THE STRUCTURAL ORGANIZATION OF LAYER IV IN THE
SOMATOSENSORY REGION (S I) OF MOUSE CEREBRAL CORTEX

THE DESCRIPTION OF A CORTICAL FIELD COMPOSED OF DISCRETE CYTOARCHITECTONIC UNITS* **

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INTRODUCTION

The mouse has a remarkable arrangement of cell bodies in layer IV within one area of its cerebral cortex. In Nissl preparations of sections made perpendicular to the pial surface the arrangement appears to be one of 'cellular columns' oriented perpendicularly to the pial surface and being as tall as layer IV itself; in sections made tangent to the pial surface, layer IV has the appearance of a 'cell-dense net'. Cortical evoked potential studies suggest that the area containing this cytoarchitectonic arrangement is coincidental with the somatosensory (S I) head-face region.

The structural peculiarities of the region, demonstrated by Nissl's method, were noted or illustrated by three authors early in this century, but not until 1922 had anyone proposed a structural basis for the appearance of this unusual portion of cortex. In that year, Lorente de Nó using primarily Golgi-impregnated material described glomérulos in the area and was able to identify at least seven cortical cell types associated with them. He demonstrated afferents from below with rich arborizations associated specifically with the glomérulos. In 1929, Rose used Nissl-stained coronal sections paying special attention to the appearance of layer IV to define an area, T1, with which our own later delineation of the field exhibiting the 'columnar' and the 'cell-dense net' arrangement agreed.

The present study was undertaken to more clearly define the structural organization of layer IV in this S I region of the mouse cerebral cortex. The results will be presented in three parts.

In Part I, sections made in a plane tangent to the pia mater — 'tangential sections' — and sections made in a coronal plane — 'coronal sections' — stained with Nissl or Golgi-Nissl methods, are used to clarify the cytoarchitectonic organization of the area. The resulting morphological concept is one of a multicellular cytoarchitectonic unit — ranging from about 100 μm to about 400 μm in diameter — which we termed 'barrel'. Measures of barrels and their parts are made. The anatomical concept of the barrel is discussed with emphasis on correlation with earlier observations made by other workers and by ourselves. Some questions are raised about the possible cellular constituents and connections of the barrels.

In Part II, the recognition of barrels in Nissl-stained tangential sections is used in an attempt to define the precise position, extent and area of the field containing the barrels, the number of barrels in the field, and the constancy of shape and of arrangement of barrels from one hemisphere to the other and from one mouse to another. Briefly, the possible significance of these findings is discussed in relatiion to the several cytoarchitectonic maps of the mouse cerebral cortex in relation to what is known about the physiological organization of the cerebral cortex of the mouse and the rat.

In Part III, we discuss a subfield — the posteromedial barrel subfield (PMBSF) — in which the shape, size, organization and number of the barrels is particularly clear and constant. On the basis of the suggestive similarity between the arrangements of mystacial vibrissae on the muzzle and of PMBSF barrels in the cortex, and on the basis of the evoked potential maps in the mouse and of recent microelectrode studies.

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of rat cortex it is proposed that barrels in the PMBSF are the cortical correlates of the mystacial vibrissae — more specifically, that one barrel represents one vibrissa.

In a general discussion it is suggested that *barrels* are the morphological manifestation in layer IV of the *functional cortical columns* described by neurophysiologists.

**MATERIAL AND HISTOLOGIC METHODS**

*Animals.* Ten adult C57 mice* and one adult Swiss mouse** were used. Weights ranged between 27 and 34.5 g. Ten were males.

*Fixation.* (a) Formalin (7 specimens). Animals were anesthetized with pentobarbital-sodium and briefly perfused through the heart with 0.9% saline solution at room temperature. Perfusion was continued with 10% formalin, which had been neutralized over Ca(CO₃)₂, in 0.9% saline. The brains were removed several days after perfusion and kept in the 10% formalin solution for about three weeks prior to dehydration and embedding. (b) Golgi-Cox (4 specimens). Animals were anesthetized and allowed to die by exsanguination. The fresh brains were removed immediately, care being taken not to touch the cortical surface, and were placed in a Golgi-Cox solution for 30 days prior to dehydration and embedding.

*Dehydration and embedding.* All tissues were dehydrated in a graded ethanol series and embedded in Ceducol. § §

*Sectioning.* Twelve hemispheres sectioned tangentially were cut at 100 μm, 70 μm or 50 μm. Six hemispheres sectioned coronally were cut at either 50 μm or 30 μm.

*Staining.* The formalin-fixed sections were stained with methylene blue-Cl. Cox-fixed preparations were treated to reveal the impregnated cells and counterstained with methylene blue-Cl by a recent modification of the combined Golgi-Nissl method.

*Data collection and reduction.* Methods of data collection and reduction are presented with related observations.

**PART I. A MULTI-CELLULAR CORTICAL UNIT: THE BARREL**

*Observations*

*A. General appearance*

In layer IV there are multi-cellular cytoarchitectonic units which because of their three-dimensional character we choose to call *barrels*. The first four photomicrographs (Figs. 1–4) illustrate the barrels and their component parts.

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* Obtained from the Roscoe B. Jackson Laboratories, Bar Harbor, Me. 04609.
** Obtained from Hazleton–Carbia, Inc., Burtonsville, Md. 20730.
§ Ceducol is a purified collodion wool manufactured by Merck, Darmstadt, W. Germany (U.S. agents: Brinkman Instruments, Inc., Westbury, N.Y. 11590).
§ § After embedment, brains had shrunk to exactly 80% (linear) of the size they had while the animals were still alive, or dying from the effects of anesthesia and complete calvarium removal. This percentage is an average based upon measurement of three distances, made before and after shrinkage, between pairs of standard points in each of four animals.

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Fig. 1. Photomicrograph of a tangential section of layer IV in mouse S I cortex: the anterior part of barrel field (see lower inset to Fig. 10 for location; quadrangle 1). Illustration serves to show barrel components: each barrel (B) is made up of a ring of high cell density, the side (stippling), which surrounds a less cellular area, the hollow (h). A septum (arrowhead) separates adjacent barrels; septal junctions (..) occur where three or more septa meet. A wall (between hollow arrows) consists of the sides of two adjoining barrels and the intervening septum. Notice that barrel profiles are all about 100 μm in diameter and that they are roughly circular. Formalin fixation, methylene blue-Cl, 50 μm thick section. Bar = 100 μm.

Fig. 2. Photomicrograph of a tangential section of layer IV, taken from the posteromedial barrel subfield (PMBSF) (see lower inset to Fig. 10 for location; quadrangle 2). Illustration serves to show barrels and their components. Symbols are the same as those used in Fig. 1. Notice that the general features are the same as those shown in Fig. 1, but that the barrels are (a) bigger, (b) roughly elliptical, and (c) separated by septa perpendicular to the short barrel axis that are about the same width as septa in the anterior barrel field, and by much wider septa perpendicular to the long axis. The broken line shown at edges indicates the approximate perpendicular intersection with the section depicted in Fig. 4. Approximate orientation of section shown in Fig. 2 is indicated by line drawn at edges of Fig. 4. Formalin fixation, methylene blue-Cl, 50 μm thick section. Bar = 100 μm.
Fig. 3. Photomicrograph of a tangential section of layer IV, taken from the PMBSF. Symbols are the same as those used in Fig. 1. This is a Cox-fixed, Golgi-Nissl-stained preparation which shows the unusual staining property of the hollows (light stipple) which sometimes is obtained by this method. Notice that septa stand out. 70 μm thick section. Bar = 100 μm.

(1) **Appearance in tangential sections.** Fig. 1 which is taken from the antero-lateral part of the cortical field in which barrels occur and Fig. 2 which is taken from the posteromedial part, show the basic plan. (See Fig. 10 for location of Figs. 1 and 2 in the barrel field.) In both figures the plane of the section coincides with the horizontal plane of layer IV.

Each unit shows a dense ring of cell bodies which has, roughly, the shape of a circle or an ellipsoid. This ring represents the side of the barrel and surrounds an area of lesser cell density which we name the hollow. Each barrel is separated from its neighbors by a clear, nearly acellular area (fewer cells than in the hollow) which is the septum. In properly oriented sections, a septum can be seen to separate a barrel from its neighbors. The point at which three or more septa join is the septal junction. That the barrel is a unit, side and hollow inclusive, is emphasized in Fig. 3 in which the peculiar staining property that these units sometimes exhibit in the combined Golgi-Nissl method is demonstrated. In this preparation a substance in the barrel which is absent from septa and adjacent layers stains light blue; the septa in this preparation stand out very clearly.

Under some conditions such as low power microscopy, oblique orientation of sides in the section, or very thick sections, the septum between two barrels cannot be visualized; the two sides blend together. We call the apparent structure produced by the seeming disappearance of the septum, the wall. A wall always includes the adjacent sides of two neighboring barrels and the intervening septum even though these finer details may not readily be appreciated. The overall pattern of walls produced by many barrels crammed together is one of a cell dense net (see Fig. 10).

(2) **Appearance in coronal sections.** Fig. 4 is a coronal section through the posterior portion of the area showing the appearance of barrels in a plane perpendicular to the pia mater. Because of geometric probabilities the septa are less clearly
Fig. 4. Photomicrograph of a coronal section through the PMBSF. Symbols are same as those used in Fig. 1. Roman numerals indicate cortical layers. P = pia. Notice how sides diverge near ‘mid-height’ of each barrel to make barrel barrel-shaped. Note that the septa are somewhat less easily distinguishable here than in Figs. 1 and 2. The line shown at edges indicates approximate perpendicular intersection with the section depicted in Fig. 2. Approximate orientation of section of Fig. 4 is indicated by line drawn at edges of Fig. 2. Formalin fixation, methylene blue-CI, 30 μm thick section. Bar = 100 μm.

seen in this kind of preparation. Commonly sides with intervening septum blend to form a wall. Nevertheless, sides and septa may be appreciated in portions of the section shown. Hollows stand out well. A geometric detail frequently observed in coronal sections is that barrels are tapered at both top and bottom. Consequently, septa are wider at upper and lower ends of layer IV than they are at ‘mid-height’.

B. Barrel dimensions

(1) Measurement of components. Measurement of barrels and their component parts was undertaken to ascertain approximate sizes and to obtain some idea of size variations within the barrel field itself. These measurements were made directly in the microscope using a 40 × objective, a 12 × ocular, and an ocular reticle (unit of measurement 2.4 μm). Formalin-fixed tangentially cut Nissl-stained material was used. Barrel and hollow diameters were obtained by orienting the reticle scale along the long axis of the barrel thus measuring its major axis (a), and by measuring the minor axis (b) at the midpoint of a (see also next section). Thickness measurements of walls, sides and septa were made by orienting the reticle perpendicularly to the wall at a point.
TABLE I
RANGES OF DIMENSIONS OF BARRELS AND BARREL COMPONENTS*

<table>
<thead>
<tr>
<th>Barrel component**</th>
<th>Tangential sections</th>
<th>Coronal sections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior (μm)</td>
<td>PMBSF (μm)</td>
</tr>
<tr>
<td>Barrel (axes)***</td>
<td>150 -70</td>
<td>300 -70</td>
</tr>
<tr>
<td>Hollow (axes)***</td>
<td>120 -60</td>
<td>290 -60</td>
</tr>
<tr>
<td>Side</td>
<td>10 - 5</td>
<td>12.5- 7.5</td>
</tr>
<tr>
<td>Septum</td>
<td>7.5- 2.5</td>
<td>20 – 2.5</td>
</tr>
<tr>
<td>Wall</td>
<td>20 -10</td>
<td>40 -15</td>
</tr>
</tbody>
</table>

* Uncorrected for shrinkage due to fixation. Larger values rounded off to 10 μm; smaller values rounded off to 2.5 μm.
** Approximately 15 measurements were made to determine each range.
*** Ranges presented for tangential sections include both major and minor axes.

where sides and septum were clearly visible. Measurements were made from sections taken from several hemispheres. The sections were chosen for optimal orientation — i.e., precisely tangential to the anterior and posterior parts of the field, respectively. For reasons given below (Part III), the measurements from PMBSF are segregated from those of the anterior part of the barrel field. Measurements were also made from coronal sections which were taken from the PMBSF because, when sections are cut perpendicularly to the pial surface, the barrel components appear clearer in the PMBSF.

In spite of possible differences in fixation, shrinkage, animal size, etc., there was good correspondence in the ranges of measurements obtained from sections of different specimens. The ranges of dimensions are summarized in Table I. (As our measurements are preliminary and few, we have elected to give ranges rather than more sophisticated statistical indices.)

As would be expected from inspection of the barrel field as a whole (Figs. 10, 11, 14), major axes of barrels (a) are greater in PMBSF than in the anterior part of the field*. In PMBSF, minor axes of barrels are similar in length to those measured in the anterior part of the field. Major barrel axes in PMBSF differ greatly from minor axes, whereas in the anterior part of the field they are of the same order of magnitude. Since the coronal sections are cut approximately along the minor axes (b) of barrels in PMBSF, one would expect the measurements in the coronal sections to be in the range of anterior barrel diameters and indeed they are. The ranges of barrel axes measured directly on the specimen (shown in Table I) are comparable with the ranges of barrel axes determined on photomicrographs of two whole fields. Measured on the

* We have chosen to speak of posteromedial barrel subfield (PMBSF) as a specific region of the barrel field for (1) strictly morphological reasons (see Part III), and (2) a likely functional correlation. We have refrained from naming the rest of the field in the absence of (1) distinct morphological criteria, and (2) a clearer understanding of the functional relationships of barrels there. More sophisticated studies will probably allow future parcelation of the 'anterior part of the barrel field'. A naming would only be complicated by a pre-existing less rational terminology.

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micrographs, the dimensions \(a\) and \(b\) of the largest barrel are 380 \(\mu m\) and 170 \(\mu m\), respectively, and of the smallest barrel 100 \(\mu m\) and 50 \(\mu m\), respectively (Fig. 5).

Because a hollow only lacks the thickness of two sides (about 5 \(\mu m\) each), one may expect that the range of hollow diameters will be about 10 \(\mu m\) less than that of the barrel diameters. Sides in all areas are about the same thickness. They are usually only slightly thicker than the average diameter of a single cell body that lies within them, since sides are composed of cells stacked not quite exactly one on top of the other. The width of the wall fluctuates mainly with the width of the septum. In the anterior barrel field, and in PMBSF (perpendicular to the minor axes \(b\)) septa are commonly very narrow — less than 5 \(\mu m\). These septa measure less than the diameter of most of the cell bodies seen in layer IV. In the PMBSF, the septa perpendicular to the major axis \(a\) are much wider — up to 20 \(\mu m\). The measurements were not made in the septal junctions. The discrepancies noted when measured and derived data are compared (e.g., barrel = hollow + 2 sides; wall = septum + 2 sides) are no doubt due to the smallness of the sample.

The thickness of layer IV as a whole, as measured in the coronal sections, is about 100 \(\mu m\) throughout the barrel field.

(2) Distribution of barrels according to relative size. The sizes of all barrels in two complete barrel fields were measured and plotted.

Measurements were made from the photomicrographs shown in Figs. 10 and 11. We have made the assumption that, in tangential sections, barrels are roughly either elliptical or circular and that their relative sizes can better be expressed by a two-dimensional parameter related to area, rather than by a one-dimensional parameter, e.g., a single axis. The measurements were made in the following manner: (1) a rule was moved about on the photograph until the maximum barrel axis \(a\) was found; (2) the midpoint of \(a\) was determined; and (3), at this midpoint, the axis \(b\) perpendicular to \(a\) was measured. The product, \(ab\), is used as the index of relative barrel size. The expression is a function of area; it is also an approximate measure of volume, the height of layer IV being nearly the same throughout the barrel field.

In Fig. 5, we have indicated the population distributions of the products \(ab\) of the two barrel fields. Again we have chosen to plot the data from the PMBSF's separately. There is a striking similarity in the frequency distributions from the two fields with the exception that the values for 9L are approximately one bin greater than those for 10L (see Discussion Part I). Notice that the barrels not in the PMBSF seem to have a normal distribution in both specimens. All larger barrels are from the PMBSF.

Discussion

A. Review of previous terminology

There have been observations in the literature on mouse neocortex which are related to the concept of the barrel. The literature review which follows is summarized in Table II. The translations into English are our own. The original terms are placed in the table (in all cases we used the authors’ own phrases).

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De Vries\textsuperscript{12,13} described ‘small islands’, or ‘small elliptical, or square fields’ in layer IV of his field H\textsuperscript{2}. A likely explanation of his observation is that his technique, toluidine blue stain after fixation in alcohol, stained the barrels. Although his technique is different from our own, De Vries shows (ref. 13, Fig. 10) a drawing in which these fields closely resemble the appearance of barrels in many of our Golgi–Nissl preparations. (De Vries’ whole field H, characterized by a thick, intensely staining layer IV, corresponds to our barrel field. H\textsuperscript{2} closely corresponds to our PMBSF!)

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TABLE II

COMPARISON OF BARREL-RELATED TERMS USED IN PRECEDING STUDIES, WITH DEFINITIONS USED BY US

<table>
<thead>
<tr>
<th>Author</th>
<th>Terms used by author</th>
<th>‘Equivalent’ used by us</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Vries 12,*</td>
<td>kleine Felder von elliptischer oder quadratricher Form; Inselchen</td>
<td>barrels (or, perhaps, only hollows?)</td>
</tr>
<tr>
<td>Droogleever Fortuyn 14,**</td>
<td>clouds; deeply stained spots narrow strips with . . . light tint</td>
<td>barrels</td>
</tr>
<tr>
<td>Lorente de Nö 31</td>
<td>glomérulo; pléyade segmentos claros (de forma cuadrada) columns (verticales)</td>
<td>barrel</td>
</tr>
<tr>
<td>In Nissl-image</td>
<td>massas celulares bandas claras (verticales) espacio interglomerular</td>
<td>hollows</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>knüeluartige Anordnung der Kornerzellen</td>
<td>walls</td>
</tr>
<tr>
<td>Rose 47</td>
<td>kniuelartige Anordnung der Kornerzellen</td>
<td>walls</td>
</tr>
<tr>
<td>Van Erp Taalman Kip 54,***</td>
<td>clouds cell-dense columns; clumps cell-dense net; network holes; ‘clear’ spaces</td>
<td>hollows</td>
</tr>
<tr>
<td>Woolsey 68</td>
<td></td>
<td>walls</td>
</tr>
<tr>
<td>Labedsky and Lierse 30</td>
<td>Nester</td>
<td>barrels (or, perhaps, only hollows?)</td>
</tr>
</tbody>
</table>

* A German version appeared one year later 13.
** An English version of this work was published three years later 15.
*** An English version of this work was published one year later 3.

Droogleever Fortuyn 14,15 noticed ‘deeply stained spots’ or ‘clouds’ in layer IV of his area j. Although he does not provide pictures of them in either report, we must conclude from his careful description, that he was staining entire barrels. His method, we believe, led to preparations which appeared similar to the one depicted in our Fig. 3. He assumed that the ‘extraordinarily deep stain’ was due to a high density of fibers; it could be obtained only after formalin fixation (which was started ‘. . . in every case within 24 hours . . .’ after death of the animal). Droogleever Fortuyn also measured his clouds; they were ‘1/5-1/10 mm in diameter*. He remarked: ‘They are separated by extremely narrow strips with the usual light tint of the background’ and concluded that in this ‘dimorphous’ cortex these ‘narrow strips’ (our septa) form the continuous phase whereas the ‘clouds’ (our barrels) are separate entities. Apparently, Droogleever Fortuyn did not distinguish hollow from side.

Two papers of Rose are pertinent to this discussion. In the first 46 ‘walls’ are depicted although no mention is made of them.

Lorente de Nö 31 recognized the ‘small islands’ of De Vries, or ‘clouds’ of Droogleever Fortuyn. He thoroughly analyzed them, in both Nissl- and Golgi-preparations.

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As stated above, he described these structural entities as *glomérulos*. The reader is referred to Table II for an item by item comparison of Lorente de Nó's nomenclature with that presented by us.

Rose, in a second paper — a classic atlas of the mouse cortex
describes, in layer IV of his area T1, a 'clew-like arrangement of cells' (clew = ball of yarn) which probably refers to the pattern suggested by walls when seen en face in coronal sections. In one of his plates (ref. 47, plate 10, in area T1, at label 'K') some of his walls were optimally cut so that they are shown to be comprised of two sides separated by a septum! In another illustration (plate 11), a typical 'clew-like arrangement' is seen. In view of the distinction we make between PMBSF (with large barrels) and anterior part of the barrel field (with small barrels), it is interesting to note that Rose, in the section by section account of mouse cortex, writes that the clew-like arrangement is more prominent in the posterior sections through T1 than in the anterior section!

Van Erp Taalman Kip used Droogleever Fortuyn's field j — in the course of a comparative, quantitative investigation of the neocortex of a small group of mammals — partly because its clouds made recognition of the field in some of the animals used relatively easy. For him, clouds were 'lighter patches with less cells'; his clouds, we believe, were hollows.

The cell-dense columns and the cell-dense net proposed in a more recent description of the barrel field can now be equated with walls and with the wall pattern as revealed in tangential sections. To the favorably disposed eye, the photomicrographs (ref. 68, Figs. 5, 6, 7) demonstrate the component parts of the wall: sides and septum. On re-examination of the slides from which these figures were taken, all components described in the present paper can be seen, although not so clearly as in the presently described material.

Labedsky and Lierse have stained mouse cortex for succinic dehydrogenase (SDH). They describe loci in layer IV with increased SDH activity in a region which they identify as Rose's area T1 (which we, in turn, identify as the barrel field). In the photomicrographs accompanying their report, these loci look somewhat similar to the barrels of some of our Golgi–Nissl stained material (see Fig. 3). We had previously suggested, on the basis of similar SDH positive loci reported by Friedel in the guinea pig and the rat, that what we now call hollows in mouse cortex might be centers for increased SDH activity. The dimensions of the SDH positive loci in Labedsky and Lierse's paper suggest that the barrels, or perhaps the hollows, are the areas of elevated SDH activity. A counterstaining method demonstrating perikarya would be necessary to confirm this impression.

**B. Dimensions**

When measuring barrel diameters, at least two kinds of artifacts may be involved. The first is the result of making tangential sections which are not exactly parallel to the pia. Since the plane of layer IV is curved, the plane of section in a series of sections from one specimen is never tangential for all of the barrels measured. If we assume that in a perfectly tangential section the cross-section of a barrel is
circular, it follows that a non-tangential section will intercept the barrel obliquely and result in an elliptical profile. In general, the cross-sectional area of a barrel will be greater the more oblique the plane of intersection. Consequently, the barrel areas will be systematically greater than they would have been had it been possible to derive the data from sections exactly tangential for every barrel. Secondly, since the barrel is tapered at top and bottom its greatest ("true") diameter in the exact tangential plane can only be obtained when the "mid-height" region of the barrel is included in the section. This does not cause a serious problem if the section includes all, or almost all, of layer IV — such as in specimen 9L (see Fig. 11) where the sections are 100 μm — since one will see the widest portion of the barrel. But if one takes thinner sections — 50 μm, as in 10L — one frequently measures the tapered diameter at either the top or the bottom rather than the widest, "mid-height" diameter. The effect of this second bias is opposite to the first, in that it yields cross-sectional barrel areas which are systematically less than the maximum tangential barrel area.

Both of these effects may be at play in producing the slight but definite discrepancy in the population distributions shown in Fig. 5. It is difficult to say which one of the two effects has the greater influence. Of course, the differences may also be due to something cruder in nature, such as a fixation artifact or simply variation in brain size, rather than to these geometric subtleties.

C. Bruegel's barrel

We shall now consider several questions which the barrel concept raises.

First, do sheets of cell bodies separate hollows from adjacent cortical layers: in other words, do the barrels have tops and bottoms? Tops — that is, a sheet of cells separating the hollow from layer III — are somewhat evident in coronal sections (Fig. 4) although they certainly are not striking. As one approaches layer IV from the pia in tangential sections, patches of greater cell density foreshadow the appearance of subjacent hollows (see Fig. 9). The absence of similar dense zones separating hollows from layer V suggests that there are no bottoms. This point awaits careful analysis of perikaryal arrangements in and about individual barrels.

Second, what may be the cellular make-up of the barrels? High magnification observations with the light microscope suggest that both neurons and neuroglia comprise the cellular populations of sides and hollows. We are presently looking at Golgi and Golgi-Nissl preparations to answer some questions about the types of neurons found in the barrels, and plan to apply glia stains.

Third, what are the synaptic contacts of the various neuronal elements of these multi-cellular cortical units? Lorente de Nó 31 has supplied data which suggest that barrel neurons have intimately entangled axonal and/or dendritic fields confined to a barrel and that there are cells in adjoining cortical layers and in remoter areas of the brain (thalamus!) which have projections arborizing specifically within barrels. Certainly, it is likely that there is exchange of information between neurons in a given barrel but the specific nature of such exchange as well as its anatomical substrate has yet to be determined. Proof that loci of high SDH activity found by Labedsky and Lierse 30 are identical to barrels or hollows would be a further contribution to

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the hypothesis that barrels have a high synaptic density, since it has been shown in
cerebellar glomeruli that there is a direct correlation between SDH activity and
density of synapses identified by electron microscopy. In Part III, part of this question
is considered in relation to recent physiological data and formulation.

In concluding Part I, we should like to emphasize the three-dimensional struc-
ture of the multi-cellular unit we have described (see Figs. 2 and 4). Fig. 6 is a re-
production of a small part of an etching made of (the elder) Bruegel's now dis-
appeared work 'The Fair of St. George's Day'. This 17th century representation of
the barrel is a geometrically adequate rendition of the unit. The cortical barrel may
have undergone some distortions from Bruegel's model in various places in the field
but the basic elements, sides and hollows, are shared by all. The field as a whole —
discussed in Part II — may be thought of as being composed of many barrels placed
tightly side by side much as, in Bruegelian dimensions, might happen at a brewery.

PART II. THE BARREL FIELD

Observations

A. Position of barrel field

(1) Determination of reference planes. Early in this study it became apparent
that a system was needed which would enable us to exactly describe the location
of the barrel field in respect to the whole brain. In the absence of obvious and constant
surface landmarks in the lissencephalic mouse brain, it is difficult to locate a point
to which tangential sections can be made. The reference planes described here
comprise a useful system by which to orient three-dimensionally the plane con-
taining the barrel field to the whole brain. We defined three reference planes (Figs.
7A, B) onto which the barrel field was projected and to which the predominant ori-
entation of the field was related by known angles (see Fig. 7). By holding the specimen
so that the microtome knife passes in the plane of that predominant orientation,
\textit{i.e.}, the 'best' plane (see below), it is possible to obtain, in only a few 50 or 100 \(\mu\)m
sections, the entire barrel field (see Figs. 7C, D). That 'best' plane can be described
by its relations to any two of the three reference planes. In Fig. 7 we have drawn

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Fig. 7. Drawings to show the barrel field and its relationship to three reference planes and three standard projection views of the mouse brain. A, The three standard projection views of the brain: anterior, dorsal and lateral. Lines indicate reference planes: sagittal (S), horizontal (H) and coronal (C). Lateral projection view shows 'high points', x and y, of the dorsal aspect of the hemisphere used to determine the horizontal plane (H). B, Drawing of whole mouse brain showing spatial relations of reference planes to specimen. Planes bear same labels as in A. C, Same projection views as in A to which are now added the location of the barrel field (hatched area). Angles drawn in the anterior and lateral projection views (interrupted lines) are those used together in the coronal and sagittal planes, respectively, to obtain the 'best' plane of section (T) when the barrel field is to be approached tangentially. D, Whole specimen with reference planes (S, H, E), barrel field (hatched), and 'best' plane of tangential section (T-darkly stippled) placed according to the angles given in C.

our reference planes in relation to a specimen and shown the relation of the 'best' tangential plane to the whole brain and to the reference planes. Details of the placement of these planes are given in the legend to Fig. 7. The importance of specimen orientation for taking sections that will optimally demonstrate the barrels cannot be overemphasized.

(2) Projection of the barrel field onto the cortical surface. Figs. 7C and D, and the upper insets to Figs. 10 and 11, show the surface projections of the barrel field. The long axis of the field deviates from the sagittal plane so that the anterior portion is more lateral than the posterior. Also, the field follows the rather sharp lateral curvature of the hemisphere so that the plane of the field is domed. However, most of the barrel field is present on the gently arcing dorsal surface of the hemisphere. (A happy consequence of this is that only one or two 100 μm sections oriented tangenti-
B. Boundaries of the barrel field

(1) Description of the boundaries. The general shape of the field can be appreciated from Figs. 8, 10, 11 and 14. Three parts of the perimeter are of interest here: posterior, anterior and a 'notch' midway along the medial border.

The PMBSF has been segregated from the rest of the barrel field in some of the analyses in Part I. Its boundaries are among the characteristics that help set it apart. They are always sharp and without ambiguity. The boundaries of PMBSF form the posterior, posteromedial and posterolateral borders of the total barrel field.

The anterior borders are not clear. There is always doubt where the barrels stop and the homogeneous layer IV begins. Fig. 8 illustrates this point.

Midway along the medial border there is a gap in the continuity of the field. This 'notch' in which no barrels are found indents the field for about 1000 μm; it is about 200 μm wide and borders the PMBSF anteromedially. Extending anterolaterally, it halfway bisects the field. A possible cause of this notch could have been the slight indentation of the cortex under the frontoparietal suture. The indentation of the cortex at this point would take layer IV out of a section in which barrels are seen anterior and posterior to the notch, but would place layer IV in subsequent, deeper, sections. We have looked and did not see any barrels and therefore conclude that the gap in the continuity of the field is real.

(2) Two difficulties encountered in boundary determination. The presence of barrels makes identification of the barrel field simple. However, it is difficult to be...
more precise than 150 \mu m in defining any border that bounds a portion of the barrel field not the PMBSF. There are at least two reasons for ambiguity: one technical and the other biological.

With regard to the first point: any process that obscures individual barrels tends to constrict the field observed since we depend upon the clear-cut appearance of barrels to identify the field. In thick sections (100 \mu m), barrels are obscured as the plane of section becomes less tangential to the pia. Since the plane of the barrel field is domed, the (non-PMBSF) boundaries will be lost in any specimen in which the plane of section is tangent to the center of the field. Similarly, if one makes the plane of section tangential to the pia over one border, with the aim of seeing that border more clearly, the opposite border will be lost.

We have circumvented this problem by using many specimens and by altering slightly the plane of section in each subsequent specimen. In Fig. 8, the outline of the barrel field as determined in a specimen in which the plane of section was tangent to the center of the field has been drawn. That portion of the barrel field to which the plane of section is truly tangent will be the first to appear in the series. The locations of those areas approached truly tangentially have been indicated by hatching. Most of the field has been approached tangentially since there is little area without any hatching. Therefore, we think it is not for technical reasons that the boundaries are vague.

The problem of boundary determination can also be alleviated by making thinner sections (50 \mu m) since there is less 'cell-body interference' from layer IV in places where that layer becomes less parallel to the plane of section.

The second reason for the difficulties experienced in identifying the borders is biological. As we could determine in sections cut tangentially to the pia over the anterior border region, these anterior borders simply are not sharp; barrels lose their definition: they 'fade out' into a homogeneous layer IV.

C. Barrel field reconstructions

Two techniques were used to reconstruct barrel fields: camera lucida drawings and photomicrographic collages. From these reconstructions we (a) compared field morphology in different animals, (b) measured field area, (c) counted the numbers of barrels in the field, (d) assessed variations of barrel shape and arrangement, and (e) made the measurements of barrel diameters presented in Part I.

1) Camera lucida drawings. Drawings reconstructing barrel fields from nine cerebral hemispheres were made at a magnification of 73.5 \times with the use of a Wild drawing apparatus attached to a Zeiss GFL microscope. The technique was to take the first (most superficial) tangential section having barrels and to carefully draw the barrel sides. Prominent blood vessels were drawn to serially relate the sections (see Fig. 9, arrows). The following (deeper) section was aligned by the use of the vessels to the drawing of the preceding section. The barrel sides from this deeper section were added to the drawing and new vessels were drawn in, if necessary. The same procedure was applied to subsequent sections in which barrels appeared, until all barrels were drawn. The use of small vessels is complicated slightly by the

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Fig. 9. Photomicrographs of three serial tangential sections for hemisphere 10L. Orientation: anterior left; posterior, right; medial, up; lateral, down. A is the most superficial, C is the deepest of the three Sections illustrate the clear and picturesque display of barrels that one observes in such preparations. From sections such as these the photographic collages and the camera lucida drawings of entire barrel fields were made. Arrows point to some of the vessels which, appearing in subsequent sections, are commonly used to spatially relate serially cut sections to one another. Formalin fixation, methylene blue-Cl, 50 μm thick sections. Bar = 2 mm.
Fig. 10. Photomicrographic collage reconstructed from tangential sections to show the complete barrel field of hemisphere 10L. Orientation: anterior, left; posterior, right; medial, up; lateral, down. Cortical location of barrel field is indicated, in upper inset, by stipple. Lower inset is camera lucida drawing of the same barrel field; circular and ellipsoid profiles represent barrels (the rectangles indicate location of photographs shown in Figs. 1 and 2). Notice that the large barrels of the PMBSF (at the right in the figure) are sharply delineated from the adjacent homogeneous layer IV while this is not so for the anterior portions of the barrel field. A complete summary of the differences between PMBSF and rest of barrel field appears in Table III. Medially (top of figure), a notch without barrels is found indenting the field so as to nearly bisect it. (In the collage, this notch appears to extend farther laterally than it actually does — cf. lower inset — because of the shortcomings of the photography.) The barrel field shown here is nearly identical to that shown in the collage of Fig. 11. See also the barrel field drawings in Fig. 14. Approximately 70 photomicrographs went into the making of this illustration. Formalin fixation, methylene blue-Cl, 50 μm
Fig. 11. Photomicrographic collage reconstructed from tangential sections to show the complete barrel field of hemisphere 9L. Orientation: anterior, left; posterior, right; medial, up; lateral, down. Location of barrel field is indicated, in upper inset, by stipple. Lower inset is a camera lucida drawing of the same barrel field. Observe same features as pointed out in Fig. 10 and the near identity of this barrel field with that of 10L. Formalin fixation, methylene blue–C.I. 100 µm thick sections. Bar = 500 µm.
cortical vascular pattern, which converges toward the center of the brain from the pia. (E.g., the four marking vessels of Fig. 9 are closer together in the section of Fig. 9C than they are in the section of Fig. 9A. Fortunately, discrepancies such as these are slight and can be overcome by approximation.)

All drawings are shown in Fig. 14. The appearance of barrels and their arrangement in the camera lucida drawings was very similar to the photographic collages presented in Figs. 10 and 11.

(2) Photographic reconstructions. Photomicrographs of each section were made at 210 ×. The portion having barrels was photographed systematically by moving the slide with a mechanical stage, always being careful to preserve a slight overlap between adjacent frames. Collages were assembled according to the same principles which were applied to the camera lucida drawings. Low power pictures were made of each section so that the higher resolution frames could be properly oriented and so that barrels from the serial sections could be properly related (in Fig. 9 are samples of these low power photographs). In making the collages shown in Figs. 10 and 11, that section of the series was chosen which contained the greatest portion of the barrel field. All of the photographs were pieced together to give the complete picture of that section (in the case of the specimen depicted in Fig. 10, this section is the one shown in Fig. 9C). Over this 'first' collage were placed the photographs taken from adjacent sections. These latter photographs, once accurately oriented by use of small vessels, were pieced together and glued to the composite underneath so that the best pictures of the barrels in the field were assembled in the collage. For example, in making Fig. 10, photographs from the section in Fig. 9B were pieced over these from the section in Fig. 9C; and then those of Fig. 9A over the assembly of pictures from Figs. 9B and 9C. Similarly, photographs from deeper sections were pieced together and glued to the collage until all of the photographed barrels appeared on the collage in their proper relationship to one another. In still deeper sections no barrels were seen and no barrels could be photographed. In the collages, the barrel fields are surrounded by homogeneous layer IV. Throughout the procedure, the low power photographs of the entire sections were used for orientation purposes.

The orderly appearance, in Figs. 10 and 11, of the barrels packed together is as pleasing as it is striking. Stippling on the inset brain photographs shows the approximate cortical location of the field; inset line drawings indicate where barrels are.

Although the two collages were made from sections of different thickness and from hemispheres of different animals there is a nearly exact likeness. This high degree of similarity is also typical of the nine drawn barrel fields shown in Fig. 14.

The features mentioned in the description of the boundaries are clearly visible.

It will be noted that generally the anterior and lateral barrels are circular, smaller, fall within a narrow size range, and are apparently randomly packed. A subtle difference is that barrels in the middle of the field are slightly larger and appear to have a more orderly arrangement than those farther forward. While not so clear as in the PMBSF, the organization is suggestive of rows or concentric arcs with their centerpoint in the middle of the lateral border of the barrel field. The possible meaning of this arrangement is considered in the General Discussion.

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D. Area of barrel field

Approximations of the total area of the barrel field and of the PMBSF area were obtained as follows:\textsuperscript{18} Seven of the nine camera lucida drawings* of the barrel fields analyzed (1 cm = 136 $\mu$m; smaller versions appear in Fig. 14) were surrounded by a line circumventing its perimeter. Another line was drawn in, separating PMBSF from non-PMBSF. These contours were transposed on millimeter paper and the areas defined above were determined with a unit of measurement of (136 $\mu$m)$^2$.

The following list summarizes the area measurements. The ranges of the areas are rounded off to (0.1 mm)$^2$.

\begin{align*}
\text{Total field area} & = 2.10-2.76 \text{ mm}^2 \\
\text{PMBSF area} & = 0.95-1.09 \text{ mm}^2 \\
\text{The means are: Total field area} & = 2.52 \text{ mm}^2 \\
\text{PMBSF area} & = 1.03 \text{ mm}^2
\end{align*}

Averagely, the PMBSF area constitutes 40.9 $\%$ of the total barrel field area\textsuperscript{**}.

It should be pointed out that the mean of total barrel field areas is too small for three reasons. (1) The uncertainty of the \textit{anterior} borders of the barrel field (discussed above) leads to a lower estimate of the area of the anterior part of the barrel field. Consequently, the actual value

\[
\frac{\text{PMBSF area} \times 100}{\text{total area}}
\]

is smaller than the calculated 40.9 $\%$. (2) The flattening of the domed layer IV (which had occurred in the process of making the camera lucida drawings) reduces the calculated areas. (3) The foreshortening of the field by approaches from its various boundaries reduces the area included in the drawing.

E. Number of barrels per barrel field

The results of barrel counts using the camera lucida drawings are shown in the histogram in Fig. 12. The clear bars represent the total number of barrels counted in a hemisphere while the shaded portions indicate the number of barrels found in the PMBSF.

There is moderate variation in the total number of barrels per hemisphere: the number of barrels ranges between 170 and 210. For reasons stated above regarding difficulties in determining borders, the real number of barrels per hemisphere cannot be found by averaging individual counts. The real number is greater than any one of the values appearing in the histogram and could be arrived at by some accurate method of combining different fields. However, the underestimation cannot be more

\textsuperscript{*} See legend to Fig. 12 for explanation of why two of the barrel field drawings could not be included in these determinations.

\textsuperscript{**} In connection with a correlation presented in the General Discussion we also determined the areas of the total barrel fields including the notch (medial border of the latter being defined consistently but somewhat arbitrarily). Range: 2.44–3.02 mm$^2$; mean: 2.79 mm$^2$. The PMBSF area constitutes 36.9 $\%$ of this larger total barrel field area.

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Fig. 12. Bar-chart summarizing barrel counts from the nine hemispheres whose barrel fields are shown in Fig. 14. Each bar denotes the total number of barrels counted in the correspondingly labeled barrel field of Fig. 14. The stippled portion of a bar indicates the number of barrels in the field’s PMBSF. Notice that barrel counts made in the PMBSFs are nearly identical; the totals are more variable (see text). Stars indicate incomplete counts: in the case of 7R, a section is missing from the series used for the drawing; in 11R, the tangential approach was such that the most medial portion of the field was hit first; this approach was so ‘eccentric’ that a large number of anterolateral barrels could not be counted. (PMBF in figure = PMBSF.)

than 20–30 small barrels participating in the uncertain anterior boundaries (the stippled boundaries in Fig. 8). The barrels of the PMBSF can be more exactly identified. There is correspondingly a very good agreement of the barrel counts in this area for all hemispheres (34–40).

Discussion

A. Study of cortex in tangential sections

By tradition, the use of sections tangent to the pia in the study of the cerebral cortex has been infrequent but it is not new. Retzius (Table II, Fig. 7)⁴⁶ and Cajal (Fig. 339)⁷ used, besides countless sections cut perpendicularly to the pial surface, a few tangential sections to study the neurons of the first layer of the cerebral cortex in immature dog, and in mature cat, respectively. Klotz and Clark²⁹ used tangential sections of rat neocortex in an attempt at quantification of cytoarchitectonic characteristics*. Van der Loos⁵¹ used the tangential approach to quantitatively analyze dendritic morphology and interconnections of individual cortical neurons and Colonnier⁹ and Wong⁶⁶ used tangential sections to study dendrite field orientation in cat visual and acoustic cortex, respectively. To our knowledge, our study represents the first attempt to delineate a cytoarchitectonic field by the tangential approach. The barrels of the cortex of the mouse perhaps uniquely pre-dispose that cortex to

* Somewhat startlingly, these authors concluded, among other things, that the graphs resulting from their analysis ‘were valueless as anatomical descriptions’.

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investigation by the tangential method. Although the cytoarchitectonic area so defined does not differ much from earlier identifications (see part B of this Discussion), the delineation of the field has been considerably easier and more accurate with the use of tangential sections. Also, we believe, the identification of the individual barrel as a unit could not have been made as convincingly as it was done in tangential sections. Thus, by changing the plane of section, much has been learned about the organization of layer IV of this cortex; at the same time a price has been paid by the loss of the relationship among the individual layers.

B. Earlier cytoarchitectonics

The findings in the present study do not appreciably alter the interpretations of earlier cytoarchitectonic studies. Fields approximately corresponding to the barrel field are Isenschmid's field b86, De Vries' field H12,13, Droogleever Fortuyn's field j14,15, Rose's (1912) fields 1, 2, 3, 5, 7 and 2246, Rose's (1929) field T147, and T. A. Woolsey's field of the cell-dense net88. (Cf. ref. 68, Discussion, Section B, and Fig. 9 for a discussion of the basis for the 'equivalence' of these fields with what we now call the barrel field).

C. Physiology

The relation of the barrel field to cortical localization patterns in the mouse as previously defined68 essentially has not been altered by the present investigation. The placement of the field in relation to cortical sensory projections is shown in Fig. 13. The barrel field is confined to the head and possibly distal forelimb regions of S I. In the General Discussion we shall consider the questions why the barrel

Fig. 13. Diagram of mouse brain to show the relation of various cortical sensory areas determined by evoked potential techniques, to the barrel field. The S I musculus has been revised from an earlier drawing (ref. 68, Fig. 8) in accordance with recent microelectrode findings in the rat67,88. Notice relation of the whole of S I to the barrel field and, particularly, the relation of the vibrissal rows (lines on the musculus' face) to the approximate location of the PMBSF.

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Fig. 14. Camera lucida drawings of barrel fields from nine mouse cerebral hemispheres. Letters R and L indicate barrel field from right or left hemisphere, respectively. Orientation: medial, up; lateral, down; the anterior aspects of the field are facing the middle of the figure; the posterior aspects, the sides. For the sake of clarity, septa are somewhat widened. Barrels of the PMBSF have been blackened. Notice that the PMBSF barrels are arranged in five distinct rows, are generally elliptical, and are larger than other barrels. Observe the similarity of the barrel fields. They compare well with the barrel field appearances in the collages of Figs. 10 and 11. Cf. legend to Fig. 12 for an explanation of the truncated appearance of the fields from hemispheres 7R and 11R.
PART III. SPECIAL PROPERTIES OF THE POSTEROMEDIAL BARREL SUBFIELD (PMBSF)

Throughout Parts I and II we have segregated the posteromedial barrel subfield (PMBSF) because of its special characteristics. In this part we shall demonstrate the special qualities of this subfield and, referring to what is known of the physiology of the area, we shall suggest that it has a special function. We believe that each one of the barrels of the PMBSF is the cortical correlate of a contralateral mystacial vibrissa. Mystacial vibrissae* are associated with the important and highly specialized peripheral tactile organs which many mammals, including the mouse, have on their muzzles.

Observations

A. Boundaries of the PMBSF

A characteristic of the PMBSF is that it is sharply separated from surrounding homogeneous layer IV on three sides. The subfield provides the posterior and posteromedial boundaries of the barrel field. Its anteromedial aspect contributes the posterior border of the medial notch. The PMBSF, then, is in contact with the rest of the barrel field only at its anterolateral aspect. Fig. 14 demonstrates this.

B. Pattern of rows

The barrels of the PMBSF are arranged in a highly organized pattern of five rows. This is shown clearly in both the camera lucida drawings of all of the specimens (Fig. 14) and in the photographic collages of two of them (Figs. 10, 11). In the drawings, we have blackened only those barrels which we judge belong to the PMBSF. In every instance there is a distinct five row pattern regardless of the plane of tangential approach. Notice that four large barrels on the posteromedial border straddle the five rows.

C. Size, shape, number and separation of barrels in PMBSF

Barrels in the PMBSF are the largest in the barrel field (see Table I and Fig. 5). There is a broad size spectrum and a progressive increase in size from anterolateral to posteromedial, resulting primarily from a lengthening of major axes. Minor axes are quite constant and are very similar to the axes of circular barrels. Most barrels in the PMBSF have a distinctive ellipsoid shape (Figs. 2, 3 and 14). This is not a

* A mystacial vibrissa is a sinus hair which we operationally separate from all other sinus hairs because of (1) its location on the upper lip (moustache), (2) its very large size, (3) its movements (whisking).

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### Table III

**Contrasting Properties of Barrels from the Anterior Part of the Barrel Field and from PMBSF**

<table>
<thead>
<tr>
<th>Property</th>
<th>Anterior</th>
<th>PMBSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Field boundaries</td>
<td>Unclear — not resolved to less than one barrel (100 μm)</td>
<td>Sharp — always resolved to barrel sides</td>
</tr>
<tr>
<td>2. Organization</td>
<td>'Random' packing</td>
<td>Arranged in five rows*</td>
</tr>
<tr>
<td>3. Size</td>
<td>Smallest to medium — long axis 100–150 μm</td>
<td>Medium to largest — long axis 150–380 μm</td>
</tr>
<tr>
<td>4. Shape</td>
<td>Roughly circular on tangent section</td>
<td>Frequently elliptical on tangent section</td>
</tr>
<tr>
<td>5. Separation</td>
<td>5 μm septa in all directions</td>
<td>5 μm septa within a row; 20 μm septa between rows</td>
</tr>
<tr>
<td>6. Numbers</td>
<td>Approximately 160</td>
<td>34–40</td>
</tr>
</tbody>
</table>

* The five rows are straddled at their medial ends by four large barrels.

The geometrical artifact: the ellipsoid shape is obtained in precisely tangential approaches (Fig. 14: 111L). The number of barrels — 34–40 — in the PMBSF is quite constant (Fig. 12) which in part may be the result of the certainty with which they could be identified. Finally, barrels in adjacent rows are separated by a septum much wider than that found between barrels in the same row or between barrels in other parts of the field (Table I). It is by this greater separation that the organization into rows is emphasized (cf., e.g., Fig. 10). In Table III, these properties are summarized and contrasted with the properties of barrels of the rest of the field.

### Discussion

**A. Vibrissae**

Because many of the unusual features of the PMBSF can be explained by assuming that a relationship exists between the barrels in the PMBSF and the mystacial vibrissae on the muzzle, we shall summarize some of the available information related to mouse and rat vibrissae.

1. **Mystacial organization.** The mystacial vibrissae in the mouse and in the rat are organized in five rows nearly parallel to the bridge of the nose (see Fig. 15A). Each row has 4–7 easily identified large vibrissae. The caudal ones are long and thick. The vibrissae gradually diminish in both dimensions as they are located more rostrally. Four large vibrissae straddle the caudal ends of the five rows. Rostrally the diminution in size makes it difficult on gross inspection to be certain where the vibrissal rows stop. In our counts we are consistently able to identify 25–27 large mystacial vibrissae in the mouse; there are about 35 in the rat.

2. **Structure.** To date, the best characterization of the structures of mystacial vibrissae is Vincent’s 1913 study of the rat. A more recent, less detailed study has shown the vibrissal structure in the mouse to be essentially identical. Vincent noted that vibrissae are distinguished by: (a) large hair size, (b) dense connective tissue isolating the follicle, (c) extensive blood supply, blood sinuses (hence ‘sinus hair’)

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and erectile tissue, (d) striated muscles which attach to the follicular capsule (apparently responsible for the complex whisking movements described by Welker60), and (e) multiple innervation consisting of: (i) a main sensory nerve containing '... 150 or more large medullated nerve fibers', (ii) free sensory endings at the skin surface around the hair, and (iii) extensive varicose nerves associated with the vessels (presumably having an autonomic function). Vincent concluded: 'Histological and structural studies show that the tactile hair is a powerful organ of touch ...'. She speculated on how each of the structural features could contribute to the great sensitivity of the sinus hair as a tactile organ.

Recently, Vincent's observation that some nerve endings are associated with Merkel cells has been confirmed electron microscopically41. Andres2 made a more comprehensive ultrastructural survey of nerve endings in the rat vibrissa. He described the following types which, with one exception, are associated with the main myelinated sensory nerve of the follicle: (a) axons which branch and make contact with several Merkel cells; (b) thickest axons which have lancet-shaped endings occurring (i) as single straight terminals, (ii) as multiply branched terminals, and (iii) — associated with the skin nerve — as multiple, circularly placed terminals; (c) axons ending in encapsulated corpuscles. Axons with free endings are associated with the largely unmyelinated dermal nerve.

3 Some receptive field properties of vibrissae. Recently Zucker70 and Zucker and Welker71 have examined the receptor properties of single units in the trigeminal ganglion of the rat. They have found: (a) A majority of units studied responded to stimulation of vibrissae; few units responded to stimulation of surrounding patches of common hair. (b) All units responding to deflection of vibrissae never responded to manipulation of more than one vibrissa or to surrounding common hair. (c) First order neurons from a vibrissa are 'capable of coding the following aspects of mechanical stimuli: (i) peripheral location, (ii) deflection direction, (iii) onset, (iv) termination, (v) amplitude, (vi) velocity, (vii) duration, (viii) repetition, and (ix) temporal pattern'. (d) Stimulation of the facial nerve to simulate protraction ('whisking' of the vibrissae) elicited a response in only 50% of the units studied but by imposing a barrier across the path of the protracting vibrissae almost all units could be driven. Clearly the rat, and probably the mouse, has a great deal of specific information coded even at the level of the single first order neuron. The morphological basis for much of this specificity may be found in the four different kinds of receptors, each having different geometric dispositions, as elegantly described in Andres' electron microscopical study2 of sinus hair innervation.

B. Physiology

1 Evoked potential studies in the mouse. We have noted that in the macro-electrode evoked potential maps of the mouse cortex the area of the whole barrel field is within the head and face region of S168 (see Fig. 13). Although we previously had felt that the vibrissal representation was in the midportion of what we now call the 'barrel field', the physiological evidence was not sufficiently fine-grained to definitely support that belief. (It should be remembered that the overall dimensions of
the barrel field are 1100 × 2800 μm, and that the electrode tip diameter of 300 μm used in the evoked potential study is, in comparison, quite large.

Largely based upon recent microelectrode studies in the rat\textsuperscript{57,58}, a re-appraisal of the figurine maps of the earlier study\textsuperscript{68} was made. The re-appraisal showed the original data to be compatible with the belief that the vibrissae are represented in the posterior half of the barrel field. The musculus in Fig. 13 has been modified to convey this belief.

(2) Microelectrode maps of Sm 1 in the rat. The macroelectrode cortical localization patterns in the mouse are quite comparable to those in the rat\textsuperscript{67}. In the beautiful studies of Carol Welker\textsuperscript{58}, Sm 1 has been delineated by the microelectrode unit cluster technique. What interests us here is the representation of the mystacial vibrissae in the rat cortex.

C. Welker has found that in the cerebral cortex of the rat each vibrissa is individually represented; the organization is in rows which are slightly oblique to the coronal plane; the more dorsal vibrissae are represented posteriorly and the more ventral vibrissae are anterior; more caudal vibrissae are represented medially while the rostral ones are lateral. She has estimated\textsuperscript{59} that a 'microfield' for the representation of only one large mystacial vibrissa is about 0.6 × 0.6 mm, which is quite comparable with one large PMBSF barrel if one allows for shrinkage. Most unit clusters were found in layer IV.

C. Behavior

Along with her morphological studies, Vincent\textsuperscript{55} studied the importance of vibrissae in the behavior of white rats as determined by their ability to run mazes. She concluded that the mystacial vibrissae significantly influenced the performance of rats in mazes. Without vibrissae the animals appeared lost in their environment, and ran the maze more slowly. They held their bodies close to the floor, apparently depending for information on increased contact of their mouth parts and ventral surfaces with the maze. If vibrissae were removed from one side of the snout the rats could run the maze almost as fast as with all vibrissae present. However, they then preferred to stay with their intact side close to the maze edges or walls.

W. Welker\textsuperscript{60} has used high speed motion picture techniques to study the 'whisking cycle' in rats. Whisking movements are repetitive, high frequency (up to 7/sec) movements (successive waves of vibrissal protraction and retraction) of the entire mystacial pad which are apparently involved in the rat's active investigation of the environment. Although many mammals and apparently all rodents have prominent mystacial vibrissae not all actively 'whisk'. All of the other animals in which 'barrels' have been seen — gerbil\textsuperscript{53}, rat\textsuperscript{15,54,57}, chinchilla\textsuperscript{59} and mouse — are whisking rodents, except the guinea pig\textsuperscript{15,19,54}.

D. 'Differentiation of structure means differentiation of function' (Hughlings Jackson)\textsuperscript{20}

(1) The PMBSF is the cortical correlate of the contralateral mystacial vibrissae. The rationale for the belief that the PMBSF is directly related to the vibrissae follows two lines of reasoning: morphological and physiological.

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Fig. 15. Figure summarizing the one PMBSF barrel—one mystacial vibrissa hypothesis. A, Photograph of the group of right mystacial vibrissae from a mouse. Vibrissae were painted before photography. Dots were placed, on the photograph, over points where vibrissae are implanted. Nose points downward, mouth is to the left. Notice that the vibrissae are arranged in five rows of 4–7 vibrissae each and that 4 vibrissae straddle the caudal ends of the rows. B, Collage of a left PMBSF. Anterior, left; posterior, right; medial, up; lateral, down. A is contralateral to B. Inset shows barrels in B definitely identified as PMBSF barrels.

(a) The mystacial vibrissae are the biggest and most complex tactile structures in the mouse (e.g., structure, information encoding, importance in behavior). The biggest and most distinctive barrels are in the PMBSF.

(b) The mystacial vibrissae are arranged in five rows. The barrels of the PMBSF are arranged in five rows. Four barrels straddle the ends of the five barrel rows just as four vibrissae straddle the ends of five rows of vibrissae (Fig. 15).

(c) There are about 25–27 easily identified mystacial vibrissae — about 4–7 per row. There are 34–40 barrels in the PMBSF, 4–9 per row. Since our counts on the face may not include the smallest mystacial vibrissae, and our PMBSF barrel counts may include small-vibrissa barrels, the ratio of barrels to the vibrissae may well be one to one.

(d) Microelectrode studies in the rat show that each vibrissa is represented separately in the cortex in areas of 600 μm by 600 μm — dimensions which are in the range of our axis measurements. The functional organization of the rows of mystacial vibrissae is very similar in location, orientation and number to the barrels in the PMBSF (compare C. Welkers Fig. 2 with our Figs. 10 and 11).

(e) Macroelectrode studies in the mouse are compatible with the belief that vibrissae are represented in the area of the PMBSF.

For these five reasons we propose (a) that the PMBSF is indeed the cortical anatomical correlate of the mystacial vibrissae; (b) that the dorsal and ventral vibrissae are represented in the posterolateral and anterior portions of the PMBSF, respectively, while the rostral and caudal vibrissae are represented in the lateral and postero-
medial aspects of the field, respectively; and (c) that there is a one to one relationship between the big barrels in the PMBSF and the mystacial vibrissae on the muzzle. In Fig. 15 we have summarized this hypothesis by placing photographs of mystacial vibrissae and of the PMBSF side by side.

(2) Anatomical segregation of vibrissa-related neurons at subcortical stations. Perhaps, the barrels in the PMBSF represent the terminal stations in a chain of anatomically distinct representations of individual vibrissae. Welker and co-workers\textsuperscript{27,62,63} have shown such anatomically segregated representation in dorsal column nuclei, thalamus, and cerebral convolutions, of the highly sensitive volar surface of the raccoon forepaw and digits. Although in the rat detailed studies have been made on the organization of the trigeminal ganglion\textsuperscript{71}, of nuclei of termination of sensory trigeminus\textsuperscript{39,40}, and of thalamic relay nuclei\textsuperscript{17}, evidence for anatomical segregation of single-vibrissa related regions has not been found at any of these subcortical stations. This absence of anatomical segregation may, in part, reflect the lack of directed anatomical attention given to this problem. To our knowledge, no such information is available for the mouse, either.

GENERAL DISCUSSION

A. Why the term barrel?

As we have stated before (cf. Table I) we believe that the cytoarchitectonic units which we call barrels are essentially equivalent to the 'clouds', or 'deeply stained spots', of Droogleever Fortuyn\textsuperscript{14} and to the \textit{glomerulos} described by Lorente de Nó\textsuperscript{31}. In spite of this we have three reasons for changing the earlier terminology: (1) In comparison with 'cloud' or 'glomerulo', the term barrel gives a more accurate impression of the three-dimensional structures described in this paper. (2) 'Glomerulus' is a term that has been applied to other well studied areas of the CNS — of the cerebellum and of the olfactory bulb — as well as to extracranial (e.g., renal) structural entities. The term 'barrel' prevents confusion with these other structures. (3) The word 'glomerulus' describing CNS morphology has been applied to areas rich in nerve cell processes but devoid of perikarya. Our hollow is an area relatively devoid of perikarya; the \textit{barrel}, however, is more: a hollow surrounded by a side, the latter consisting mainly of perikarya. The term 'barrel' emphasizes the inclusion of cell bodies in our concept of the barrel as a cytoarchitectonic unit.

B. How much periphery does the barrel field represent?

If it is agreed that the PMBSF is the site of representation of the large mystacial vibrissae, then what may the other barrels represent? C. Welker, as part of her rat study\textsuperscript{58}, has done a careful depilation of the rat head to determine the number of sinus hairs. Not only are there the large mystacial vibrissae but there are smaller hairs about the nares, along the upper lip, inside the mouth on what C. Welker calls the furry buccal pad, and along the lower lip. There are also long sinus hairs at the angle of the jaw, over the eyes and at the wrist. The total counted by C. Welker in the rat is about 220. This is in surprisingly close agreement with our barrel counts (see Fig. 12).

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C. Welker found\textsuperscript{57,58} also that the cortical area which represents the mystacial vibrissae is about 35\% of the total S I head region. We found the PMBSF area to be 40.9\% of the total barrel field area. If one, however, includes the area of the medial notch in the total barrel field area, the PMBSF occupies 36.9\% of that area — a remarkable fit with the physiologically derived percentage.

Summarizing, there is close correlation between the barrel field location and the S I head representation in the mouse; there is good agreement of barrel counts in the mouse and sinus hair counts in the rat; and there is similarity between the ratios PMBSF/whole barrel field in mouse, and mystacial vibrissa area/total head area in rat. All of these points support two conclusions: (1) The barrel field represents part of the head. (2) Our specific hypothesis, \textit{i.e.} 'one PMBSF barrel represents one large contralateral mystacial vibrissa', can be extended to 'one barrel represents one contralateral sinus hair'. This extended hypothesis may explain several features of the barrel field described in Part II. The slightly larger barrels in the middle portion of the field which seem to be arranged in radiating rows and arcs are probably related to the sinus hairs of the upper lip; and the medial notch may be related to some of the mouth parts.

The problem remains as to where the common hairy skin of the head, and especially that at the base of the sinus hairs, is represented cortically. The same problem pertains to lips and mouth parts. Several possibilities exist: (1) the representation is in the barrel field; (2) the representation is beside the barrel field (maybe in the notch); (3) there is no representation. Precise microelectrode analyses are necessary to settle this point.

\textbf{C. Barrels emphasize two principles of cortical organization}

\textit{(1) The concept of vertical or columnar cortical organization.} Over a century ago, the earliest histological study of the cerebral cortex revealed a radial pattern of fibers and cell bodies\textsuperscript{4}. These patterns were amply confirmed in countless, subsequent cytoarchitectonic and myeloarchitectonic studies.

Lorente de Nó's classic and comprehensive paper, of 30 years ago, on cerebral cortex structure\textsuperscript{32} contains an early, clear statement of the hypothesis that cerebral cortex is organized in vertical columns composed of interconnected cells extending through the full thickness of gray matter. We found it interesting that Lorente de Nó's hypothesis was largely based on observations made in the mouse, partly in what we now call the barrel field.

Mountcastle, on the basis of microelectrode studies of S I cortex of the cat, was the first to propose \textit{functional} 'columnar' organization of the cerebral cortex\textsuperscript{85}. A clear-cut morphologic correlate of the columns was not found. Recently Mountcastle and Darian-Smith\textsuperscript{36} summarized this concept as follows:

'1. When microelectrode penetrations are made normal to the cortical surface and parallel to the vertical columns of cells, all neurons encountered are of the same modality type. 2. Neurons encountered in vertical penetrations are related not only to the same modality but also to nearly identical peripheral receptive fields. 3. When peripheral stimuli activating each \ldots cortical neuron are carefully positioned at the'}
most sensitive loci in their receptive fields, the latencies of response of cells in the
different cortical layers fall within a narrow range, within 2 to 4 msec of each other.
Cells of layers III and IV are activated earliest by the thalamocortical volley.

Mountcastle felt, on the basis of penetrations not normal to the pia, that these
functional columns were at most 500 μm in diameter. Since 1957, the functional columnar
organization of cerebral cortex has been confirmed in several systems in several species:
S I and S II of monkey, S II and S III of cat, M I of cat, V I of cat, V II of cat; it has been hesitantly proposed for A I of cat. The
diameters of functional columns are reported to be between 100 and 500 μm.

An attempt to find the morphological correlates of the columns in monkey
S I only confirmed observations made many years earlier. Studies of tangential
sections through cat V I cortex and cat A I, A II and auditory association cortex have been revealing only in that the tangential extents of the pyramids’ dendrite
systems are of the same order of magnitude as functional columns. They have failed
to demonstrate a substrate for functional columns. Colonnier devoted a paper

Recently, Hubel and Wiesel placed small lesions in one layer of the lateral
geniculate body, and found bouton degeneration in monkey VI. The pattern of de-
geneneration, when seen from the pia, looked like a mosaic of parallel 350 μm wide
‘stripes’ which the authors interpreted as corresponding to their ‘eye-preference
columns’. Degeneration was primarily in layer IV.

The range of barrel diameters is fully within the range of the diameters of the
functional columns physiologically demonstrated in the studies listed above.

On the basis of the preceding arguments we believe that the barrels of the S I
cortex of the mouse are the morphological manifestation, in layer IV, of functional
columns defined by electrophysiological means.

The concept of laminar cortical organization. Barrels are found only in
layer IV. We now turn to evidence which refines the ‘columnar concept’ and which
suggests that, while all cortical layers in a S I column are related to the same modality
and locus, it is layer IV which appears to be the first receiving station. Layers above
and below, it seems, are involved in the subsequent manipulation of information.

(a) Morphologically, all of the primary sensory cortices are distinguished by
their highly developed layer IV.

(b) Specific thalamo-cortical afferents terminate in layer IV (Cajal — Golgi
analysis; Polyak — Marchi degeneration study; Nauta — Nauta degeneration
study; Hubel and Wiese — Fink and Heimer degeneration study).

(c) Barrels in the mouse cortex apparently receive specific thalamic afferents.

(d) Physiologically, it is likely that input from the thalamus is primarily to
layer IV.

(i) C. Welker’s clusters of units, found under light Nembutal anesthesia, are all
from layer IV. Such anesthesia depresses spontaneous cortical activity, and reduces
intracortical synaptic transmission.

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(ii) Using very light or no anesthesia, Powell and Mountcastle, as well as Hubel and Wiesel, found a greater density of driven units in the middle layers (III and IV) than in other layers.

(iii) In their investigation of cat area 17, and especially of monkey area 17, Hubel and Wiesel were able to find only 'simple' units in what they determined to be layer IV. There were 'simple' units above and below layer IV, but also 'complex' and 'hypercomplex' units. Their interpretation was that the 'simple' units represented the first cortical station while 'complex' units seemed to be the result of further neuronal interaction. They noted that the stratification on the basis of 'simple' and 'complex' units emphasizes laminar cortical organization within a given column.

(iv) Mountcastle and Darian-Smith stated: 'Cells of layers III and IV are activated earliest by the thalamo-cortical volley while those of the deeper layers discharge with larger latencies.' (see their point 3, above).

**D. Why do mice have barrels?**

We have emphasized that technical details (specimen orientation, plane of section, section thickness, etc.) have been important factors in the elucidation of barrel structure and in the delineation of the barrel field. Here, we shall consider how the nature of the sensory surface and the nature of the mouse itself may be responsible for the prominence of barrels in mouse cerebral cortex.

(1) The nature of the sensory surface and sense organs. The sinus hairs of the rodent (and other mammals) are markedly different from a continuous two-dimensional sensory surface such as skin and retina. Sinus hairs, taken together, form a punctate rather than a continuous sensory surface. They are clearly separated on the periphery and the mouse may not require the mechanism of afferent inhibition to refine localization. The precise pre-determined location of first order neuron receptive fields such as those associated with sinus hairs would permit a greater degree of order in central neuronal populations such as in barrels to form a more efficient neuronal circuitry.

For a given cortical locus, functional cortical columns in monkey and cat S I cortex represent only one of several 'submodalities'. Presumably, the information concerning all 'submodalities' for a given locus is integrated elsewhere (e.g., pre-cruciate gyrus of the cat). However, as Zucker and Welker have demonstrated (see above), the receptors associated with a single vibrissa of the rat are capable of encoding information which may be regarded as representing many 'submodalities'. The morphological substrates of these 'submodalities' probably are the four receptor types and the patterns of their arrangement described by Andres. The projection of many 'submodalities' to a single S I cortical locus — which the 'one barrel-one sinus hair' hypothesis implies — could emphasize local cortical cytoarchitectonic organization by permitting early cortical integration of the different 'submodalities'.

(2) Possible factors making barrels prominent in mouse cortex. First, it may be that in mice sinus hairs are relatively more important in gathering information than they are in other mammals having whiskers. Concomitant with their importance could be a relatively greater development of corresponding cortex. Second, but not neces-
sarily complimentary to the first point, the size of the mouse cerebral cortex may approach a minimum. In line with this thought, we hesitantly reason that neurons in layer IV of the mouse cerebral cortex are perhaps just barely enough for the cortical manipulation of sensory data. Bigger animals have more than such a marginally low number of layer IV neurons in S I; indeed, their entire cortex, S I included, is more voluminous. The increase in the number of elements may tend to obscure the basic pattern; one cannot see the grooves for the forest.

E. Future studies

Our hypothesis that specific and constant cortical structures are related to specific and constant peripheral receptors can be tested in several ways. We have begun sensory deprivation experiments. A study of animals in which there is a genetically determined variation in the number of sense organs\textsuperscript{16} is planned.

A number of problems which have hitherto successfully resisted a fine-grain analysis may now be investigated. These problems are related to the general questions: what are the basic elements of sensory cortex circuitry, and how does the cerebral cortex process sensory data?

The hypothesized vibrissa-barrel relationship may make an integrated approach to these questions possible. Correlated analysis by means of Golgi and electron microscopic techniques should clarify sensory cortex circuitry; microelectrode studies of the barrel field should shed light on the functional aspects of cortical information processing; combined ontogenetic histochemical and behavioral studies could correlate the level of synaptic activity with the appearance of certain patterns of behavior.

We anticipate that after the interdisciplinary pursuit of some of the above ideas we shall have occasion to paraphrase the Bard who sings\textsuperscript{46}:

'But mice, rats and gerbils and such small deer
Were Tom’s and Hendrik’s food for many a long year'.

SUMMARY

1. Formalin-fixed, Nissl-stained and Cox-fixed, Golgi–Nissl-stained preparations of sections cut \textit{coronally} and \textit{tangentially} to the pia have been used to elucidate the organization of layer IV in mouse S I cerebral cortex.

2. A multicellular cortical cytoarchitectonic unit is described which is as tall as layer IV, roughly cylindrical, 100–400 μm in diameter, with its center line normal to the pia. Because of their characteristic shape we call these units \textit{barrels}. Each barrel is composed of a ring of cells, the \textit{side}, which surrounds a less cellular central \textit{hollow}. The nearly acellular area surrounding each barrel and separating adjacent barrels is called the \textit{septum}. Ranges of measurements of the barrels and their components are given and the size distribution of the barrels in two barrel containing regions is given. The unit is discussed in relation to observations reported in several earlier accounts of the mouse cortex.

3. The cytoarchitectonic region which contains the barrels has been determined by a tangential approach. Its exact place on the cortical surface, its outline, appearance,
area (ca. 2.52 mm$^2$) and the number of barrels which it contains (approximately 200) are given. The most distinguishing feature of the barrel field is its relative constancy in all measures from one hemisphere to the next and from one specimen to the next. The barrel field is discussed in relation to earlier cytoarchitectonic studies and to physiologic data.

4. A particularly striking and constant portion of the barrel field, the postero-medial barrel subfield (PMBSF) is presented. Barrels in the PMBSF are characterized by their greater size, elliptical shape, organization into five distinct rows and by their constant number. On the basis of morphological and physiological considerations it is postulated that each barrel in the PMBSF is the cortical correlate of a contralateral mystacial vibrissa.

5. On the basis of area measurement, total barrel counts, and counts of all facial sinus hairs in the rat, the 'one barrel-one vibrissa' hypothesis is expanded to read: each barrel is the cortical correlate of a contralateral sinus hair.

6. A general hypothesis is proposed which states that barrels are the morphological manifestation in layer IV of the functional columns of physiologists.

7. It is suggested that barrels offer excellent opportunities for integrated studies of a sensory cerebral cortex at a degree of resolution heretofore inaccessible.

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REFERENCES

1 ABELES, M., AND GOLDSTEIN, M. H., JR., Functional architecture in the cat’s primary auditory cortex: columnar organization and organization according to depth, J. Neurophysiol., in press.
3 ANGEVINE, J. B., JR., Time of neuron origin in the hippocampal region; an autoradiographic study in the mouse, Exp. Neurol., Suppl. 2 (1965) 1–70.
4 BERLIN, R., Beitrag zur Structurlehre der Grosshirnwindungen, Inauguralab., Erlangen, 1858.
6 CAJAL, S. RAMÓN Y, Studien über die Hirnrinde der Menschen, Heft 1: Die Sehrinde, Barth, Leipzig, 1900, 77 pp.

Brain Research, 17 (1970) 205–242


48 Shakespeare, W., *King Lear*, Act III, Scene IV.


58 Welker, C., Microelectrode delineation of fine grain somatotopic organization of Sm I cerebral neocortex in albino rat, in preparation.

59 Welker, C., Personal communications.


61 Welker, W. I., Personal communication.


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THE STRUCTURAL ORGANIZATION OF LAYER IV IN THE
SOMATOSENSORY REGION (S I) OF MOUSE CEREBRAL CORTEX

THE DESCRIPTION OF A CORTICAL FIELD COMPOSED OF DISCRETE
CYTOARCHITECTONIC UNITS**

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INTRODUCTION

The mouse has a remarkable arrangement of cell bodies in layer IV within the area of its cerebral cortex. In Nissl preparations of sections made perpendicular to the pial surface the arrangement appears to be one of "cellular columns" oriented perpendicular to the pial surface and being as tall as layer IV itself; in sections made tangential to the pial surface, layer IV has the appearance of a "cell-dense net". Cortical evoked potential studies suggest that the area containing this cytoarchitectonic arrangement is coincident with the somatosensory (SI) head-face region.\(^8\)

The structural peculiarities of the region, demonstrated by Nissl's method, were noted or illustrated by three authors early in this century,\(^13\),\(^14\),\(^44\), but not until 1922 had anyone proposed a structural basis for the appearance of this unusual portion of the cortex. In that year, Lorente de Nó\(^4\) using primarily Golgi-impregnated material described glioméricos in the area and was able to identify at least seven cortical cell types associated with them. He demonstrated afferents from below with rich arborizations associated specifically with the glioméricos. In 1929, Rose\(^4\) used Nissl-stained coronal sections paying special attention to the appearance of layer IV to define an area, T1, with which our own later delineation of the field exhibiting the "columnar" and the "cell-dense net" arrangement agreed.\(^4\)

The present study was undertaken to more clearly define the structural organization of layer IV in this SI region of the mouse cerebral cortex. The results will be presented in three parts.

In Part I, sections made in a plane tangent to the pia mater — "tangential sections" — and sections made in a coronal plane — "coronal sections" — stained with Nissl or Golgi-Nissl methods, are used to clarify the cytoarchitectonic organization of the area. The resulting morphological concept is one of a multilayered, cytoarchitectonic unit — ranging from about 100 µm to about 400 µm in diameter — which we termed "barrel". Measures of barrels and their parts are made. The anatomical concept of the barrel is discussed with emphasis on correlation with earlier observations made by other workers and by ourselves. Some questions are raised about the possible cellular constituents and connections of the barrels.

In Part II, the recognition of barrels in Nissl-stained tangential sections is used in an attempt to define the precise position, extent and area of the field containing the barrels, the number of barrels in the field, and the constancy of shape and of arrangement of barrels from one hemisphere to the other and from one mouse to another. Briefly, the possible significance of these findings is discussed in relation to the several cytoarchitectonic maps of the mouse cerebral cortex in relation to what is known about the physiological organization of the cerebral cortex of the mouse and the rat.

In Part III, we discuss a subfield — the postmedian barrel subfield (PMBSF) — in which the shape, size, organization and number of the barrels is particularly clear and constant. On the basis of the suggestive similarity between the arrangements of mystacial vibrissae on the muzzle and of PMBSF barrels in the cortex, and on the basis of the evoked potential maps in the mouse and of recent microelectrode studies

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of rat cortex\(^8\) it is proposed that barrels in the PMBSF are the cortical correlates of the mystacial vibrissae — more specifically, that one barrel represents one vibrissa.

In a general discussion it is suggested that barrels are the morphological manifestation in layer IV of the functional cortical columns described by neurophysiologists.

MATERIAL AND HISTOLOGIC METHODS

**Animals.** Ten adult C57 mice* and one adult Swiss mouse** were used. Weights ranged between 27 and 34.5 g. Ten were males.

**Fixation.** (a) Formalin (7 specimens). Animals were anesthetized with pentobarbital-sodium and briefly perfused through the heart with 0.9% saline solution at room temperature. Perfusion was continued with 10% formalin, which had been neutralized over CaCO\(_3\), in 0.9% saline. The brains were removed several days after perfusion and kept in the 10% formalin solution for about three weeks prior to dehydration and embedding. (b) Golgi-Cox (4 specimens). Animals were anesthetized and allowed to die by exsanguination. The fresh brains were removed immediately, being care taken not to touch the cortical surface, and were placed in a Golgi-Cox solution for 30 days prior to dehydration and embedding.\(^5\)

**Dehydration and embedding.** All tissues were dehydrated in a graded ethanol series and embedded in Celloidin.\(^4\)

**Sectioning.** Twelve hemispheres sectioned tangentially were cut at 100 µm, 70 µm or 50 µm. Six hemispheres sectioned coronally were cut at either 50 µm or 30 µm.

**Staining.** The formalin-fixed sections were stained with methyl blue—C.I. Cox-fixed preparations were treated to reveal the impregnated cells and counterstained with methyl blue—C.I. by a recent modification\(^6\) of the combined Golgi—Nissl methods.\(^4\)

Data collection and reduction. Methods of data collection and reduction are presented with related observations.

PART I. A MULTI-CELLULAR CORtical UNIT: THE BARREL

**Observations.**

A. General appearance

In layer IV there are multi-cellular cytoarchitectonic units which because of their three-dimensional character we choose to call barrels. The first four photomicrographs (Figs. 1-4) illustrate the barrels and their component parts.

* Obtained from the Roseo B. Jackson Laboratories, Bar Harbor, Me. 04609.
** Obtained from Hadley-Clark, Inc., Burlington, Md. 20750.
\(^4\) Celloidin is a purified colloid used manufactured by Merck, Darmstadt, W. Germany (U.S. agent: Brinkman Instruments, Inc., Westbury, N. Y. 11590).
\(^5\) After embalming, brains had shrunk to exactly 80% (linear) of the size they had but while the animals were still alive, or dying from the effects of anesthesia and complete curarization amanol. This percentage is an average based upon measurement of three distances, made before and after shrinkage, between pairs of standard points in each of four animals.

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Fig. 1. Photomicrograph of a tangential section of layer IV in mouse S I cortex: the interior part of barrel field (see lower inset to Fig. 10 for location: quadrangle 1). Illustration serves to show barrel components: each barrel (b) is made up of a ring of high cell density, the side (stippling), which surrounds a less cellular area, the hollow (h). A septum (arrowhead) separates adjacent barrels; septal junction (c) occur where three or more septa meet. A wall (between hollow arrow) consists of the sides of two adjoining barrels and the intervening septum. Notice that barrel profiles are all about 100 μm in diameter and that they are roughly circular. Formalin fixation, methylene blue-CI, 50 μm thick section. Bar = 100 μm.

Fig. 2. Photomicrograph of a tangential section of layer IV, taken from the posteroangular barrel subfield (PMBA) (see lower inset to Fig. 10 for location: quadrangle 2). Illustration serves to show barrels and their components. Symbols are the same as those used in Fig. 1. Notice that the general features are the same as those shown in Fig. 1, but that the barrels are (a) bigger, (b) roughly elliptical, and (c) separated by septa perpendicular to the short barrel axis that are about the same width as septa in the anterior barrel field, and by much wider septa perpendicular to the long axis. The broken line shown at edges indicates the approximate perpendicular intersection with the sections depicted in Fig. 4. Approximate orientation of section shown in Fig. 3 is indicated by line drawn at edges of Fig. 4. Formalin fixation, methylene blue-CI, 50 μm thick section. Bar = 100 μm.

(1) Appearance in tangential sections. Fig. 1 which is taken from the anterolateral part of the cortical field in which barrels occur and Fig. 2 which is taken from the posterolateral part, show the basic plan. (See Fig. 10 for location of Figs. 1 and 2 in the barrel field.) In both figures the plane of the section coincides with the horizontal plane of layer IV.

Each unit shows a dense ring of cell bodies which has, roughly, the shape of a circle or an ellipsoid. This ring represents the sides of the barrel and surrounds an area of lesser cell density which we name the hollow. Each barrel is separated from its neighbors by a clear, nearly acellular area (fewer cells than in the hollow) which is the septum. In properly oriented sections, a septum can be seen as separate a barrel from its neighbors. The point at which three or more septa join is the septal junction. That the barrel is a unit, side and hollow inclusive, is emphasized in Fig. 3 in which the peculiar staining property that these units sometimes exhibit in the combined Golgi-Nissl method is demonstrated. In this preparation a substance in the barrel which is absent from septa and adjacent layers stains light blue; the septa in this preparation stand out very clearly.

Under some conditions such as low power microscopy, oblique orientation of sides in the section, or very thick sections, the septum between two barrels cannot be visualized; the two sides blend together. We call the apparent structure produced by the seeming disappearance of the septum, the wall. A wall always includes the adjacent sides of two neighboring barrels and the intervening septum even though these finer details may not readily be appreciated. The overall pattern of walls produced by many barrels crammed together is one of a cell dense net (see Fig. 10).

(2) Appearance in coronal sections. Fig. 4 is a coronal section through the posterior portion of the area showing the appearance of barrels in a plane perpendicular to the pin mater. Because of geometric probabilities the septa are less clearly
where sides and septum were clearly visible. Measurements were made from sections taken from several hemispheres. The sections were chosen for optimal orientation—i.e., precisely tangential to the anterior and posterior parts of the field, respectively. For reasons given below (Part III), the measurements from PMBSF are segregated from those of the anterior part of the barrel field. Measurements were also made from coronal sections which were taken from the PMBSF because, when sections are cut perpendicularly to the pial surface, the barrel component appears clearer in the PMBSF.

In spite of possible differences in fixation, shrinkage, animal size, etc., there was good correspondence in the ranges of measurements obtained from sections of different specimens. The ranges of dimensions are summarized in Table I. (As our measurements are preliminary and few, we have elected to give ranges rather than more sophisticated statistical indices.)

As would be expected from inspection of the barrel field as a whole (Figs. 10, 11, 14), major axes of barrels \((a)\) are greater in PMBSF than in the anterior part of the field *. In PMBSF, minor axes of barrels are similar in length to those measured in the anterior part of the field. Major barrel axes in PMBSF differ greatly from minor axes, whereas in the anterior part of the field they are of the same order of magnitude. Since the coronal sections are cut approximately along the minor axes \((b)\) of barrels in PMBSF, one would expect the measurements in the coronal sections to be in the range of anterior barrel diameters and indeed they are. The ranges of barrel axes measured directly on the specimen (shown in Table I) are comparable with the ranges of barrel axes determined on photomicrographs of two whole fields. Measured on the

* We have chosen to speak of postero medial barrel subfield (PMBSF) as a specific region of the barrel field for (1) strictly morphological reasons (see Part III), and (2) a likely functional correlation. We have refrained from naming the rest of the field in the absence of (1) distinct morphological criteria, and (2) a clear understanding of the functional relationships of barrels there. More sophisticated studies will probably allow future delineation of the 'anterior part of the barrel field'. A naming would only be complicated by a pre-existing less rational terminology.

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** Table I **

<table>
<thead>
<tr>
<th>Barrel Component</th>
<th>Tangential Sections</th>
<th>PMBSF (um)</th>
<th>Coronal Sections</th>
<th>PMBSF (um)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrel (axes)***</td>
<td>120 - 70</td>
<td>300 - 70</td>
<td>40 - 40</td>
<td></td>
</tr>
<tr>
<td>Hollow (axes)***</td>
<td>120 - 60</td>
<td>200 - 40</td>
<td>40 - 80</td>
<td></td>
</tr>
<tr>
<td>Sides</td>
<td>10 - 5</td>
<td>13.5 - 5.5</td>
<td>15 - 5</td>
<td></td>
</tr>
<tr>
<td>Septum</td>
<td>7.5 - 2.5</td>
<td>20 - 2.5</td>
<td>12.5 - 2.5</td>
<td></td>
</tr>
<tr>
<td>Wall</td>
<td>20 - 10</td>
<td>40 - 15</td>
<td>35 - 15</td>
<td></td>
</tr>
</tbody>
</table>

* Uncorrected for shrinkage due to fixation. Larger values rounded off to 10 um; smaller values rounded off to 2.5 um.
** Approximately 15 measurements were made to determine each range.
*** Ranges presented for tangential sections include both major and minor axes.

---

Fig. 4. Photomicrograph of a coronal section through the PMBSF. Symbols are same as those used in Fig. 1. Roman numerals indicate cortical layers. P = pia. Notice how the sulci are somewhat less easily distinguishable here than in Figs. 1 and 2. The line drawn at edges indicates approximate perpendicular intersection with the section depicted in Fig. 2. Approximate orientation of section of Fig. 4 is indicated by a line drawn at edges of Fig. 2. Formalin fixation, methylene blue-Alcian blue, 30 microns thick sections. Bar = 100 um.
micrographs, the dimensions a and b of the largest barrel are 380 \(\mu m\) and 170 \(\mu m\), respectively, and of the smallest barrel 100 \(\mu m\) and 30 \(\mu m\), respectively (Fig. 5).

Because a hollow only lacks the thickness of two sides (about 5 \(\mu m\) each), one may expect that the range of hollow diameters will be about 10 \(\mu m\) less than that of the barrel diameters. Sides in all areas are about the same thickness. They are usually only slightly thicker than the average diameter of a single cell body that lies within them, since sides are composed of cells stacked not quite exactly one on top of the other. The width of the wall fluctuates markedly with the width of the septum. In the anterior barrel field, and in PMBSF (perpendicular to the minor axis b) septa are commonly very narrow — less than 5 \(\mu m\). These septa measure less than the diameter of most of the cell bodies seen in layer IV. In the PMBSF, the septa perpendicular to the major axis a are much wider — up to 23 \(\mu m\). The measurements were not made in the septal junctions. The discrepancies noted when measured and derived data are compared (e.g., barrel = hollow + 2 sides; wall = septum + 2 sides) are no doubt due to the smallness of the sample.

The thickness of layer IV as a whole, as measured in the coronal sections, is about 100 \(\mu m\) throughout the barrel field.

2. Distribution of barrels according to relative size. The sizes of all barrels in two complete barrel fields were measured and plotted.

Measurements were made from the photomicrographs shown in Figs. 10 and 11. We have made the assumption that, in tangential sections, barrels are roughly either elliptical or circular and that their relative sizes can better be expressed by a two-dimensional parameter related to area, rather than by a one-dimensional parameter, e.g., a single axis. The measurements were made in the following manner: (1) a rule was moved about on the photograph until the maximum barrel axis a was found; (2) the midpoint of a was determined; and (3) at this midpoint, the axis b perpendicular to a was measured. The product, ab, is used as the index of relative barrel size. The expression is a function of area; it is also an approximate measure of volume, the height of layer IV being nearly the same throughout the barrel field.

In Fig. 5, we have indicated the population distributions of the products ab of the two barrel fields. Again we have chosen to plot the data from the PMBSF’s separately. There is a striking similarity in the frequency distributions from the two fields with the exception that the values for 9L are approximately one bin greater than those for 10L. (see Discussion Part III).

Notice that the barrels not in the PMBSF seem to have a normal distribution in both specimens. All larger barrels are from the PMBSF.

Discussion

A. Review of previous terminology

There have been observations in the literature on mouse neocortex which are related to the concept of the barrel. The literature review which follows is summarized in Table II. The translations into English are our own. The original terms are placed in the table (in all cases we used the author’s own phrases).

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<table>
<thead>
<tr>
<th>Author</th>
<th>Terms used by author</th>
<th>Equivalent used by us</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Vries'**</td>
<td>kleine Felder von ellipsoider oder quadratischer Form; isolierte</td>
<td>barrels (or, perhaps, only hollows?)</td>
</tr>
<tr>
<td>Dr. Olof Forsythe***</td>
<td>clouds; deeply stained spots</td>
<td>barrels</td>
</tr>
<tr>
<td>Lorente de Nó*</td>
<td>glomérulos, plástic</td>
<td>barrel</td>
</tr>
<tr>
<td>In Nissl-image</td>
<td>segmentos claros de forma quadrada</td>
<td>hollows</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>columnas (verticales)</td>
<td>walls</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>masas calloso</td>
<td>hollows, (l.'barrels?')</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>bandas claras (verticales)</td>
<td>walls</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>espacios interglomerulares</td>
<td>walls</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>demaranzamiento</td>
<td>pattern or wall?</td>
</tr>
<tr>
<td>In Golgi-image</td>
<td>de la corteza lámbrica</td>
<td>wall</td>
</tr>
<tr>
<td>Van Epp Taalman Kip***</td>
<td>clouds</td>
<td>hollows</td>
</tr>
<tr>
<td>Woolsey</td>
<td>cell-dense columns; clumps</td>
<td>walls</td>
</tr>
<tr>
<td>Labedsky and Liers*</td>
<td>cell-dense net; nestwork</td>
<td>wall pattern</td>
</tr>
<tr>
<td>Labelsky and Liers*</td>
<td>holes; 'clear' spaces</td>
<td>hollows</td>
</tr>
<tr>
<td>**</td>
<td></td>
<td>barrels (or, perhaps, only hollows?)</td>
</tr>
</tbody>
</table>

* A German version appeared one year later**.
** An English version of this work was published three years later***.
*** An English version of this work was published one year later**.

Dr. Olof Forsythe noticed 'deeply stained spots' or 'clouds' in layer IV of his area J. Although he does not provide pictures of them in either report, we must conclude from his careful description, that he was staining entire barrels. His method, we believe, led to preparations which appeared similar to the one depicted in our Fig. 3. He assumed that the 'extraordinarily deep stain' was due to a high density of fibers; it could be obtained only after formalin fixation (which was started '... in every case within 24 hours...'. after death of the animal). Dr. Olof Forsythe also measured his clouds; they were '1/3-1/2 mm in diameter'. He remarked: 'They are separated by extremely narrow strips with the usual light tint of the background and concluded that in this 'differente' cortex these 'narrow strips' (or septa) form the continuous phase whereas the 'clouds' (our barrels) are separate entities. Apparently, Dr. Olof Forsythe did not distinguish hollow from side.

Two papers of Rose are pertinent to this discussion. In the first* 'walls' are depicted although no mention is made of them.

Lorente de Nó recognized the 'small islands' of De Vries, or 'clouds' of Dr. Olof Forsythe. He thoroughly analyzed them, in both Nissl- and Golgi-preparations.

As stated above, he described these structural entities as glomérulos. The reader is referred to Table II for an item by item comparison of Lorente de Nó's nomenclature with that presented by us.

Rose, in a second paper — a classic atlas of the mouse cortex — describes, in layer IV of his area T1, a 'cleft-like arrangement of cells' (cleft — ball of yarn) which probably refers to the pattern suggested by walls when seen in face in coronal sections. In one of his plates (ref. 47, plate 10, in area T1, at labe 'K') some of his walls were optimally cut so that they are shown to be comprised of two sides separated by a septum! In another illustration (plate 11), a typical 'cleft-like arrangement' is seen. In view of the distinction we make between PMBSF (with large barrels) and anterior part of the barrel field (with small barrels); it is interesting to note that Rose, in the section by section account of mouse cortex, writes that the cleft-like arrangement is more prominent in the posterior sections through T1 than in the anterior section!

Van Epp Taalman Kip used Dr. Olof Forsythe's field J — in the course of a comparative, quantitative investigation of the neocortex of a small group of mammals — partly because its clouds made recognition of the field in some of the animals used relatively easy. For him, clouds were 'lighter patches with less cells'; his clouds, we believe, were hollows.

The cell-dense columns and the cell-dense net proposed in a more recent description of the barrel field can now be equated with walls and with the wall pattern as revealed in tangential sections. To the favorably disposed eye, the photomicrographs (ref. 68, Figs. 5, 6, 7) demonstrate the component parts of the walls and septum. On re-examination of the slides from which these figures were taken, all components described in the present paper can be seen, although not so clearly as in the presently described material.

Labedsky and Lierse have stained mouse cortex for succinic dehydrogenase (SDH). They describe loci in layer IV with increased SDH activity in a region which they identify as Rose's area T1 (which we, in turn, identify as the barrel field). In the photomicrographs accompanying their remarks, these loci look somewhat similar to the barrels of some of our Golgi-Nissl stained material (see Fig. 3). We had previously suggested, on the basis of similar SDH positive loci reported by Friede in the guinea pig and the rat, that what we now call hollows in mouse cortex might be centers for increased SDH activity. The dimensions of the SDH positive loci in Labedsky and Liers's paper suggest that the barrels, or perhaps the hollows, are the areas of elevated SDH activity. A countercartaining method demonstrating perikarya would be necessary to confirm this impression.

B. Dimensions

When measuring barrel diameters, at least two kinds of artifacts may be involved. The first is the result of making tangential sections which are not exactly parallel to the pia. Since the plane of layer IV is curved, the plane of section in a series of sections from one specimen is never tangential for all of the barrels measured. If we assume that in a perfectly tangential section the cross-section of a barrel is...
circular, it follows that a non-tangential section will intercept the barrel obliquely and result in an elliptical profile. In general, the cross-sectional area of a barrel will be greater the more oblique the plane of intersection. Consequently, the barrel areas will be systematically greater than they would have been had it been possible to derive the data from sections exactly tangential for every barrel. Secondly, since the barrel is tapered at top and bottom its greatest (= 'true') diameter in the exact tangential plane can only be obtained when the 'mid-height' region of the barrel is included in the section. This does not cause a serious problem if the section includes all, or almost all, of layer IV — such as in specimen 9L (see Fig. 11) where the sections are 100 μm — since one will see the widest portion of the barrel. But if one takes thinner sections — 50 μm, as in 10L — one frequently measures the tapered diameter at either the top or the bottom rather than the widest, 'mid-height' diameter. The effect of this second bias is opposite to the first, in that it yields cross-sectional barrel areas which are systematically less than the maximum tangential barrel area.

Both of these effects may be at play in producing the slight but definite discrepancy in the population distributions shown in Fig. 5. It is difficult to say which one of the two effects has the greater influence. Of course, the differences may also be due to something cruder in nature, such as a fixation artifact or simply variation in brain size, rather than to these geometric subtleties.

C. Bruegel's barrel

We shall now consider several questions which the barrel concept raises.

First, do sheets of cell bodies separate hollows from adjacent cortical layers? In other words, do the barrels have tops and bottoms? Tops — that is, a sheet of cells separating the hollow from layer III — are somewhat evident in coronal sections (Fig. 4) although they certainly are not striking. As one approaches layer IV from the pia in tangential sections, patches of greater cell density foreshadow the appearance of subjacent hollows (see Fig. 9). The absence of similar dense zones separating hollows from layer V suggests that there are no bottoms. This point awaits careful analysis of perikaryal arrangements in and about individual barrels.

Second, what may be the cellular make-up of the barrels? High magnification observations with the light microscope suggest that both neurons and neuropils comprise the cellular populations of sides and hollows. We are presently looking at Golgi and Golgi-Nissl preparations to answer some questions about the types of neurons found in the barrels, and plan to apply glia stains.

Third, what are the synaptic contacts of the various neuronal elements of these multi-cellular cortical units? Lorente de Nó's has supplied data which suggest that barrel neurons have intimately entangled axonal and/or dendritic fields confined to a barrel and that there are cells in adjoining cortical layers and in remoter areas of the brain (thalami?) which have projections arborizing specifically within barrels. Certainly, it is likely that there is exchange of information between neurons in a given barrel but the specific nature of such exchange as well as its anatomical substrate has yet to be determined. Proof that loci of high SDH activity found by Labecky and Liersch are identical to barrels or hollows would be a further contribution to

**Fig. 6. Schematic representation summarizing the three-dimensional appearance of the multi-cellular cortical cytoarchitectonic unit of the hand region of mouse S1, the barrel. Compare with Figs. 1–4. Drawing is a detail from a 176th century etching after Bruegel's painting 'Fair of St. George's Day'29. This 17th century representation of the barrel is a geographically adequate rendition of the unit. The cortical barrel may have undergone some distortions from Bruegel's model in various places in the field but the basic elements, sides and hollows, are shared by all. The field as a whole — discussed in Part II — may be thought of as being composed of many barrels placed tightly side by side much as, in Bruegelian dimensions, might happen at a brewery.**

**PART II. THE BARREL FIELD**

**Observations**

**A. Position of barrel field**

(1) Determination of reference planes. Early in this study it became apparent that a system was needed which would enable us to exactly describe the location of the barrel field in respect to the whole brain. In the absence of obvious and constant surface landmarks in the somatosensory mouse brain, it is difficult to locate a point to which tangential sections can be made. The reference planes described here comprise a useful system by which to orient three-dimensionally the plane containing the barrel field to the whole brain. We defined three reference planes (Figs. 7A, B) onto which the barrel field was projected and to which the predominant orientation of the field was related by known angles (see Fig. 7). By holding the specimen so that the microtome knife passes in the plane of that predominant orientation, i.e. the 'best' plane (see below), it is possible to obtain, in only a few 50 or 100 μm sections, the entire barrel field (see Figs. 7C, D). That 'best' plane can be described by its relations to any two of the three reference planes. In Fig. 7 we have drawn

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Fig. 7. Drawings to show the barrel field and its relationship to three reference planes and three standard projection views of the mouse brain. A, The three standard projection views of the brain: anterior, dorsal and lateral. Lines indicate reference planes: sagittal (S), horizontal (H) and coronal (C). Lateral projection views show 'high points', x and y, of the dorsal aspect of the hemisphere used to determine the horizontal planes (H). B, Drawing of a rat skull brain showing spatial relations of reference planes to specimens. Planes have same labels as in A, C, Same projection views as in A to which are now added the location of the barrel field (hatched area). Angles drawn in the anterior and lateral projection views (interlaced lines) are those used together with the coronal and sagittal planes, respectively, to obtain the 'best' plane of section (T) when the barrel field is to be approached tangentially. D, Whole specimen with reference planes (S, H, E), barrel field (hatched), and 'best' plane of tangential section (T-darkly stippled) placed according to the angles given in C.

our reference planes in relation to a specimen and shown the relation of the 'best' tangential plane to the whole brain and to the reference planes. Details of the placement of these planes are given in the legend to Fig. 7. The importance of specimen orientation for taking sections that will optimally demonstrate the barrels cannot be overemphasized.

(2) Projection of the barrel field onto the cortical surface. Figs. 7C and D, and the upper insets to Figs. 10 and 11, show the surface projections of the barrel field. The long axis of the field deviates from the sagittal plane so that the anterior portion is more lateral than the posterior. Also, the field follows the rather sharp lateral curvature of the hemisphere so that the plane of the field is domed. However, most of the barrel field is present on the gently arcing dorsal surface of the hemisphere. (A happy consequence of this is that only one or two 100 μm sections oriented tangen-
more precise than 150 μm in defining any border that bounds a portion of the barrel field not the PMBSF. There are at least two reasons for ambiguity: one technical and the other biological.

With regard to the first point: any process that obscures individual barrels tends to confine the field observed since we depend upon the clear-cut appearance of barrels to identify the field. In thick sections (100 μm), barrels are obscured as the plane of section becomes less tangential to the pia. Since the plane of the barrel field is domed, the (non-PMBSF) boundaries will be lost in any specimen in which the plane of section is tangential to the center of the field. Similarly, if one makes the plane of section tangential to the pia over one border, with the aim of seeing that border more clearly, the opposite border will be lost.

We have circumvented this problem by using many specimens and by altering slightly the plane of section in each subsequent specimen. In Fig. 8, the outline of the barrel field as determined in a specimen in which the plane of section was tangential to the center of the field has been drawn. That portion of the barrel field to which the plane of section is truly tangential will be the first to appear in the series. The locations of those areas approached truly tangentially have been indicated by hatching. Most of the field has been approached tangentially since there is little area without any hatching. Therefore, we think it is not for technical reasons that the boundaries are vague.

The problem of boundary determination can also be alleviated by making thinner sections (50 μm) since there is less ‘cell-body interference’ from layer IV in places where that layer becomes less parallel to the plane of section.

The second reason for the difficulties experienced in identifying the borders is biological. As we could determine in sections cut tangentially to the pia over the anterior border region, these anterior borders simply are not sharp; barrels lose their definition: they ‘fade out’ into a homogeneous layer IV.

C. Barrel field reconstructions

Two techniques were used to reconstruct barrel fields: camera lucida drawings and photomicrographic collages. From these reconstructions we (a) compared field morphology in different animals, (b) measured field area, (c) counted the numbers of barrels in the field, (d) assessed variations of barrel shape and arrangement, and (e) made the measurements of barrel diameters presented in Part I.

(1) Camera lucida drawings. Drawings reconstructing barrel fields from nine cerebral hemispheres were made at a magnification of 73.5 X with the use of a Wild drawing apparatus attached to a Zeiss CFL microscope. The technique was to take the first (most superficial) tangential section having barrels and to carefully draw the barrel sides. Prominent blood vessels were drawn to serially relate the sections (see Fig. 9, arrows). The following (deeper) section was aligned by the use of the vessels to the drawing of the preceding section. The barrel sides from this deeper section were added to the drawing and new vessels were drawn in, if necessary. The same procedure was applied to subsequent sections in which barrels appeared, until all barrels were drawn. The use of small vessels is complicated slightly by the
Fig. 10. Photomicrographic collage reconstructed from tangential sections to show the complete barrel field of hemisphere 10L. Orientation: anterior, left; posterior, right; medial, up; lateral, down. Cortical location of barrel field is indicated, as upper inset, by stipple. Lower inset is camera lucida drawing of the same barrel field; circular and ellipsoid profiles represent barrels (the rectangles indicate location of photographs shown in Figs. 1 and 2). Notice that the large barrels of the PMSBF (at the right in the figure) are sharply delineated from the adjacent homogeneous layer IV while this is not so for the anterior portions of the barrel field. A complete summary of the differences between PMSBF and rest of barrel field appears in Table III. Medially (top of figure), a notch without barrels is found indenting the field so as to nearly bisect it. (In the collage, this notch appears to extend farther laterally than it actually does — cf. lower inset — because of the shortening of the photographs.) The barrel field shown here is nearly identical to that shown in the collage of Fig. 11. See also the barrel field drawings in Fig. 14. Approximately 76 photomicrographs went into the making of this illustration. Formalin fixation, methylene blue–Cl, 50 μm thick sections. Bar = 500 μm.

Fig. 11. Photomicrographic collage reconstructed from tangential sections to show the complete barrel field of hemisphere 9L. Orientation: anterior, left; posterior, right; medial, up; lateral, down. Location of barrel field is indicated, as upper inset, by stipple. Lower inset is a camera lucida drawing of the same barrel field. Observe same features as pointed out in Fig. 10 and the near identity of this barrel field with that of 10L. Formalin fixation, methylene blue–Cl. 100 μm thick sections. Bar = 500 μm.
cortical vascular pattern, which converges toward the center of the brain from the pia. (E.g., the four marking vessels of Fig. 5 are closer together in the section of Fig. 9) than they are in the section of Fig. 9). Fortunately, discrepancies such as these are slight and can be overcome by approximation.

All drawings are shown in Fig. 14. The appearance of barrels and their arrangement in the camera lucida drawings was very similar to the photographic collages presented in Figs. 10 and 11.

(2) Photographic reconstructions. Photomicrographs of each section were made at 210 ×. The portion having barrels was photographed systematically by moving the slide with a mechanical stage, always being careful to preserve a slight overlap between adjacent frames. Collages were assembled according to the same principles which were applied to the camera lucida drawings. Low power pictures were made of each section so that the higher resolution frames could be properly oriented and so that barrels from the serial sections could be properly related (in Fig. 9 are samples of these low power photographs). In making the collages shown in Figs. 10 and 11, that section of the series was chosen which contained the greatest portion of the barrel field. All of the photographs were pieced together to give the complete picture of that section (in the case of the specimen depicted in Fig. 10, this section is the one shown in Fig. 9C). Over this first collage were placed the photographs taken from adjacent sections. These latter photographs, once accurately oriented by use of small vessels, were pieced together and glued to the composite underneath so that the best pictures of the barrels in the field were assembled in the collage. For example, in making Fig. 10, photographs from the section in Fig. 9B were placed over these from the section in Fig. 9C; and then those of Fig. 9A over the assembly of pictures from Figs. 9B and 9C. Similarly, photographs from deeper sections were pieced together and glued to the collage until all of the photographed barrels appeared on the collage in their proper relationship to one another. In still deeper sections no barrels were seen and no barrels could be photographed. In the collages, the barrel fields are surrounded by homogeneous layer IV. Throughout the procedure, the low power photographs of the entire sections were used for orientation purposes.

The orderly appearance, in Figs. 10 and 11, of the barrels packed together is as pleasing as it is striking. Stippling on the islet brain photographs shows the approximate cortical location of the field; inset line drawings indicate where barrels are.

Although the two collages were made from sections of different thickness and from hemispheres of different animals there is a nearly exact likeness. This high degree of similarity is also typical of the nine drawn barrel fields shown in Fig. 14.

The features mentioned in the description of the boundaries are clearly visible.

It will be noted that generally the anterior and lateral barrels are circular, smaller, fall within a narrow size range, and are apparently randomly packed. A subtle difference is that barrels in the middle of the field are slightly larger and appear to have a more orderly arrangement than those farther forward. While not so clear as in the PMBSF, the organization is suggestive of rows or concentric areas with their centerpoint in the middle of the lateral border of the barrel field. The possible meaning of this arrangement is considered in the General Discussion.

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STRUCTURE OF LAYER IV IN MOUSE S I CORTEX

D. Area of barrel field

Approximations of the total area of the barrel field and of the PMBSF area were obtained as follows: Seven of the nine camera lucida drawings* of the barrel fields analyzed (1 cm × 136 μm); smaller versions appear in Fig. 14) were surrounded by a line circumventing its perimeter. Another line was drawn in, separating PMBSF from non-PMBSF. These contours were transposed on millimeter paper and the areas defined above were determined with a unit of measurement of (136 μm)².

The following list summarizes the area measurements. The ranges of the areas are rounded off to (0.1 mm)².

Total field area = 2.10-2.76 mm²
PMBSF area = 0.95-1.09 mm²
The means are: Total field area = 2.52 mm²
PMBSF area = 1.03 mm²

Averagely, the PMBSF area constitutes 40.9% of the total barrel field area**.

It should be pointed out that the mean of total barrel field areas is too small for three reasons. (1) The uncertainty of the anterior borders of the barrel field (discussed above) leads to a lower estimate of the area of the anterior part of the barrel field. Consequently, the actual value

PMBSF area × 100

is smaller than the calculated 40.9%. (2) The flattening of the domed layer IV (which had occurred in the process of making the camera lucida drawings) reduces the calculated areas. (3) The foreshortening of the field by approaches from its various boundaries reduces the area included in the drawing.

E. Number of barrels per barrel field

The results of barrel counts using the camera lucida drawings are shown in the histogram in Fig. 12. The clear bars represent the total number of barrels counted in a hemisphere while the shaded portions indicate the number of barrels found in the PMBSF.

There is moderate variation in the total number of barrels per hemisphere: the number of barrels ranges between 170 and 210. For reasons stated above regarding difficulties in determining borders, the real number of barrels per hemisphere cannot be found by averaging individual counts. The real number is greater than any one of the values appearing in the histogram and could be arrived at by some accurate method of combining different fields. However, the underestimation cannot be more

* See legend to Fig. 13 for explanation of why two of the barrel field drawings could not be included in these determinations.

** In connection with a correlation presented in the General Discussion we also determined the areas of the total barrel fields including the notch (medial border of the latter being defined consistently but somewhat arbitrarily). Range: 2.44-4.02 mm²; mean: 2.79 mm². The PMBSF area constitutes 36.9% of this larger total barrel field area.

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Discussion

A. Study of cortex in tangential sections

By tradition, the use of sections tangent to the pia in the study of the cerebral cortex has been infrequent but it is not new. Retzius (Table II, Fig. 7) and Cajal (Fig. 339) used, besides countless sections cut perpendicularly to the pial surface, a few tangential sections to study the neurons of the first layer of the cerebral cortex in immature dog, and in mature cat, respectively. Klötzl and Clark used tangential sections of rat neocortex in an attempt at quantification of cytoarchitectonic characteristics. Van der Loos used the tangential approach to quantitatively analyze dendritic morphology and interconnections of individual cortical neurons and Colonnier and Wong used tangential sections to study dendrite field orientation in cat visual and acoustic cortex, respectively. To our knowledge, our study represents the first attempt to delineate a cytoarchitectonic field by the tangential approach. The barrels of the cortex of the mouse perhaps uniquely pre-dispose that cortex to

* Somewhat startlingly, these authors concluded, among other things, that the graphs resulting from their analysis ‘were valuable as anatomical descriptions’.

B. Earlier cytoarchitectonics

The findings in the present study do not appreciably alter the interpretations of earlier cytoarchitectonic studies. Fields approximately corresponding to the barrel field are Iesenschmid’s field b, De Vries’ field H, Droogeleer Fortuin’s field j, Rose’s (1912) fields 1, 2, 3, 5, 7 and 23, Rose’s (1929) field T, and T. A. Woolsey’s field of the cell-dense net (Cf. ref. 68, Discussion, Section B, and Fig. 9 for a discussion of the basis for the ‘equivalence’ of these fields with what we now call the barrel field).

C. Physiology

The relation of the barrel field to cortical localization patterns in the mouse as previously defined essentially has not been altered by the present investigations. The placement of the field in relation to cortical sensory projections is shown in Fig. 13. The barrel field is confined to the head and possibly distal forelimb regions of S I. In the General Discussion we shall consider the questions why the barrel...
field may represent a portion of SI, and how the morphologic details described in Part II—boundaries, number of barrels, areas, the anterolateral part of the barrel field, the PMBSF, the medial notch, and the barrel arrangement—may be related to physiologically derived data.

PART III. SPECIAL PROPERTIES OF THE POSTEROMEDIAL BARREL SUBFIELD (PMBSF)

Throughout Parts I and II we have segregated the posteromedial barrel subfield (PMBSF) because of its special characteristics. In this part we shall demonstrate the special qualities of this subfield and, referring to what is known of the physiology of the area, we shall suggest that it has a special function. We believe that each one of the barrels in the PMBSF is the cortical correlate of a contralateral mystacial vibrissa. Mystacial vibrissae* are associated with the important and highly specialized peripheral tactile organs which many mammals, including the mouse, have on their muzzles.

Observations

A. Boundaries of the PMBSF

A characteristic of the PMBSF is that it is sharply separated from surrounding homogeneous layer IV on three sides. The subfield provides the posterior and posteromedial boundaries of the barrel field. Its anteromedial aspect contributes the posterior border of the medial notch. The PMBSF, then, is in contact with the rest of the barrel field only at its anterolateral aspect. Fig. 14 demonstrates this.

B. Pattern of rows

The barrels of the PMBSF are arranged in a highly organized pattern of five rows. This is shown clearly in both the camera lucida drawings of all of the specimens (Fig. 14) and in the photographic collages of two of them (Figs. 10, 11). In the drawings, we have blackened only those barrels which we judge belong to the PMBSF. In every instance there is a distinct five row pattern regardless of the plane of tangential approach. Notice that four large barrels on the posteromedial border straddle the five rows.

C. Size, shape, number and separation of barrels in PMBSF

Barrels in the PMBSF are the largest in the barrel field (see Table I and Fig. 5). There is a broad size spectrum and a progressive increase in size from anterolateral to posteromedial, resulting primarily from a lengthening of major axes. Minor axes are quite constant and are very similar to the axes of circular barrels. Most barrels in the PMBSF have a distinctive ellipsoid shape (Figs. 2, 3 and 14). This is not a

* A mystacial vibrissa is a sinus hair which we operationally separate from all other sinus hairs because of (1) its location on the upper lip (moustache), (2) its very large size, (3) its movements (whisking).

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TABLE III

<table>
<thead>
<tr>
<th>Property</th>
<th>Anterior</th>
<th>PMBSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Field boundaries</td>
<td>Unclear — not resolved to less</td>
<td>Sharp — always resolved to</td>
</tr>
<tr>
<td></td>
<td>than one barrel (106 μm)</td>
<td>barrel sides</td>
</tr>
<tr>
<td>2. Organization</td>
<td>‘Random’ packing</td>
<td>Arranged in five rows*</td>
</tr>
<tr>
<td>3. Size</td>
<td>Smallest to medium — long</td>
<td>Medium to largest — long</td>
</tr>
<tr>
<td></td>
<td>axis 100–180 μm</td>
<td>axis 150–380 μm</td>
</tr>
<tr>
<td>4. Shape</td>
<td>Roughly circular on tangential</td>
<td>Frequently elliptical on</td>
</tr>
<tr>
<td></td>
<td>section</td>
<td>tangential section</td>
</tr>
<tr>
<td>5. Separation</td>
<td>5 μm septa in all directions</td>
<td>5 μm septa within a row;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 μm septa between rows</td>
</tr>
<tr>
<td>6. Numbers</td>
<td>Approximately 150</td>
<td>34–40</td>
</tr>
</tbody>
</table>

* The five rows are straddled at their medial ends by four large barrels.

geometrical artifact: the ellipsoidal shape is obtained in precisely tangential approaches (Fig. 12) which in part may be the result of the certainty with which they could be identified. Finally, barrels in adjacent rows are separated by a septum much wider than that found between barrels in the same row or between barrels in other parts of the field (Table I). It is by this greater separation that the organization into rows is emphasized (cf., e.g., Fig. 10). In Table III, these properties are summarized and contrasted with the properties of barrels of the rest of the field.

Discussion

A. Vibrissae

Because many of the unusual features of the PMBSF can be explained by assuming that a relationship exists between the barrels in the PMBSF and the mystacial vibrissae on the muzzle, we shall summarize some of the available information related to mouse and rat vibrissae.

(1) Mystacial organization. The mystacial vibrissae in the mouse and in the rat are organized in five rows nearly parallel to the bridge of the nose (see Fig. 15A). Each row has 4–7 easily identified large vibrissae. The caudal ones are long and thick. The vibrissae gradually diminish in both dimensions as they are located more rostrally. Four large vibrissae straddle the caudal ends of the five rows. Rostrally the diminution in size makes it difficult on gross inspection to be certain where the vibrissal rows stop. In our counts we are consistently able to identify 25–27 large mystacial vibrissae in the mouse; there are about 35 in the rat.67

(2) Structure. To date, the best characterization of the structures of mystacial vibrissae is Vincent’s 1913 study of the rat.64 A more recent, less detailed study has shown the vibrissal structure in the mouse to be essentially identical.64 Vincent noted that vibrissae are distinguished by: (a) large hair size, (b) dense connective tissue isolating the follicle, (c) extensive blood supply, blood sinuses (hence ‘sinus hair’). and erectile tissue, (d) striated muscles which attach to the follicular capsule (apparently responsible for the complex whisking movements described by Welker69), and (e) multiple innervation consisting of: (i) a main sensory nerve containing . . . 150 or more large modulated nerve fibers, (ii) free sensory endings at the skin surface around the hair, and (iii) extensive varicose nerves associated with the vessels (presumably having an autonomic function). Vincent concluded: ‘Histological and structural studies show that the tactile hair is a powerful organ of touch . . . ’. She speculated on how each of the structural features could contribute to the great sensitivity of the sinus hair as a tactile organ.

Recently, Vincent’s observation that some nerve endings are associated with Merkel cells has been confirmed electron microscopically.65 Andree6 made a more comprehensive ultrastructural survey of nerve endings in the rat vibrissae. He described the following types which, with one exception, are associated with the main myelinated sensory nerve of the follicle: (a) axons which branch and make contact with several Merkel cells; (b) thickest axons which have lanceolate-shaped endings occurring (i) as single straight terminals, (ii) as multiply branched terminals, and (iii) — associated with the skin nerve — as multiple, circularly placed terminals; (c) axons ending in encapsulated corpuscles. Axons with free endings are associated with the largely unmyelinated dermal nerve.

(3) Some receptive field properties of vibrissae. Recently Zucker68 and Zucker and Welker19 have examined the receptor properties of single units in the trigeminal ganglion of the rat. They have found: (a) A majority of units studied responded to stimulation of vibrissae; few units responded to stimulation of surrounding patches of common hair. (b) All units responding to deflection of vibrissae never responded to manipulation of more than one vibrissa or to surrounding common hair. (c) First order neurons from a vibrissa are capable of coding the following aspects of mechanical stimuli: (i) peripheral location, (ii) deflection direction, (iii) onset, (iv) termination, (v) amplitude, (vi) velocity, (vii) duration, (viii) repetition, and (ix) temporal pattern. (d) Stimulation of the facial nerve to simulate protraction (‘whisking’ of the vibrissae) elicited a response in only 50% of the units studied but by imposing a barrier across the path of the protracting vibrissae almost all units could be driven. Clearly the rat, and probably the mouse, has a great deal of specific information coded even at the level of the single first order neuron. The morphological basis for much of this specificity may be found in the four different kinds of receptors, each having different geometric dispositions, as elegantly described in Andree’s electron microscopical study6 of sinus hair innervation.

B. Physiology

(3) Evoked potential studies in the mouse. We have noted that in the macro-electrode evoked potential maps of the mouse cortex the area of the whole barrel field is within the head and face region of SI69 (see Fig. 13). Although we previously had felt that the vibrissal representation was in the midportion of what we now call the ‘barrel field’, the physiological evidence was not sufficiently fine-grained to definitely support that belief. (It should be remembered that the overall dimensions of

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the barrel field are 1100 × 2800 μm, and that the electrode tip diameter of 300 μm used in the evoked potential study is, in comparison, quite large.)

Largely based upon recent microelectrode studies in the rat(21,28), a reappraisal of the figureine maps of the earlier study(41) was made. The reappraisal showed the original data to be compatible with the belief that the vibrissae are represented in the posterior half of the barrel field. The musculus in Fig. 13 has been modified to convey this belief.

(2) Microelectrode maps of Sm I in the rat. The microelectrode cortical localization patterns in the mouse are quite comparable to those in the rat(45). In the beautiful studies of Carol Welker(45), Sm I has been delineated by the microelectrode unit cluster technique. What interests us here is the representation of the mystacial vibrissae in the rat cortex.

C. Welker has found that in the cerebral cortex of the rat each vibrissa is individually represented; the organization is in rows which are slightly oblique to the coronal plane; the more dorsal vibrissae are represented posteriorly and the more ventral vibrissae are anterior; more caudal vibrissae are represented mediolaterally while the rostral ones are lateral. She has estimated(45) that a microfield for the representation of only one large mystacial vibrissa is about 0.6 × 0.6 mm, which is quite comparable with one large PMBSF barrel if one allows for shrinkage. Most unit clusters were found in layer IV.

C. Behavior

Along with her morphological studies, Vincent(48) studied the importance of vibrissae in the behavior of white rats as determined by their ability to run mazes. She concluded that the mystacial vibrissae significantly influenced the performance of rats in mazes. Without vibrissae the animals appeared lost in their environment, and ran the maze more slowly. They held their bodies close to the floor, apparently depending for information on increased contact of their mouth parts and ventral surfaces with the maze. If vibrissae were removed from one side of the snout the rats could run the maze almost as fast as with all vibrissae present. However, they then preferred to stay with their intact side close to the maze edges or walls.

W. Welker(50) has used high speed motion picture techniques to study the ‘whisking cycle’ in rats. Whisking movements are repetitive, high frequency (up to 7/sec) movements (successive waves of vibrissal protrusion and retraction) of the entire mystacial pad which are apparently involved in the rat’s active investigation of the environment. Although many mammals and apparently all rodents have prominent mystacial vibrissae not all activity ‘whisk’. All of the other animals in which ‘barrels’ have been seen — gerbil(15,44,57), chinchilla(9) and mouse — are whisking rodents, except the guinea pig(19,24).

D. ‘Differentiation of structure means differentiation of function.’ (Hughekins Jackson)(39)

(1) The PMBSF is the cortical correlate of the contralateral mystacial vibrissae. The rationale for the belief that the PMBSF is directly related to the vibrissae follows two lines of reasoning: morphological and physiological.

(a) The mystacial vibrissae are the biggest and most complex tactile structures in the mouse (e.g., structure, information encoding; importance in behavior). The biggest and most distinctive barrels are in the PMBSF.

(b) The mystacial vibrissae are arranged in five rows. The barrels of the PMBSF are arranged in five rows. Four barrels straddle the ends of the five barrel rows just as four vibrissae straddle the ends of five rows of vibrissae (Fig. 15).

(c) There are about 25-25 easily identified mystacial vibrissae — about 4-7 per row. There are 34-40 barrels in the PMBSF, 4-9 per row. Since our counts on the face may not include the smallest mystacial vibrissae, and our PMBSF barrel counts may include small-vibrissa barrels, the ratio of barrels to the vibrissae may well be one to one.

(d) Microelectrode studies in the rat(51) show that each vibrissa is represented separately in the cortex in areas of 600 μm by 600 μm — dimensions which are in the range of our axis measurements. The functional organization of the rows of mystacial vibrissae is very similar in location, orientation and number to the barrels in the PMBSF (compare C. Welker(51) Fig. 2 with our Figs. 10 and 11).

(e) Microelectrode studies in the mouse(51) are compatible with the belief that vibrissae are represented in the area of the PMBSF.

For these reasons we propose (a) that the PMBSF is indeed the cortical anatomical correlate of the mystacial vibrissae; (b) that the dorsal and ventral vibrissae are represented in the postero-lateral and anterior portions of the PMBSF, respectively, while the rostral and caudal vibrissae are represented in the lateral and poste-
medial aspects of the field, respectively; and (c) that there is a one to one relationship between the big barrels in the PMBSF and the mystacial vibrissae on the muzzle. In Fig. 15 we have summarized this hypothesis by placing photographs of mystacial vibrissae and of the PMBSF side by side.

(2) Anatomical segregation of vibrissae-related neurons at subcortical stations. Perhaps, the barrels in the PMBSF represent the terminal stations in a chain of anatomically distinct representations of individual vibrissae. Walker and co-workers have shown such anatomically segregated representation in dorsal column nuclei, thalamus, and cerebral convolutions, of the highly sensitive volar surface of the raccoon forepaw and digits. Although in the rat detailed studies have been made on the organization of the trigeminal ganglion, of nuclei of termination of sensory trigeminal trigemini, and of thalamic relay nuclei, evidence for anatomical segregation of single-vibrissae related regions has not been found at any of these subcortical stations. This absence of anatomical segregation may, in fact, reflect the lack of directed anatomical attention given to this problem. To our knowledge, no such information is available for the mouse, either.

GENERAL DISCUSSION

A. Why the term barrel?

As we have stated before (cf. Table I) we believe that the cytoarchitectonic units which we call barrels are essentially equivalent to the ‘clouds’, or ‘deeply stained spots’, of Droogierev Fortuny and to the glomerulae described by Lorente de Nó. In spite of this we have three reasons for changing the earlier terminology: (1) In comparison with ‘cloud’ or ‘glomerula’, the term barrel gives a more accurate impression of the three-dimensional structures described in this paper. (2) ‘Glomerula’ is a term that has been applied to other well studied areas of the CNS — of the cerebellum and of the olfactory bulb — as well as to extracranial (e.g., renal) structural entities. The term ‘barrel’ prevents confusion with these other structures. (3) The word ‘glomerula’ describing CNS morphology has been applied to areas rich in nerve cell processes but devoid of perikarya. Our hollow is an area relatively devoid of perikarya; the barrel, however, is more: a hollow surrounded by a side, the latter consisting mainly of perikarya. The term ‘barrel’ emphasizes the inclusion of cell bodies in our concept of the barrel as a cytoarchitectonic unit.

B. How much periphery does the barrel field represent?

If it is agreed that the PMBSF is the site of representation of the large mystacial vibrissae, then what may the other barrels represent? C. Walker, as part of her rat study, has done a careful depletion of the rat head to determine the number of sinus hairs. Not only are there the large mystacial vibrissae but there are smaller hairs about the nares, along the upper lip, inside the mouth on what C. Walker calls the furry buccal pad, and along the lower lip. There are also long sinus hairs at the angle of the jaw, over the eyes and at the wrist. The total counted by C. Walker in the rat is about 220. This is in surprisingly close agreement with our barrel counts (see Fig. 12).

C. Walker found also that the cortical area which represents the mystacial vibrissae is about 35% of the total S I head region. We found the PMBSF area to be 40.9% of the total barrel field area. If one, however, includes the area of the medial notch in the total barrel field area, the PMBSF occupies 36.9% of that area — a remarkable fit with the physiologically derived percentage.

Summarizing, there is close correlation between the barrel field location and the S I head representation in the mouse; there is good agreement of barrel counts in the mouse and sinus hair counts in the rat; and there is similarity between the ratios PMBSF/whole barrel field in mouse, and mystacial vibrissae area/total head area in rat. All of these points support two conclusions: (1) The barrel field represents part of the head. (2) Our specific hypothesis, i.e. ‘one PMBSF barrel represents one large contralateral mystacial vibrissa’, can be extended to ‘one barrel represents one contralateral sinus hair’. This extended hypothesis may explain several features of the barrel field described in Part II. The slightly larger barrels in the middle portion of the field which seem to be arranged in radiating rows and area are probably related to the sinus hairs of the upper lip; and the medial notch may be related to some of the mouth parts.

The problem remains as to where the common hairy skin of the head, and especially that at the base of the sinus hairs, is represented cortical. The same problem pertains to lips and mouth parts. Several possibilities exist: (1) the representation is in the barrel field; (2) the representation is beside the barrel field (payne in the notch); (3) there is no representation. Precise microelectrode analyses are necessary to settle this point.

C. Barrels emphasize two principles of cortical organization

(1) The concept of vertical or columnar cortical organization. Over a century ago, the earliest histological study of the cerebral cortex revealed a radial pattern of fibers and cell bodies. These patterns were amply confirmed in countless, subsequent cytoarchitectonic and myeloarchitectonic studies.

Lorente de Nó’s classic and comprehensive paper, of 30 years ago, on cerebral cortex structure contains an earlier, clear statement of the hypothesis that cerebral cortex is organized in vertical columns composed of interrelated cells extending through the full thickness of gray matter. We found it interesting that Lorente de Nó’s hypothesis was largely based on observations made in the mouse, partly in what we now call the barrel field.

Mountcastle, on the basis of microelectrode studies of S I cortex of the cat, was the first to propose functional ‘columnar’ organization of the cerebral cortex. A clear-cut morphologic correlate of the columns was not found. Recently Mountcastle and Darian-Smith summarized this concept as follows:

1. When microelectrode penetrations are made normal to the cortical surface and parallel to the vertical columns of cells, all neurons encountered are of the same modality type. 2. Neurons encountered in vertical penetrations are related not only to the same modality but also to nearly identical peripheral receptive fields. 3. When peripheral stimuli activating each... cortical neuron are carefully positioned at the
most sensitive loci in their receptive fields, the latencies of response of cells in the different cortical layers fall within a narrow range, within 2 to 4 msec of each other. Cells of layers III and IV are activated earliest by the thalamocortical volley . . .

Mountcastle's hypothesis, on the basis of generations not normal to the pia, that these functional columns were all 200 μm in diameter. Since 1957, the functional columns of cerebral cortex have been confirmed in several systems in several species: S I and S II of monkey; S II and S III of cat; I of cat; I of cat; II of cat; and of monkey; V I of cat; and of monkey; II of cat; it has been hitherto proposed for A I of cat. The diameters of functional columns are reported to be between 100 and 500 μm.

An attempt to find the morphological correlates of the columns in monkey S I only confirmed observations made many years earlier. Studies of tangential sections through cat V I cortex and cat A I II and auditory association cortex have been revealing only in that the tangential extents of the pyramidal dendrite systems are of the same order of magnitude as functional columns. They have failed to demonstrate a substrate for functional columns. Colonnier devoted a paper to the demonstration of the morphological basis of the functional vertical columns. In that study, many interesting arguments converged but the demonstration of clear morphological correlates of the physiologically columns was not made.

Recently, Hubel and Wiesel placed small lesions in one layer of the lateral geniculate body, and found bouton degeneration in monkey V I. The pattern of degeneration, when seen from the pia, looked like a mosaic of parallel 300 μm wide 'stripes' which the authors interpreted as corresponding to their 'eye-preference columns'. Degeneration was primarily in layer IV.

The range of barrel diameters is fully within the range of the diameters of the functional columns physiologically demonstrated in the studies listed above.

On the basis of the preceding arguments we believe that the barrels of the S I cortex of the mouse are the morphological manifestation, in layer IV, of functional columns defined by electrophysiological means.

(2) The concept of laminar cortical organisation. Barrels are found only in layer IV. We now turn to evidence which refutes the 'columnar concept' and which suggests that, while all cortical layers in a S I column are related to the same modality and locus, it is layer IV which appears to be the first receiving station. Layers above and below, it seems, are involved in the subsequent manipulation of information.

(a) Morphologically, all of the primary sensory cortices are distinguished by their highly developed layer IV.

(b) Specific thalamo-cortical afferents terminate in layer IV (Cajal — Golgi analysis; Polyak — Marchi degeneration study; Nauta — Nauta degeneration study; Hubel and Wiesel — Fink and Heilbronn degeneration study).

(c) Barrels in the mouse cortex apparently receive specific thalamic afferents.

(d) Physiologically, it is likely that input from the thalamus is primarily to layer IV.

(i) C. Weller's clusters of units, found under light Nembutal anesthesia, are all from layer IV. Such anesthesia depresses spontaneous cortical activity, and reduces intracortical synaptic transmission.

(ii) Using very light or no anesthesia, Powell and Mountcastle, as well as Hubel and Wiesel, found a greater density of driven units in the middle layers (III and IV) than in other layers.

(iii) In their investigation of cat area 17, and especially of monkey area 17, Hubel and Wiesel were able to find only 'simple' units in what they determined to be layer IV. There were 'simple' units above and below layer IV, but also 'complex' and 'hypercomplex' units. Their interpretation was that the 'simple' units represented the first cortical station while 'complex' units seemed to be the result of further neuronal interaction. They noted that the stratification on the basis of 'simple' and 'complex' units emphasizes laminar cortical organization within a given column.

(iv) Mountcastle and Darian-Smith stated: 'Cells of layers III and IV are activated earliest by the thalamo-cortical volley while those of the deeper layers discharge with larger latencies. (see their point 3, above).

D. Why do mice have barrels?

We have emphasized that technical details (specimen orientation, plane of section, section thickness, etc.) have been important factors in the elucidation of barrel structure and in the delineation of the barrel field. Here, we shall consider how the nature of the sensory surface and the nature of the mouse itself may be responsible for the prominence of barrels in mouse cerebral cortex.

(1) The nature of the sensory surface and nerve organs. The sinus hairs of the rodent (and other mammals) are markedly different from a continuous two-dimensional sensory surface such as skin and retina. Sinus hairs, taken together, form a punctate rather than a continuous sensory surface. They are clearly separated on the peripheral and the mouse may not require the mechanism of afferent inhibition to refine localization. The precise pre-determined location of first order neuron receptive fields such as those associated with sinus hairs would permit a greater degree of order in central neuronal populations such as in barrels to form a more efficient neuronal circuitry.

For a given cortical locus, functional cortical columns in monkey and cat S I cortex represent only one of several 'submodalities'. Presumably, the information concerning all 'submodalities' for a given locus is integrated elsewhere (e.g., precentral gyros of the cat). However, as Zucker and Weller, have demonstrated (see above), the receptors associated with a single vibrissa of the rat are capable of encoding information which may be regarded as representing many 'submodalities'. The morphological substrates of these 'submodalities' probably are the four receptor types and the patterns of their arrangement described by Andés. The projection of many 'submodalities' to a single S I cortical locus — which the 'one barrel-size sinus hair' hypothesis implies — could emphasize local cortical synaptic organization by permitting early cortical integration of the different 'submodalities'.

(2) Possible factors making barrels prominent in mouse cortex. First, it may be that in mice sinus hairs are relatively more important in gathering information than they are in other mammals having whiskers. Concomitant with their importance could be a relatively greater development of corresponding cortex. Second, but not nec-

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sarily complimentary to the first point, the size of the mouse cerebral cortex may approach a minimum. In line with this thought, we hesitate to reason that neurons in layer IV of the mouse cerebral cortex are perhaps just barely enough for the cortical manipulation of sensory data. Larger animals have more than such a marginally low number of layer IV neurons in S I; indeed, their entire cortex, S I included, is more voluminous. The increase in the number of elements may tend to obscure the basic pattern; one cannot see the groves for the forest.

E. Future studies

Our hypothesis that specific and constant cortical structures are related to specific and constant peripheral receptors can be tested in several ways. We have begun sensory deprivation experiments. A study of animals in which there is a genetically determined variation in the number of sensory organs is planned.

A number of problems which have hitherto successfully resisted a fine-grain analysis may now be investigated. These problems are related to the general questions: what are the basic elements of sensory cortex circuitry, and how does the cerebral cortex process sensory data?

The hypothesized vibrissa-barrel relationship may make an integrated approach to these questions possible. Correlated analysis by means of Golgi and electron microscopic techniques should clarify sensory cortex circuitry; microelectrode studies of the barrel field should shed light on the functional aspects of cortical information processing; combined ontogenetic histochimical and behavioral studies could correlate the level of synaptic activity with the appearance of certain patterns of behavior.

We anticipate that after the interdisciplinary pursuit of some of the above ideas we shall have occasion to paraphrase the Bard who sings:

'But mice, rats and gerbils and such small deer
Were Tom's and Hendrikk's food for many a long year'.

SUMMARY

1. Formalin-fixed, Nissl-stained and Cov-fixed, Golgi-Nissl-stained preparations of sections cut coronally and tangentially to the pia have been used to elucidate the organization of layer IV in mouse S I cerebral cortex.

2. A multicellular cortical cytoarchitectonic unit is described which is as tall as layer IV, roughly cylindrical, 100–400 μm in diameter, with its center line normal to the pia. Because of their characteristic shape we call these units barrels. Each barrel is composed of a ring of cells, the side, which surrounds a less cellular central hollow.

3. The nearly acellular area surrounding each barrel and separating adjacent barrels is called the septum. Ranges of measurements of the barrels and their components are given and the size distribution of the barrels in two barrel containing regions is given. The unit is discussed in relation to observations reported in several earlier accounts of the mouse cortex.

4. The cytoarchitectonic region which contains the barrels has been determined by a tangential approach. Its exact place on the cortical surface, its outline, appearance,
UNIT RESPONSES AND CONVERGENCE OF SENSORY STIMULI IN THE HYPOTHALAMUS

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INTRODUCTION

Electrophysiological studies using both the evoked potentials and unit recording have demonstrated the projection of sensory inputs into the hypothalamus. Though some topographic differences were observed for the distribution of the short latency evoked potentials in various regions, many of the units examined in the ventromedial and posteromedial hypothalamus have been found to respond to more than one sensory modality. It is therefore the purpose of this report to describe in detail the results of an individual statistical analysis of the responsiveness, the changes in firing rate and the convergence of sensory stimuli, in a large number of single cells in the hypothalamus. Furthermore, an analysis was made of the common properties of the units in intact cats and in animals with different brain lesions.

MATERIALS AND METHODS

The data presented in this report are based upon experiments performed on 138 cats weighing between 2.5 and 3.5 kg. Out of these animals chronic bilateral brain lesions were performed on 34 cats: in 6 animals in the globus pallidus, in 17 animals in the caudate nucleus and in 11 animals in the midbrain reticular formation. The operative procedures were performed under pentobarbital anesthesia and the same operative and recording techniques and the evaluation of data were used as in our previous studies. Extracellular unit activity was recorded with stainless steel microelectrodes with a tip of 1-3 μm. Statistical analysis of a total of 141 units was performed in the region of the anterior (F 12-13; L 0.5-1.5; H 3-5) and posterior (F 8.5-9; L 0.5-1; H 2-3) hypothalamus: 1084 cells in intact animals and 327 cells in animals with extrahypothalamic brain lesions. Single, photo, acoustic and sciotic stimuli were delivered every 2 sec, at the beginning of each oscilloscope sweep, and their effects on the spontaneous activity of the units was studied during 2000 msec after the stimulus. Only units which were tested for a period of 200 sec were included in this study. Single sciotic and brain stimuli were delivered by a Grass...
the barrel field are 1100 × 2800 μm, and that the electrode tip diameter of 300 μm used in the evoked potential study is, in comparison, quite large.)

Largely based upon recent microelectrode studies in the rat17,18, a re-appraisal of the figure maps of the earlier studies18 was made. The re-appraisal showed the original data to be compatible with the belief that the vibrissae are represented in the posterior half of the barrel field. The musculus in Fig. 13 has been modified to convey this belief.

(2) Microelectrode maps of Sm 1 in the rat. The microelectrode cortical localization patterns in the mouse are quite comparable to those in the rat17. In the beautiful studies of Carol Welker19, Sm 1 has been delineated by the microelectrode unit cluster technique. What interests us here is the representation of the mystacial vibrissae in the rat cortex.

C. Welker has found that in the cerebral cortex of the rat each vibrissa is individually represented; the organization is in rows which are slightly oblique to the coronal plane; the more dorsal vibrissae are represented posteriorly and the more ventral vibrissae are anterior; more caudal vibrissae are represented medially while the rostral ones are lateral. She has estimated that a 'microfield' for the representation of only one large mystacial vibrissa is about 0.6 × 0.6 mm, which is quite comparable with one large PMBSF barrel if one allows for shrinkage. Most unit clusters were found in layer IV.

C. Behavior

Along with her morphological studies, Vincent16 studied the importance of vibrissae in the behavior of white rats as determined by their ability to run mazes. She concluded that the mystacial vibrissae significantly influenced the performance of rats in mazes. Without vibrissae the animals appeared lost in their environment, and ran the maze more slowly. They hele their bodies close to the floor, apparently depending for information on increased contact of their mouth parts and ventral surfaces with the maze. If vibrissae were removed from one side of the snout the rats could not run the maze as fast as with all vibrissae present. However, they then preferred to stay with their intact side close to the maze edges or walls.

W. Welker18 has used high speed motion picture techniques to study the 'whisking cycle' in rats. Whisking movements are repetitive, high frequency (up to 7/sec) movements (successive waves of vibrissal protration and retraction) of the entire mystacial pad which are apparently involved in the rat's active investigation of the environment. Although many mammals and apparently all rodents have prominent mystacial vibrissae not all active 'whisk'. All of the other animals in which 'barrels' have been seen — gerbil19, rat18,25,57, chinchilla and mouse — are whisking rodents, except the guinea pig18,23,19,54.

D. 'Differentiation of structure means differentiation of function'? (Hughlings-Jackson)18

(1) The PMBSF is the cortical correlate of the contralateral mystacial vibrissae. The rationale for the belief that the PMBSF is directly related to the vibrissae follows two lines of reasoning: morphological and physiological.

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(1) Appearance in tangential sections. Fig. 1 which is taken from the antero-lateral part of the cortical field in which barrels occur: and Fig. 2 which is taken from the postero-medial part, show the basic plan. (See Fig. 10 for location of Figs. 1 and 2 in the barrel field.) In both figures the plane of the section coincides with the horizontal plane of layer IV.

Each unit shows a dense ring of cell bodies which has, roughly, the shape of a circle or an ellipsoid. This ring represents the side of the barrel and surrounds an area of lesser cell density which we name the hollow. Each barrel is separated from its neighbors by a clear, nearly acellular area (fewer cells than in the hollow) which is the septum. In properly oriented sections, a septum can be seen to separate a barrel from its neighbors. The point at which three or more septa join is the septal junction. That the barrel is a unit, side and hollow inclusive, is emphasized in Fig. 1 in which the peculiar staining property that these units sometimes exhibit is the combined Golgi-Nissl method is demonstrated. In this preparation a substance in the barrel which is absent from septa and adjacent layers stains light blue; the septa in this preparation stand out very clearly.

Under some conditions such as low power microscopy, oblique orientation of sides in the section, or very thick sections, the septums between two barrels cannot be visualized; the two sides blend together. We call this apparent structure produced by the seeming disappearance of the septum, the wall. A wall always includes the adjacent sides of two neighboring barrels and the intervening septum even though less fine details may not readily be appreciated. The overall pattern of walls produced by many barrels clumped together is one of a cell dense net (see Fig. 10).

(2) Appearance in coronal sections. Fig. 4 is a coronal section through the posterior portion of the area showing the appearance of barrels in a plane perpendicular to the pia mater. Because of geometric probabilities the septa are less clearly visible.

Fig. 1. Photomicrograph of a tangential section of layer IV in mouse SI cortex: the anterior part of barrel field (see lower inset to Fig. 10 for location; quadrangle 1). Illustration serves to show barrel components: (a) barrel (B) is made up of a ring of high cell density, the side (stippling), which surrounds a less cellular area, the hollow (H). A septum (arrowheads) separates adjacent barrels; septal junction (C) occurs where three or more septa intersect. A wall (between hollow arrow) consists of the sides of two adjoining barrels and the intervening septum. Notice that barrel profiles are all about 100 µm in diameter and that they are roughly circular. Formalin fixation, methylene blue-C, 50 µm thick section. Bar = 100 µm.

Fig. 2. Photomicrograph of a tangential section of layer IV, taken from the postero-medial barrel subfield (PMBMF) (see lower inset to Fig. 10 for location; quadrangle 2). Illustration serves to show barrels and their components. Symbols are the same as those used in Fig. 1. Notice that the general features are the same as those shown in Fig. 1, but that the barrels are (a) bigger, (b) roughly elliptical, and (c) separated by septa perpendicular to the short barrel axis that are about the same width as septa in the anterior barrel field, and by much wider septa perpendicular to the long axis. The broken line shown at edges indicates the approximate perpendicular intersection with the section depicted in Fig. 4. Approximate orientation of section shown in Fig. 2 is indicated by line drawn at edges of Fig. 4. Formalin fixation, methylene blue-C, 50 µm thick section. Bar = 100 µm.

Fig. 3. Photomicrograph of a tangential section of layer IV, taken from the PMBMF. Symbols are the same as those used in Fig. 1. This is a Cresyl violet-Golgi-Nissl-stained preparation which shows the cresyl staining property of the hollows (light stipple) which sometimes is obtained by this method. Notice that septa stand out. 70 µm thick section. Bar = 100 µm.
seen in this kind of preparation. Commonly sides with intervening septa blend to form a wall. Nevertheless, sides and septa may be appreciated in portions of the section shown. Hollows stand out well. A geometric detail frequently observed in coronal sections is that barrels are tapered at both top and bottom. Consequently, septa are wider at upper and lower ends of layer IV than they are at 'mid-height'.

B. Barrel dimensions

(1) Measurement of components. Measurement of barrels and their component parts was undertaken to ascertain approximate sizes and to obtain some idea of size variations within the barrel field itself. Those measurements were made directly in the microscope using a 40 x objective, a 12 x ocular, and an ocular reticle (unit of measurement 2.4 μm). Formalin-fixed tangentially cut Nissl-stained material was used. Barrel and hollow diameters were obtained by orienting the reticle scale along the long axis of the barrel thus measuring its major axis (a), and by measuring the minor axis (b) at the midpoint of a (see also next section). Thickness measurements of walls, sides and septa were made by orienting the reticle perpendicularly to the wall at a point

TABLE I

<table>
<thead>
<tr>
<th>Barrel components*</th>
<th>Tangential sections</th>
<th>Coronal sections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior (μm)</td>
<td>PMBSF (μm)</td>
</tr>
<tr>
<td>Barrel (axes)***</td>
<td>130 - 70</td>
<td>300 - 70</td>
</tr>
<tr>
<td>Hollow (axes)***</td>
<td>120 - 60</td>
<td>290 - 60</td>
</tr>
<tr>
<td>Side</td>
<td>10 - 5</td>
<td>13.5 - 7.5</td>
</tr>
<tr>
<td>Septum</td>
<td>7.5 - 3.5</td>
<td>20 - 2.5</td>
</tr>
<tr>
<td>Wall</td>
<td>20 - 10</td>
<td>40 - 15</td>
</tr>
</tbody>
</table>

* Uncorrected for shrinkage due to fixation. Larger values rounded off to 10 μm; smaller values rounded off to 2.5 μm.
** Approximately 15 measurements were made to determine each range.
*** Ranges presented for tangential sections include both major and minor axes.

where sides and septum were clearly visible. Measurements were made from sections taken from several hemispheres. The sections were chosen for optimal orientation—i.e., precisely tangential to the interior and posterior parts of the field, respectively. For reasons given below (Part II), the measurements from PMBSF are segregated from those of the anterior part of the barrel field. Measurements were also made from coronal sections which were taken from the PMBSF because, when sections are cut perpendicularly to the pal surface, the barrel components appear clearer in the PMBSF.

In spite of possible differences in fixation, shrinkage, animal size, etc., there was good correspondence in the ranges of measurements obtained from sections of different specimens. The ranges of dimensions are summarized in Table I. (As our measurements are preliminary and few, we have elected to give ranges rather than more sophisticated statistical indices.)

As would be expected from inspection of the barrel field as a whole (Figs. 10, 11, 14), major axes of barrel (a) are greater in PMBSF than in the anterior part c of the field*. In PMBSF, minor axes of barrels are similar in length to those measured in the anterior part of the field. Major barrel axes in PMBSF differ greatly from minor axes, whereas in the anterior part of the field they are of the same order of magnitude. Since the coronal sections are cut approximately along the minor axes (b) of barrels in PMBSF, ones would expect the measurements in the coronal sections to be in the range of anterior barrel diameters and indeed they are. The ranges of barrel axes measured directly on the specimen (shown in Table I) are comparable with the ranges of barrel axes determined on photomicrographs of the whole fields. Measured on the

* We have chosen to speak of postero-medial barrel subfield (PMBSF) as a specific region of the barrel field for (1) strictly morphological reasons (see Part III), and (2) a likely functional correlation. We have refrained from naming the rest of the field other than (1) distinct morphological criteria, and (2) a clearer understanding of the functional relationships of barrels there. More sophisticated studies will probably allow future parcellation of the 'anterior part of the barrel field'. A naming would likely be complicated by a pre-existing nos ecological terminology.
more precise than 150 μm in defining any border that borders a portion of the barrel field not the PMBSF. There are at least two reasons for ambiguity: one technical and the other biological.

With regard to the first point: any process that obscures individual barrels tends to constrict the field observed since we depend upon the clear-cut appearance of barrels to identify the field. In thick sections (100 μm), barrels are obscured as the plane of section becomes less tangential to the pia. Since the plane of the barrel field is domed, the (non-PMBSF) boundaries will be lost in any specimen in which the plane of section is tangential to the center of the field. Similarly, if one makes the plane of section tangential to the pia over one border, with the aim of seeing that border more clearly, the opposite border will be lost.

We have circumvented this problem by using many specimens and by altering slightly the plane of section in each subsequent specimen. In Fig. 8, the outline of the barrel field is determined in a specimen in which the plane of section was tangential to the center of the field that has been drawn. That portion of the barrel field to which the plane of section is truly tangential will be the first to appear in the series. The locations of these areas are approached tangentially and have been indicated by hatching. Most of the field has been approached tangentially since there is little area without any hatching. Therefore, we think it is not for technical reasons that the boundaries are vague.

The problem of boundary determination can also be alleviated by making thinner sections (50 μm) since there is less 'cell-body interference' from layer IV in places where that layer is less parallel to the plane of section.

The second reason for the difficulties experienced in identifying the borders is biological. As we could determine in sections cut tangentially to the pia over the anterior border region, these anterior borders simply are not sharp; barrels lose their definition: they fade out into a homogeneous layer IV.

C. Barrel field reconstructions

Two techniques were used to reconstruct barrel fields: camera lucida drawings and photomicrographic collages. From these reconstructions we (a) compared field morphology in different animals, (b) measured field area, (c) counted the number of barrels in the field, (d) assessed variations of barrel shape and arrangement, and (e) made the measurements of barrel diameters presented in Part I.

(1) Camera lucida drawings. Drawings reconstructing barrel fields from thionin-stained hemispheres were made at a magnification of 150 X with the use of a Wild drawing apparatus attached to a Zeiss GFL microscope. The technique was to take the first (most superficial) tangential section having barrels and to carefully draw the barrel sides. Prominent blood vessels were drawn to serially relate the sections (see Fig. 9, arrows). The following (deeper) section was aligned by the use of the vessels to the drawing of the preceding section. The barrel sides from this deeper section were added to the drawing and new vessels were drawn in, if necessary. The same procedure was applied to subsequent sections in which barrels appeared, until all barrels were drawn. The use of small vessels is complicated slightly by the

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**Fig. 9:** Photomicrographs of three serial tangential sections for hemisphere 10L, Orientation: anterior left; posterior, right; medial, up; lateral, down. A is the most superficial, C is the deepest of the three. Sections illustrate the clear and pictographic display of barrels that one observes in each preparation. From sections such as these the photographic collages and the camera lucida drawings of entire barrel fields were made. Arrows point to some of the vessels which, appearing in subsequent sections, are commonly used to spatially relate serially cut sections to one another. Formalin fixation; methylene blue–Cr, 50 μm thick sections. Bar = 2 mm.

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Fig. 10. Photomicrographic collage reconstructed from tangential sections to show the complete barrel field of hemisphere 1L. Orientation: anterior, left; posterior, right; medial, up; lateral, down. Cortical location of barrel field is indicated, in upper inset, by stipples. Lower inset is camera lucida drawing of the same barrel field; circular and allipoid profiles represent barrels (the rectangles indicate location of photographs shown in Figs. 1 and 2). Notice that the large barrels of the PMBSF (at the right in the figure) are sharply delineated from the adjacent homogenous layer IV while this is not so for the anterior portions of the barrel field. A complete summary of the differences between PMBSF and rest of barrel field appears in Table III. Medially (top of figure), a notch without barrels is found indenting the field so as to nearly bisect it. (In the collage, this notch appears to extend farther laterally than it actually does — cf. lower inset — because of the shortcomings of the photography.) The barrel field shown here is nearly identical to that shown in the collage of Fig. 11. See also the barrel field drawings in Fig. 14. Approximately 70 photomicrographs went into the making of this illustration. Formalin fixation, methylene blue–CI, 50 μm thick sections. Bar — 500 μm.

Fig. 11. Photomicrographic collage reconstructed from tangential sections to show the complete barrel field of hemisphere 9L. Orientation: anterior, left; posterior, right; medial, up; lateral, down. Location of barrel field is indicated, in upper inset, by stipples. Lower inset is a camera lucida drawing of the same barrel field. Observe some features as pointed out in Fig. 10 and the near identity of this barrel field with that of 10L. Formalin fixation, methylene blue–CI, 100 μm thick sections. Bar — 500 μm.