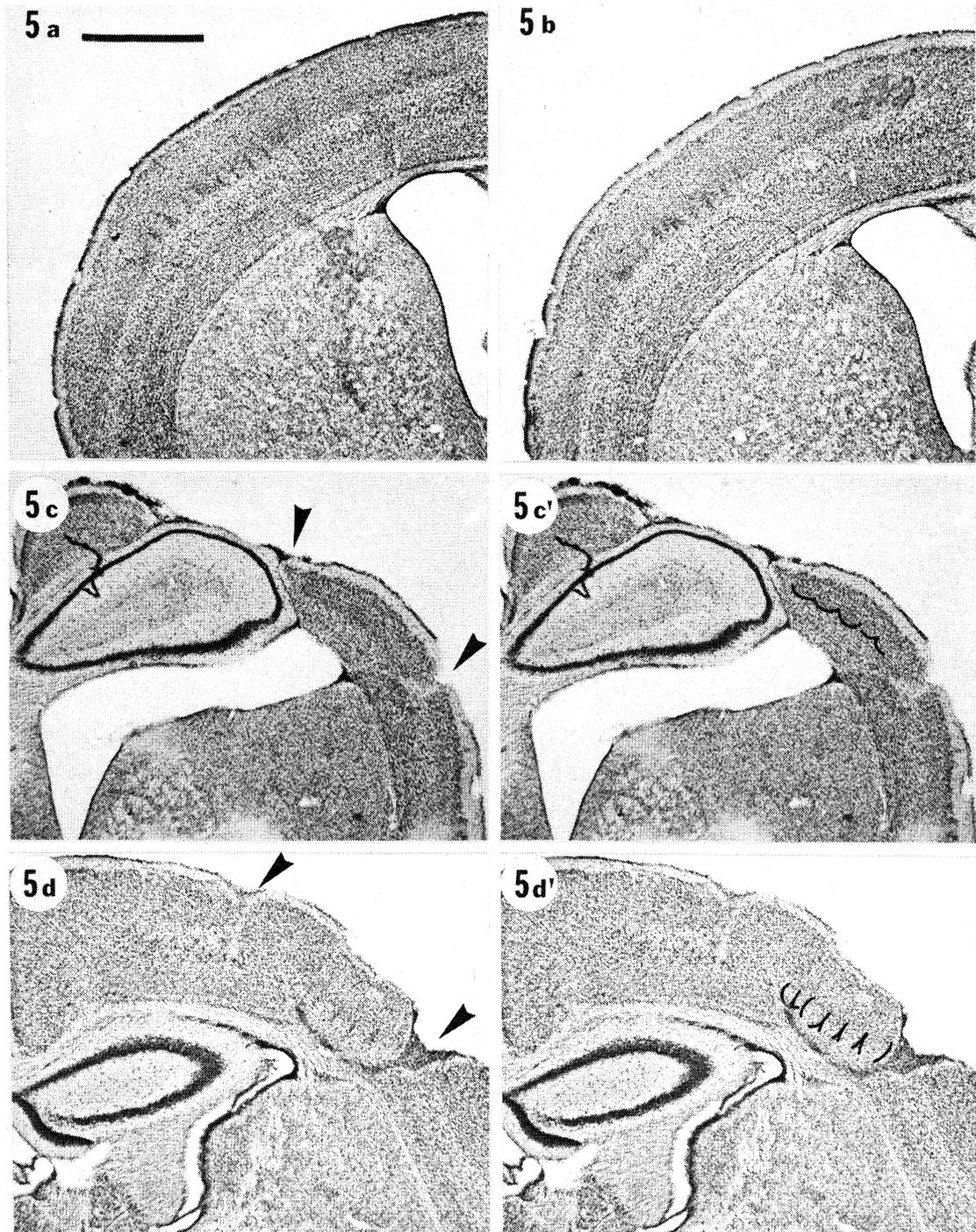


Fig. 5: Coronal sections from non-operated (a and b), and from operated cerebral hemispheres (c and d). In a and b: midline is to the right. In c and d: midline is to the left. Arrowheads in c and d indicate borders of reimplanted pieces of cortex. a) Profiles of normal barrels indicated by arrowheads are easily identifiable; b) profiles of normal barrels are difficult to identify in one section adjacent to that shown in a.; c) section through reimplanted piece of parietal cortex of case 7 operated at P3; in c' the same section is shown photographed with a transparent overlay to show profiles of barrel-like structures. d) Section through reimplanted piece of parietal cortex of case 14 operated at P8 (Fig. 2); in d', same section photographed with transparent overlay showing profiles of barrel-like structures. Scale bar the same for all pictures, is 1000μ .

Barrel-like Structures

In 14 out of the 65 cases operated with hypodermic needles, as well as in 3 out of the 11 cases operated with the "window" knife, there were barrel-like structures (see Table 1).

In the 17 cases in which barrel-like structures were found, the pieces of reimplanted cortex were reconstructed in 3-dimensions, with the computer, and the "barrelfields" drawn (cases 1-17, eight of which are represented in Fig. 2).

In cases operated up to P5, barrel-like structures are difficult to identify in coronal sections, owing to the high cellular density that exists in the hollows of those structures (i.e., their central parts). This is not observed (in the same sections) in the barrels of the contralateral non-operated side of the brain. Up to this age, the barrel-like structures also occupy different levels in the cortex.

Beyond P5 the identification of barrel-like structures is easier to make, and they are usually found in a region of the cortex that apparently corresponds of layer IV (Figs. 4c, 5c and 5d).

In two cases operated at P6, apparently normal barrels were visible outside the area of the reimplanted cortex.

All the 17 reconstructed pieces of reimplanted cortex contain "barrelfields". The majority of these "barrelfields" occupy an area that is apparently smaller than that occupied by a normal barrelfield, except in case 14. The number of barrel-like structures forming these "barrelfields" is also smaller than the number of barrels forming a normal barrelfield (Fig. 2).

When comparing a normal barrelfield with a field of barrel-like structures, it is impossible to determine the equivalence between individual barrels and barrel-like structures.

Ventrobasal Nucleus

The operations have produced variable degrees of degeneration in the ipsilateral ventrobasal nucleus and in adjacent thalamic nuclei.

In all cases operated up to P5, in two cases operated at P6, in one case operated at P8, and in two other cases operated at P9, the region of the ventrobasal nucleus is smaller on the side of the operation. In four other brains (three operated at P6, and one operated at P8) this nucleus appears to have about the same dimensions on both sides.

The rhodamine deposited under the reimplanted

cortices could be detected in the ventrobasal nucleus region of three cases. In only one of these, the manipulated piece of cortex was found, which did not contain any barrel-like structure.

The cases operated at P6 or later show an increased population of small dark cells in this nucleus. Since a specific staining has not been used, the nature of these cells is unknown, although they have features of oligodendrocytes.

Barreloids (i.e., the vibrissal follicle representatives in the ventrobasal nucleus of the thalamus, present themselves as "an array of rod-shaped domains, poor in perikaria, embedded in a lattice of high cell packing-density" /11/, were found in the ventrobasal nucleus ipsilateral to the operation in two brains operated at P2 and P3, in two operated at P6, and in two operated at P8 and P9. They were always visible on the non-operated side of the brains studied.

DISCUSSION

The experiments described here differ from those described in a previous chapter /1/, in the strain of mice used, in the ages at which operations were made, in the manner of cutting the pieces of parietal neocortex, in their dimensions (now smaller), in the manner of cutting the brains for histology (now coronally cut), and in the manner of reconstructing the barrelfields.

The results of the experiments now presented confirm the previous ones by showing that the reimplanted neocortex is capable of proceeding with its differentiation and/or of maintaining it, at least when the operations are made until P9, and the observations are made within the range of survival times used. This ability is manifested by the presence of barrel-like structures and "barrelfields" in all cases analyzed. However, in contrast to the previous ones, a homeomorphic representation of the periphery was not obtained. This is attributed to the smallness of the pieces of neocortex now cut.

Cortical Thickness

The cerebral cortex on the side of the operation was always thinner than that on the intact side.

Each operation in itself constitutes an important aggression to the still developing neocortex, aggression that leads to the necrosis and the elimination of a certain number of cells which would otherwise make part of it. Wise and Jones /12/, after making lesions in the ventrobasal nucleus of the thalamus of 1-day old rats, also found that the total thickness of the supragranular

and granular layers was significantly reduced. It is possible that in the cases presented here, the complete interruption of the connections between the thalamus and the neocortex has led to the development of a lesion in the thalamus (manifested by the variable degrees of degeneration found in the ipsilateral ventrobasal nucleus region), which in turn was later expressed at the level of the cortex by the presence of a less thick neopallium.

Lamination

The absence of layers, or the presence of an abnormal pattern of cellular lamination in the small pieces of parietal cortex of the cases operated up to P5, may be explained, at least in part, by the disruption of the pathways of neuroblast migration in the developing neocortex /4,8/, and by the peeling of the pia-mater produced in many cases /10/.

The existence of an apparently normal or quasi-normal lamination in cases operated after P5, suggests that the factors mentioned above may have played a less important role after the critical period of laminar differentiation had passed /9/; probably because most of the cells had already attained their definitive locations in the cerebral cortex. However, the absence of the molecular layer and the difficult identification of layer VI in a few of these cases, can still be attributed to the postoperative remodelling of the cortex, subsequent to the peeling of the pia-mater, and to the remodelling that certainly follows an undercutting of the cortex passing through layer VI, or very close to it.

Barrel-like Structures

The term "barrel-like structure" is used to describe the cellular arrangements found within the pieces of reimplanted cortex similar to barrels. The morphology of these structures resembles more that of barrels seen in coronal sections of normal brains of adult rats (in which the difference in cell density between the sides and the hollows is not marked), than that of the barrels of mice /9,14/. An explanation for the existence of a less clear definition of barrel-like structures may be the invasion of the cortex by a less than normal number of processes from thalamic cells, in cases in which barrels were not yet formed at the time of the operations, and to a certain degree of disorganization of those barrels that existed already at the time of the operations, due to the degeneration of the thalamic processes and subsequent remodelling.

Barrel-like structures, in cases operated up to P5,

occupy a level that apparently does not correspond to that of layer IV in the reimplanted pieces of cortex. They may, as in cases 1 (Fig. 4a), 3 and 8, be visible in a region apparently corresponding to layer V or VI. The presence of barrel-like structures in the deep part of the cortex may be ascribed to the persistence of transient thalamic projections to layers V or VI, established as early as gestational day 15 /5/. These projections would be reestablished after the operation and would remain thereafter. As Crandall and Caviness /5/ suggested, the cells of layer VI may constitute a "sufficient target for the expression of topological order".

In cases operated after P5, the barrel-like structures are less difficult to identify. This may be explained by the fact that barrels were already more or less formed at the time the operations were performed. In these cases, barrel-like structures occupy the middle third of the operated cortices.

It is not known whether the formation of barrel-like structures is due to the regeneration of surviving thalamic fibers, or to the ingrowth of new fibers that normally would have to invade the cortex after the time of the operations. It is possible that both processes took place in some of these mice.

In respect to the normal barrels found in the cortex adjacent to the implant, in two cases operated at P6 they most probably existed already at the time of the operations, since the formation of barrels begins earlier than P6. Their finding suggests that in those cases part of the barrelfield was left outside the manipulated piece of cortex (a small injection needle was used in those operations).

"Barrelfields"

The barrelfields now obtained are smaller than those obtained in a previous study /1/. This smallness can be attributed to the fact that now, the pieces of manipulated cortex were much smaller than before, and a portion of the territory where barrels were to develop was damaged by the operation and ensuing necrosis. Besides, the smallness of the viable cortex available to the ingrowing thalamic fibers may also have interfered with the development of morphologically normal barrels /7/.

The existence of a particularly large "barrelfield" in case 14 (Fig. 2) may be explained by the fact that the piece of reimplanted cortex, in this case, was relatively large (cut with the window knife), as compared with a piece cut with an hypodermic needle; also, it seems to have survived in its entirety. This "barrelfield" cannot be explained by the regeneration of, or by the growth of

new thalamocortical axons, because the survival time in this case was only of 10 days. Most probably it corresponds to the surviving parts of the barrelfield, which existed already at the time the operation was performed, i.e., at P8.

Correspondence Between "Barrelfield" and Whiskerpad

It is logical to ask whether the barrel-like structures found in the pieces of reimplanted parietal cortex, were functionally linked to the vibrissae of the mouse whiskerpad. For the present, I cannot answer this question.

There is already evidence that these structures may be functionally linked to the whiskers of the corresponding whiskerpad /12/. Wise and Jones have found that the presence of thalamocortical connections is essential for the formation of barrels.

Ventrobasal Nucleus

In normal cases, the ventrobasal nucleus of the thalamus shows barreloids (the thalamic representatives of contralateral vibrissal follicles in the muzzle). In rat, they become visible between P1 and P4 /3/. In mice, the time of their appearance has not yet been determined; however, since the development in mice is a little faster than in rats, it is possible that in the mouse, barreloids, or their primordia, exist already at P0.

The variable degree of degeneration observed in the region of the ventrobasal nucleus of the thalamus and in adjacent regions of operated animals, are probably linked to the extents of the lesions produced in the parietal cortex.

As in normal cases, one would expect to find barreloids in all the 17 cases in which "barrelfields" were found. However, barreloids were visible only in the six cases mentioned in the results. This discrepancy is difficult to explain. It cannot be attributed to a technical problem, such as a bad angle of cutting the brain sections, because barreloids were always visible (more or less clearly) in the non-operated side of the brains. A possible explanation is that barreloids, or their primordia, existed already at P0 (as suggested above), when the first operations were performed, and that they became disorganized afterwards.

CONCLUSIONS

The removal and reimplantation of small pieces of partial neocortex, made in mice of different ages from

birth up to P9, led to the following conclusions:

- 1) In cases in which the reimplanted cortex was found, its thickness was always smaller than that of the cortex in the intact side.
- 2) Cellular lamination in the manipulated pieces of cortex is normal or quasi-normal only in cases operated after P5.
- 3) Barrel-like structures can form and/or be maintained in the small pieces of cortex reimplanted at all ages up to P9.
- 4) The "barrelfields" now obtained in the pieces of reimplanted cortex are smaller than those obtained in a series of previous experiments. (This difference is attributed to the fact that now the pieces of manipulated cortex were smaller than before.)
- 5) Since barrels cannot form in the cortex without the presence of the corresponding barreloids in the thalamus /12/, the absence of the latter in most of the cases in which barrel-like structures were found in the cortex, must be attributed to their morphological disorganization after the operations.

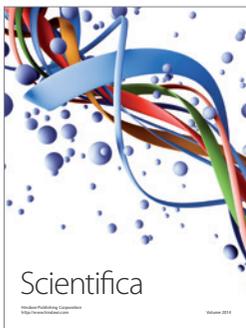
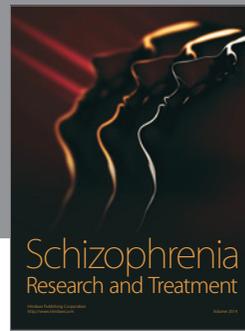
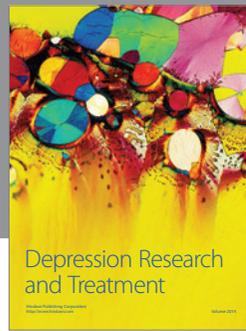
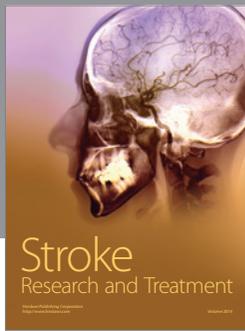
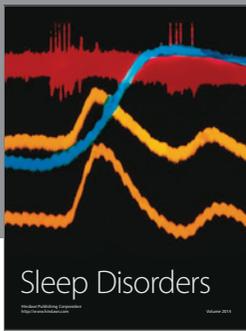
ACKNOWLEDGEMENTS

I thank Professor Van der Loos, P.G.H. Clarke, J. Dörfel, M. Gyger, P. Honegger, J.-P. Hornung and E. Welker for their critical reading of the manuscript; M.C. Cruz for help with histology; S. Daldoss and E. Brun for photography, and Ch. Vaclavik for typing. This study was supported by the Swiss National Science Foundation, grant number 3158.

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