ONTOGENY OF BARRELS IN MUROID SENSORY NEOCORTEX: TIME COURSE, RELATION TO BEHAVIORAL DEVELOPMENT, AND DEPENDENCE UPON PERIPHERAL INPUT

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TIME COURSE, RELATION TO BEHAVIORAL DEVELOPMENT, AND
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ABSTRACT

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By

William Lee Weller, Jr.

A collection of discrete barrel-shaped, multicellular subunits comprising a cytoarchitectonic field (the "barrel field") exists within the primary somatic sensory area of the cerebral cortex of Muroid rodents. A functional relationship of the "barrels" to the sinus hairs or vibrissae on the head is suggested by their spatial arrangements and by the organization of projections to the cortex from vibrissa follicles. The discrete and identifiable nature of the vibrissa follicles and the cortical barrels makes this neuroanatomical specialization particularly valuable for investigating organization, function, and ontogeny of cerebral cortex, as well as relationhips between neural and behavioral development. The present study includes fundamental observations of the time of appearance of the barrel field and the sequence of morphological changes in the barrel field during its development, placed in the context of the changing behavioral repertoire. Further, the dependence of the formation of barrels on vibrissa follicles was experimentally assessed.

The sequence of changes occurring in the barrel field in the neocortex of wild-caught white-footed mice Peromyscus leucopus during their postnatal development was determined by study of Nissl

stained sections of brains of different ages which had been sectioned tangentially to the cortical surface overlying the barrel field. Primordial barrels are first seen on the fifth postnatal day and barrels appear to be essentially mature by the eleventh postnatal day.

Observations of various behavior patterns involving vibrissae provided the behavioral context of barrel development. The time of appearance of barrels succeeds the onset of function of the tactile and olfactory senses and the appearance of nose twitching (a component of exploratory sniffing behavior); it precedes onset of function of the auditory and visual senses, the appearance of crawling, walking, and extensive grooming; it corresponds to the time of appearance of coordinated repetitive movements (whisking) of the mystacial vibrissae (another component of sniffing behavior).

The effects on the mature barrel field pattern, of cauterization of small groups of vibrissa follicles on the first and fifth postnatal days, was assessed in both Peromyscus leucopus and Mus musculus. Disruptions in the cortical barrel pattern were geometrically related to disruptions in the pattern of vibrissa follicles produced by their cauterization. This effect occurred in the barrel fields of mice subjected to lesions on the first postnatal day, but not in those subjected to lesions on the fifth day. When a follicle was absent, so was its barrel; but when a follicle was reduced in size, its barrel was either missing, reduced, or enlarged.

The development of cortical barrels of Muroids occurs over about the same period that marked changes occur in the cytology, ultrastructure, electrical activity, enzymatic activity of rat neocortex. This period

also approximates the time over which the rudiments of adult behavior patterns become established in mice and rats. The developmental changes in barrels and the barrel field suggest that each barrel owes its shape to the profuse terminal arborizations of a discrete bundle of thalamocortical axons, the geometrical relationship between neighboring bundles of axons, and the mutual physical influence of neighboring barrels. The results of peripheral lesions clearly establish a functional (morphogenetic) relationship between vibrissa follicles and cortical barrels in the same pattern suggested by analyses of morphology and somatotopic projections.

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By

William Lee Weller, Jr.

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This thesis is dedicated to my children, Jim and Amy, who like to watch the little mousies and who sacrificed more during the course of this project than perhaps anyone else.

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I want to make known, in all sincerity, my regrets over the disruption of so many mouse families.

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TABLE OF CONTENTS

LIST OF TABLES..... v

LIST OF FIGURES..... vi

INTRODUCTION..... 1

METHODS..... 4
A. Animals..... 4
B. Histological treatments..... 7
C. Cytoarchitecture..... 8
D. Behavior..... 10
E. Peripheral lesions..... 11

RESULTS..... 13
A. Definitions..... 13
1. The barrel field..... 13
2. The barrel..... 13
3. Vibrissae..... 15
B. Normal histology (P. leucopus)..... 18
1. The barrel field..... 18
2. The barrel..... 22
C. Behavior (P. leucopus)..... 23
1. Sensory modalities..... 23
2. Vibrissae movements..... 23
3. Vibrissal orienting..... 26
4. Nose movements..... 27
5. Locomotion..... 27
6. Grooming..... 28
7. Comparison with Mus..... 29
D. Peripheral lesions (P. leucopus and M. musculus). 30
1. Skin..... 30
2. Neocortex..... 30
3. Peripheral-central correlations..... 34

DISCUSSION..... 41
A. Development of barrels and other aspects of cortical ontogeny..... 41
B. Development of behavior..... 44
C. Peripheral lesions..... 45
D. Functional relationship between barrels and vibrissa follicles..... 48
E. Functional parcellation of the barrel field..... 49
F. Morphogenesis of barrels..... 53

LIST OF REFERENCES..... 56

LIST OF TABLES

Table Page

1. Age distribution of mice available for study..... 6

LIST OF FIGURES

Figure		Page
1.	Analysis of the three dimensional arrangement of layer IV neurons in muroid somatic sensory cortex.	2
2.	The arrangement of vibrissa follicles in mouse skin.....	9
3.	Descriptive parcellation of the barrel field.....	14
4.	The location and arrangement of vibrissae.....	16
5.	Histological appearance of primordial barrels in the cortex of a fifth day P. leucopus.....	20
6.	Histological appearance and reconstructed arrangements of cortical barrels in P. leucopus at different ages.....	21
7.	Summary of the development of observed behaviors.....	24
8.	Histological appearance of disruptions of the cutaneous pattern of lesioned mystacial follicles....	31
9.	Comparison of peripheral arrangement of mystacial vibrissa follicles and the cortical arrangement of barrels: first day lesion.....	32
10.	Comparison of peripheral arrangement of mystacial vibrissa follicles and the cortical arrangement of barrels: fifth day lesion.....	35
11.	The arrangement of barrels after vibrissa depilation.	36
12.	Correlation of the locations of damaged follicles and barrel field disruptions.....	37
13.	Assignment of notation of barrels.....	38
14.	Specific correlation of vibrissa lesions and barrel field disruptions.....	40

LIST OF FIGURES
(continued)

Figure | Page

15. Micromapping data and localization of a single electrode penetration within the barrel field of *P. leucopus* 50

16. A functional parcellation of the muroid barrel field: hypothesized correspondence between groups of vibrissae and groups of cortical barrels... 52

INTRODUCTION

Recently a cytoarchitectonic field in the neocortex of the mouse (Mus musculus) has been described in great detail[59]; similar fields have also been observed in the neocortex of other rodents[51]. This field is characterized most obviously by the arrangement of nerve cell bodies in layer IV, which can be considered to be a mosaic of many subunits, each of which consists of numerous cell bodies arranged in the shape of a hollow cylinder or barrel (Figure 1). This cytoarchitecture is coextensive with a portion of the head representation in the primary somatic sensory region (SmI) of the cortex[5]. Furthermore a precise correspondence exists between the spatial pattern of the larger subunits of the field ("barrels") and the spatial pattern of the follicles of the larger mystacial vibrissae. Electrophysiological investigation of a similar field in the rat suggests, in addition, the existence of a functional relationship between barrels and vibrissa follicles[51] such that there is a one-to-one correspondence between barrels and follicles.

This anatomical specialization offers unique opportunities for investigating cortical organization, cortical function, and cortical ontogeny, mainly because of the discrete and identifiable nature of the component parts (barrels) and the discrete and identifiable nature of the related peripheral structures (vibrissa follicles). It is likewise appropriate for investigation of the relationship between neural and behavioral development[13,46], since vibrissae figure prominently in the exploratory behavior of many

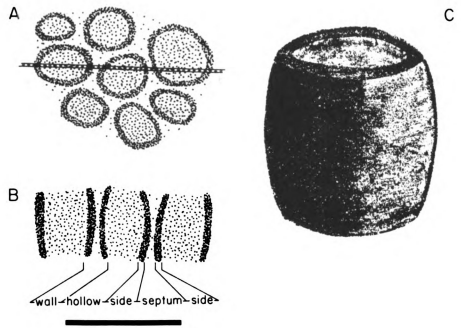

Figure 1. Analysis of the three dimensional arrangement of layer IV neurons in muroid somatic sensory cortex.

A. Schematized appearance of barrels as seen in tangential section. Cell-dense rings (sides) enclose less cell-dense interiors (hollows). Sides are separated from each other by cell-sparse septa. Dashed line indicates the relative location of the coronal plane shown at B.

B. Schematized appearance of barrels as seen in coronal section. Sides appear as two parallel cell-dense bands separated widely by a less cell-dense hollow and narrowly by a cell-sparse septum. A wall comprises two neighboring sides and the intervening septum. The hollows appear wider in the middle than at either the top (toward layer III) or bottom (toward layer V). Scale bar is 200 μm.

C. Drawing of the three dimensional shape (barrel) inferred by Woolsey and Van der Loos[59] from the arrangements of cells described in A and B.

rodents[52]. Indeed, investigation of the many aspects of the ontogenesis of neural structures is one means of establishing at least some aspects of their function[2].

This study is intended to provide information about the time of appearance of the barrel field and the sequence of morphological changes in the field which occur during development, especially in the context of the changing behavioral repertoire. In addition, this study provides a demonstration of the influence of vibrissa follicles on the development of the barrel field, a specific example of its utility for investigation of problems of neural ontogeny.

METHODS

A. Animals

Animals used in this study were laboratory mice (Mus musculus) and white-footed mice (Peromyscus leucopus novaeboracensis). While the nervous system of mice of the genus Peromyscus has been studied very little,[12,19,20,24,36,39,40] many other aspects of individuals of this genus have been studied extensively (e.g. geographical distribution, life history, behavior, psychology, physiology, anatomy, genetics: see reference 25).

Observations in this laboratory[56] of the brains of several mice of this genus (among them Peromyscus leucopus novaeboracensis) have shown the presence of cortical barrels essentially identical in description and distribution to those of Mus musculus[59].

P. leucopus is easily obtainable locally in the wild condition, which is desirable so as to avoid the possibility of inadvertently studying a distorted neural system peculiar to a particular inbred strain of laboratory animal[43]. P. leucopus is easily cared for, breeds well in captivity, and has a favorable gestation period (22 to 37 days[45]) and a reasonable litter size (1 to 7). The use of a species of Peromyscus allows the future elaboration of this study to considerations of intrageneric, interspecific differences and similarities in cortical ontogeny and function, since there are about 50 recognized species in this genus.

White-footed mice were live-trapped in wooded areas on and near the Michigan State University campus. These wild-caught animals were pair-mated and bred in the Peromyscus colony of

Professor John A. King. Females which were pregnant when trapped were mated only after their young were weaned. The 78 progeny of these wild-daught mice, designated F_1 wild-caught, constituted the group of P. leucopus available for study.

Seven pregnant albino laboratory mice (Mus musculus, Swiss Webster strain) were obtained from Spartan Research Animals, Inc., Haslett, Michigan. The 87 progeny of these females constituted the group of M. musculus available for study.

Young from each litter were sacrificed at varying ages, usually before the 21st postnatal day (weaning). The age distributions of the animals available for study are shown in Table 1.

Cages housing pregnant Peromyscus were examined at least twice daily (9am and 5pm)for the presence of litters. In the absence of contrary evidence (e.g. when parturition was in progress) young were considered to have been born at the middle of the time period between the last two examinations. Consequently there could be as much as 16 hours of uncertainty in the actual age of some specimens. To offset this uncertainty the young of each litter were sacrificed, lesioned, and/or observed at the same time of day at which they were initially found.

The cages of pregnant Mus were examined at least twice daily (9am and 9pm) but usually at other times as well (about 1pm and 5pm). The maximum uncertainty in age of any one animal was therefore twelve hours. The age of most animals, however, was known to within six hours. Experimental manipulations were performed at the same time at which the complete litter had been found.

Table 1

Age distribution of mice available for study

Postnatal Age (days)	Mus musculus	Peromyscus leucopus
1	5	3
2	5	2
3	5	3
4	5	3
5	7	3
6	7	4
7	7	3
8	7	3
9	7	3
10	7	4
11	4	9
12	6	3
13	4	3
14	2	3
15	5	3
16		3
17		4
18		2
19		2
20		1
21		1
21	2	8
Total	87	74*

*Four additional P. leucopus used for behavioral observations were not sacrificed. They were observed daily during the first nine days.

B. Histological treatments

All tissues subjected to histological analysis were fixed by immersion in formal-saline solution (one part formalin and nine parts 0.9% NaCl solution). By immersing the tissue in formalin, adequate fixation was achieved in the regions of interest (neocortex and skin) and even in more deeply lying regions of the brain. Leaving blood in the blood vessels gave texture to the otherwise featureless surface of the pallium, which was a decided advantage in orienting the brain for sectioning and in determining the position of the barrel field in relation to the pallial surface. Furthermore, small vibrissa follicles could be distinguished from pelage follicles more easily if the blood were allowed to remain within the blood sinuses encircling each vibrissa follicle[33,44,49].

After at least three days of immersion in formalin, the entire brain was removed from the skull. Each brain was then photographed in standard views and cleanly divided along the mid-sagittal plane to allow more rapid histological processing. This processing entailed dehydration in a graded series of ethanol solutions and embedment in celloidin, Serial sections of each hemisphere were cut tangentially to the cortical surface, as described by Woolsey and Van der Loos[59], at 10, 12, 15, 20, 25, or 50 μm depending on the size (age) of the brain. These sections were stained with thionin (1.0% in aqueous solution) according to a variation of the Nissl method in order to show the distribution of cell bodies.

Gross inspection and histological analysis were used to determine the spatial arrangements of vibrissa follicles in the skin. After the facial skin was dissected free from the head, the nerves, muscles, and connective tissue attached to the vibrissa follicles on the inner (dermal) side of the skin were removed to expose the dermal prominences of the large follicles. Gross inspection using a dissecting microscope (X5 -X10) and transillumination easily revealed the arrangement of the largest and some of the smaller vibrissa follicles. The blood sinuses associated with each follicle were particularly evident when filled with fixed blood, and were therefore useful indicators of the postiion of a vibrissa follicle (Figure 2).

Histological processing of the skin, as of the brains, entailed fixation in formalin, brief immersion in 5% nitric acid, dehydration in ethanol and embedment in celloidin. During the dehydration and embedment, the pieces of skin were kept flattened in order to locate most of the follicles approximately in the same plane, thus requiring less effort to reconstruct their spatial arrangement. Serial sections (75 μm) of each piece of facial skin were cut tangentially to the epidermal surface and stained with a dilute solution of thionin (0.05% in aqueous solution) employing the same method used for staining the brain sections.

C. Cytoarchitecture

The stained sections of each brain processed histologically were examined (X50) for evidences of the presence of the barrel

Figure 2. The arrangement of vibrissa follicles in mouse skin. In this depilated, unstained, transilluminated skin, the arrangement of vibrissa follicles is indicated by the location and arrangement of the associated blood sinuses. Scale bar is 2 mm.

field. Such evidence included the presence of aggregates of cells qualitatively indistinguishable from those described in the brain of the adult mouse[59] as well as the presence of spatially repetitive inhomogeneities of cell distribution in presumptive neocortical layer IV. The arrangement of barrels within each barrel field thus identified was determined by graphic superimposition of barrels present in successive sections. For brains in which barrel fields or presumptive barrel fields were identified, the degree of development of the structural components (hollows, walls, septa) of individual subunits (barrels) comprising each field was assessed at greater magnifications (X125 and X300).

D. Behavior

The time of appearance and the course of development of several behavior patterns were determined by observation of mice of different postnatal ages. The stage of development of each behavioral pattern was established only once during the lifetime of any one mouse: just prior to sacrifice. The animals from which these behavioral data derive were the same animals used for studying the normal cortical cytoarchitecture.

The behavior categories for which changes were observed include: the functionality of several sensory modalities (taction, olfaction, audition, vision); locomotion; grooming; "sniffing", which includes coordinated repetitive movements of mystacial vibrissae (whisking), nose movements, and rapid respiration[52]; response to unilateral deflection of mystacial vibrissae. These

behavioral categories include behavior patterns which are prominent components of individual adult behavior, some of which entail direct use or involvement of the mystacial vibrissae (sniffing and grooming).

The tactual modality was considered to be functional on the first day that any consistent motor response could be elicited by gentle mechanical stimulation of the skin. The olfactory modality was similarly tested for functionality by observing motor responses corresponding to the presentation of various olfactory stimuli (usually the odor of formalin which consistently elicited a strong aversive response in older mice). The functionality of the auditory modality was tested by watching for the Preyer reflex[16] (ear twitch) in response to sudden sharp noises (e.g. finger snap or hand clap). The state of functionality of the visual modality was determined solely by whether the eyelids were completely separated[48].

Locomotion was considered to be a complex behavior pattern whose description depended on several factors, but predominantly two, the posture of the animal and muscle strength.

Temporal changes in grooming behavior were best characterized by which parts of the body were groomed and whether grooming occurred "spontaneously" or only after some interference by the experimenter (e.g. handling).

E. Peripheral lesions

A small group of mystacial vibrissa follicles (usually four follicles per group) was destroyed in five litters (59 animals)

of Mus musculus and three litters (11 animals) of Peromyscus leucopus. Destruction of follicles was performed unilaterally on each animal of a single litter on the same day, on either the first or the fifth postnatal day, with a Wappler cauterizing scalpel whose "blade" was an electrolytically sharpened dissecting needle. Visual guidance of the placement of the lesions was aided by use of a dissecting microscope. The large litters of Mus allowed the sacrifice of animals at one day intervals, however, for Peromyscus animals were not sacrificed before the tenth postnatal day, except for the one animal of each litter sacrificed at the time the lesions were performed.

Since destruction of follicles necessarily caused removal of the vibrissa itself (the normal mediator of follicular stimulation), the vibrissae and fur hairs on the mystacial pad were unilaterally removed from each animal in an entire litter of Mus with a commercial cosmetic depilatory lotion each day from the first through the tenth postnatal day. Animals of this litter were likewise sacrificed at daily intervals through the tenth day.

The brains and skin of these lesioned animals were prepared for histological analysis as described above. The pattern in the cortex contralateral to the lesioned follicles was examined and compared with both the pattern of barrels in the opposite ("normal") cortex and the histologically determined pattern of follicles on the lesioned side.

RESULTS

A. Definitions

1. The barrel field

Woolsey and Van der Loos[59] declined to identify more than a single morphologically distinct subfield, the posteriomedial barrel subfield or PMBSF, reasoning that any arbitrary naming of other subfields would only confuse future subdivisions of the barrel field based on functional criteria. This point is well taken, however, without some labels description of the development of the barrel field would be most cumbersome. An interim parcellation based only on morphologically distinct groupings of barrels and their respective locations should hinder very little any future functional parcellation. With this in mind, the descriptive subdivisions used in this study, as indicated in Figure 3A, are insular group (ins), peninsular group (pen), anterolateral group (al), posteromedial group (pm). The arrangement of barrels within the posteromedial group (approximately the PMBSF) is shown in Figure 3B.

2. The barrel

Woolsey and Van der Loos[59] defined the following terms in describing the structure of individual barrels as seen in tangential and coronal sections through cortical layer IV (Figure 1A and 1B):

side - a dense ring (tangential) or band (coronal) of cell bodies,

hollow - the region of lesser cell density enclosed by a side(s),

septum - the nearly acellular area separating each barrel from its neighbors,

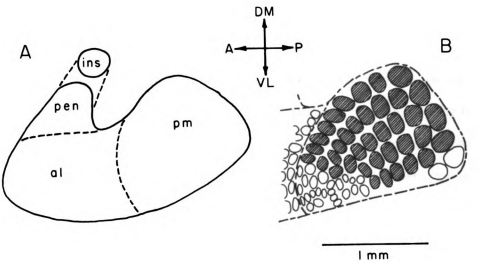

Figure 3. Descriptive parcellation of the barrel field.
A. The outline of a barrel field (left hemisphere) appears without indication of the location of individual barrels. The posteromedial division(pm) includes the posteromedial barrel subfield or PMBSF defined by Woolsey and Van der Loos[59]. The peninsular division (pen) and the insular division (ins) are sometimes joined (indicated by dashed lines). The anterolateral division (al) is comprised of the largest number of barrels, these being the smallest found within the barrel field. Arrows indicate the cardinal points of the anatomical compass: P, posterior; A, anterior; DM, dorsomedial; VL, ventrolateral.
B. The arrangement of barrels within the posteromedial division. The position and shape of each barrel seen within this division is shown; crosshatching indicates barrels in the PMBSF. The arrangements of small barrels in the anterior part of the posteromedial division (that part of pm not included in the PMBSF) is more variable than those of the large barrels in the posterior part.

septal junction - the point at which three or more septa join,

wall - the adjacent sides of two neighboring barrels, including the intervening septum (whether distinguishable or not).

3. Vibrissae

A survey of the relevant literature reveals that there has been only moderate agreement on the identification and naming of the various groups of vibrissae. The most thorough description of these groups is that of Lyne[31] who studied the distribution of vibrissae on the heads and bodies of numerous genera of marsupials. With some amendments his definitions and abbreviations are used in this study. They are listed below and indicated in Figure 4A:

1. Mystacial, My (Gk. mystax, moustache). The long "whiskers" of the snout.
2. Genal, Ge (L. gena, cheek, or broadly the lateral part of the head). Generally, vibrissae found on the cheek. One or two distinct groups may be present: When two they can usually be identified as postorbital (Pob) and postoral (Pol).
3. Supraorbital, Spo (orbit = eyesocket). Vibrissae found above the eye (also called superciliary, meaning eyebrow).
4. Labial, Lb (L. labium, lip). Vibrissae located along the upper lip, distinctly separated from the mystacial group. When not so separated they are considered part of the mystacial group.

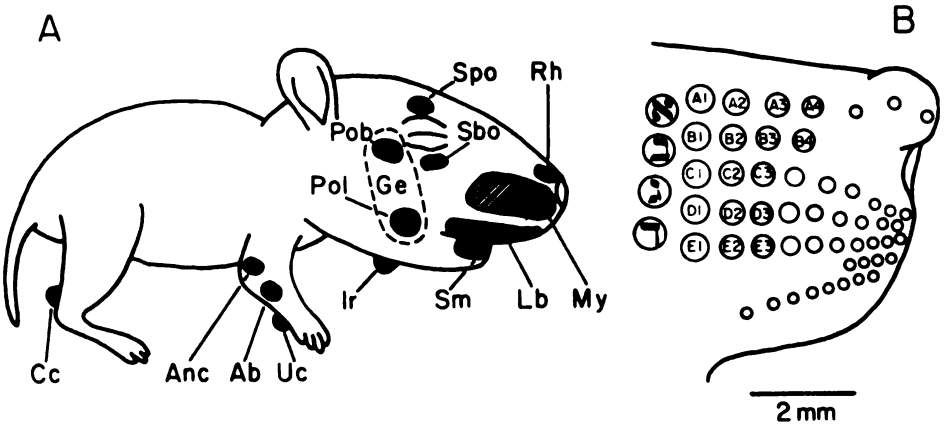

Figure 4. The location and arrangement of vibrissae.

A. Shaded regions indicate the possible locations of groups of vibrissae. These groups are rarely, if ever, all found on a single animal, rather, several or many of these groups are usually absent in any given species. The groups are named and identified with abbreviations as indicated: Rh, rhinal; My, mystacial; Lb, labial; Sm, submental; Ir, interramal; Pol, postoral; Ge, genal; Pob, postorbital; Sbo, suborbital; Spo, supraorbital; Uc, ulnocarpal; Ab, medial antebrachial; Anc, anconeal; Cc calcaneal. A labial group distinct from the mystacial group frequently is not observed. In Peromyscus and Mus these two groups are contiguous anteriorly, but are completely separated posteriorly by a strip of skin having no vibrissae at all. When a single group of vibrissae is present on the cheek, often its identification as either the postoral or the postorbital group is tenuous. Such a group is rightly called the genal group.

B. The location and size of each mystacial vibrissa follicle, except a few small anterior follicles, is indicated by a circle. Also shown are follicles of the rhinal group and some follicles of the labial group. The mystacial follicles are arranged in five, roughly posteroanterior, rows (A-E) and a single posteriorly placed dorsoventral column (א- ת). Within each row the follicles are numbered successively in the posteroanterior direction with Arabic numerals. For clarity only the larger follicles are numbered: not numbered are follicles C5-9, D5-9, and E5-10.

5. Interramal, Ir (L. ramus, branch of a tree). An unpaired group of vibrissae situated on the ventral midline between the rami of the lower jaw.
6. Submental, Sm (L. mentum, chin). Vibrissae on the chin and lower lip.
7. Suborbital, Sbo. Vibrissae found beneath the eye.
8. Rhinal, Rh (Gk. rhis, nose). Vibrissae on the dorsal aspect of the snout immediately behind the rhinarium.
9. Ulnocarpal, Uc (L. ulna, elbow; L. carpus, Gk. karpos, wrist). Vibrissae on the volar wrist.
10. Medial Antebrachial, Ab (L. antebrachium, forearm). Vibrissae on the forearm between wrist and elbow.
11. Anconeal, Anc (Gk. ankon, elbow). Vibrissae on or near the elbow.
12. Calcaneal, Cc (L. calcaneum, heel). Vibrissae on the inner aspect of the ankle.

Changes in Lyne's notation have been made only in order to provide unambiguous abbreviations.

Within some groups of vibrissae, most notably the mystacial group, the number demands (and the arrangement allows) identification of individual subgroups or individual vibrissae. The mystacial vibrissae are arranged in five antero-posterior rows and a single dorso-ventral column of four vibrissae at the posterior end of the five rows (Figure 4B). The rows, in dorsal to ventral order, have been labelled A, B, C, D, E. The ordering from dorsal to

ventral has been chosen because the dorsal rows are more easily distinguished. Likewise, within a row individual vibrissae (and follicles) have been numbered sequentially (1, 2, 3, ...) from posterior to anterior (from the largest to the smallest vibrissae). For clarity of reference, the vibrissae of the posterior column of follicles have been labelled sequentially (dorsal to ventral) with the first four characters of the Hebrew alphabet: א (aleph), ב (beth), ג (gimel), ד (daleth).

The earlier notations of Danforth[7], used more recently by Dun[11], in which the mystacial rows were identified by Roman numerals conflicts with the use of Roman numerals for identifying cortical layers which is particularly significant in the context of this study. The choice of Hebrew characters instead of the more conventional Greek characters was based on possible confusion in future studies of the relationship between vibrissae and cortical barrels in marsupials[57] whose cortical sulci have been designated with Greek letters by Ziehen[60].

The present notation is consistent with that used by Zucker and Welker[61] and by Welker[51] for the rat's vibrissae. Furthermore this notation was found to be adaptable for use with the mystacial vibrissae of the opossum[55].

B. Normal histology (P. leucopus)

1. The barrel field

The first suggestion of a barrel field can be observed in fifth day specimens: the field is evidenced by the small array of discrete, roughly circular, relatively cell-sparse regions

studding the cell-dense matrix of presumptive layer IV (Figure 5). It was not possible to reconstruct the extent of the barrel field in fifth day specimens, however. Part of the field, largely the posteromedial division, was reconstructed in sixth day specimens (Figure 6C). The barrel field as seen in successively older specimens appears gradually more distinct (Figure 6B); this is reflected in the progressively greater extents of the field which could be reconstructed (Figure 6C).

In all specimens it was difficult to determine the pattern of barrels in the insular, peninsular, and anterolateral (the anteriormost part) divisions of the field. Evidences of barrels in all parts of the barrel field were first seen in seventh day specimens. The "medial midway notch" described by Woolsey and Van der Loos[59] a barrel-free region which separates the posteromedial and the peninsular divisions, was consistently observed either as a region devoid of barrels separating two regions of barrels or as a region of apparently lesser cell density separating two regions of relatively greater cell density. This second condition was seen in fifth day specimens in which primordial barrels were found. Conclusive observations, therefore are only reported for the time of appearance of posteromedial division of the barrel field. Failure to observe the barrels of the anterior half of the field in young specimens could be a result either of a lagging schedule of development for these barrels or of the intrinsic difficulty of demonstrating the barrels of this region (which is also true in the adult brain).

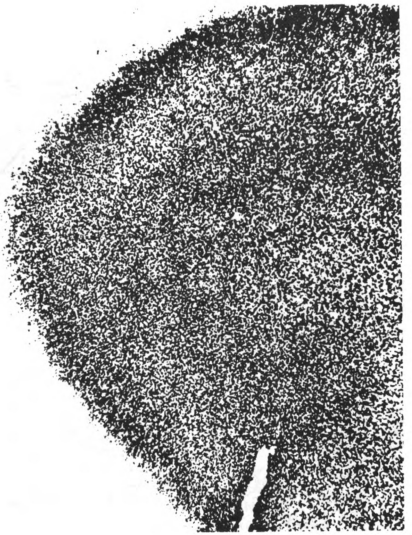

Figure 5. Histological appearance of primordial barrels in the cortex of a fifth day P. leucopus. Tangential section, 25 μm, thionin. Scale bar is 100 μm.

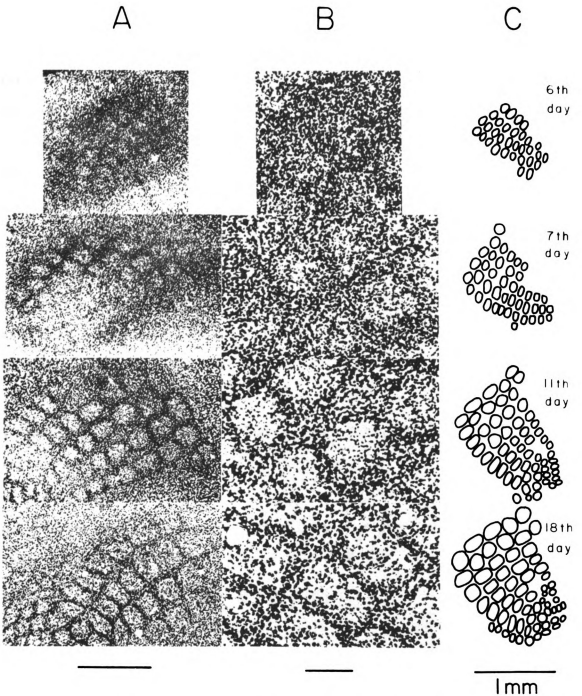

Figure 6. Histological appearance and reconstructed arrangements of cortical barrels in P. leucopus at different ages.

B. The appearance of individual barrels on the 6th, 7th, 11th, and 18th postnatal days. Scale bar is 100 μm.

A. The arrangement of barrels on the 6th, 7th, 11th, and 18th postnatal days. Scale bar is 500 μm.

C. The reconstructed arrangement of barrels within the barrel field on the 6th, 7th, 11th, and 18th postnatal days.

Tangential sections, thionin; section thicknesses: 6th day, 15 μm; 7th day, 15 μm; 11th day, 50 μm; 18th day, 25 μm.

Comparison of the reconstructions from successively older specimens shows that the earliest observed arrangement of barrels (at least within the posteromedial group) is the same as the mature pattern (Figure 6C). Following the appearance of the field, it increases gradually in size and becomes more easily distinguished from the surrounding, more homogeneous, layer IV.

2. The barrel

At the time when the barrel field can first be noted (fifth day) the cells of layer IV are not yet arranged into discrete subunits or barrels (Figure 5). Rather, the future location of each barrel appears to be marked only by a circular region slightly less cell-dense than its surround.

In seventh day specimens discrete barrels can be identified (Figure 6B). Each barrel is characterized by a well defined, relatively cell-sparse interior (the hollow) which is surrounded by a more cell-dense shell (the side) (Figure 6A). The sides of neighboring barrels appear closely apposed, yet distinguishable. They thus resemble the "walls" of Woolsey and Van der Loos[59]. Septa were observed infrequently in these specimens and usually occurred between the shorter sides of neighboring large barrels: the apposition of their longer sides appeared as walls, with no obvious intervening septa between them.

Barrels with well-defined sides, hollows, and septa can be seen in eleventh day specimens (Figure 6A). Subsequent changes in barrel appearance are largely changes in distinctness of the composite parts.

C. Behavior (P. leucopus)

1. Sensory modalities

Overt responses, (gross body movements), to tactile stimuli were found in all first day animals observed. Gentle stimulation of the perioral region resulted in less violent responses, viz. bilateral pawing at the nose. Gross or localized muscular responses to olfactory stimuli were observed in some second day animals and in all older animals. Auditory function, as judged by the presence of the Preyer reflex, was present in some twelfth day animals and in all thirteenth day animals. Visual function, as judged by complete separation of the eyelids, was present in some twelfth and thirteenth day animals and in all fourteenth day animals. While the periods of onset of function of these two sensory modalities (audition and vision) appear to overlap (Figure 7), the Preyer reflex was never absent in animals whose eyes were open. These observations on the onset of auditory and visual function fall within the ranges reported by Layne[29]; auditory, 9 - 13 days; visual, 10 - 15 days.

2. Vibrissae movements

As early as the first day small, rather subtle movements of mystacial vibrissae were seen to occur in response to their external manipulation and also in response to stroking the nose very gently. Through the third day these movements (the vibrissa reflex) were characterized as small amplitude, coordinated retractions of at least the larger more caudal mystacial vibrissae, followed by

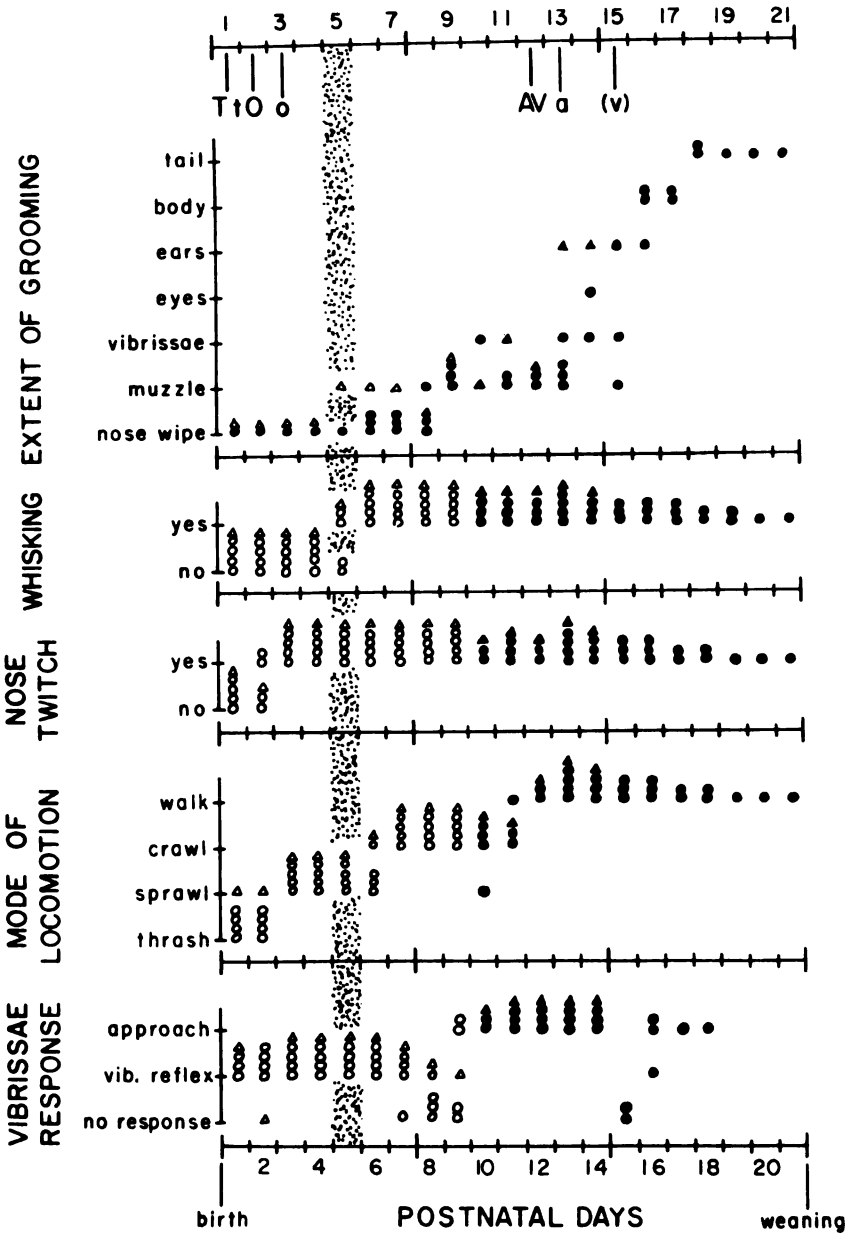

Figure 7. Summary of the development of observed behaviors. See text for description of the behavioral categories. Each solid black circle represents a different individual (Peromyscus) observed only once during its lifetime. Each of four P. leucopus from a single litter were observed once daily through the ninth day with a dissecting microscope (open circles). Each triangle represents a different individual (Mus) from the same litter. Open triangles indicate animals observed with the aid of a microscope.

a slower return to the resting position. A single vibrissa reflex followed a single stimulus; the vibrissa movements were not repetitive. These responses were often obscured by the extensive movements of the facial musculature accompanying the repetitive vocalizations emitted by most young mice removed from the nest for observation.

Some fourth day animals exhibited this vibrissa retraction reflex, while others showed protraction of vibrissae in response to the same type of stimulus. Retraction and protraction reflexes were not observed in the same animal. Protraction reflexes were present through the sixth day and were observed in some seventh and eighth day animals. A single fourth day animal showed a few successive retraction reflexes in response to a single presentation of an olfactory stimulus.

Rudimentary whisking movements of the mystacial vibrissae (i.e. repetitive, coordinated movements) were seen in some fifth day animals (Figure 7). Such whisking movements were seen in all sixth day animals observed with the aid of a dissecting microscope (they were obscured by facial movements associated with vocalizations in the first animals examined without a microscope). Whisking at this early age was of very small amplitude and occurred in brief bouts of perhaps five or six repetitions each, usually in response to the presentation of an olfactory stimulus. Whisking bouts appeared to gradually increase in duration, rate and amplitude of whisking, and incidence over the period from the sixth through the ninth days. From the tenth

through the fourteenth days (usually just before the approximate onset of auditory function) a marked increase in these characteristics of whisking was evident, so that some fourteenth and all fifteenth day animals whisked their vibrissae in a manner qualitatively indistinguishable from that of adults.

3. Vibrissal orienting

Gross body movements elicited by deflection of the mystacial vibrissae were not seen until the ninth day (Figure 7). The particular response on this day and succeeding days (through the fourteenth day) was a turning of the head toward the stimulated side. This response was altered by the interaction of visual orientation with the tactile (vibrissal) orientation from about the fifteenth day on. In these older animals the head was oriented toward the stimulus (so that the nose pointed more toward the stimulus direction) at the same time that the head was withdrawn from the stimulus, presumable to permit a good look at the disturbance. Over-reaction (strong avoidance) apparently to deflection of the mystacial vibrissae, but actually in response to abrupt stimulation of the short fur hairs on the face, was observed from the seventh through the ninth days.

The vibrissal placing response[13], observed when a mouse suspended by its tail reaches with its forepaws for an object contacting only its vibrissae, was exhibited by fourth day animals (Figure 7). Unilateral contact with the vibrissae resulted in a side-specific response (rapid flailing with the ipsilateral forepaw while the contralateral forepaw remained comparatively

motionless). Consequently it would appear that in spite of the apparent absence of overt orienting responses, the ability to orient to tactile stimulation of the mystacial vibrissae is present from the fourth day.

4. Nose movements

Specific, localized movements of the nose and nostrils (twitches) occurring independently of those of the rest of the facial musculature were first observed in some second day animals (Figure 7). These nose movements were consistently observed with the use of a microscope in third day animals. As in the case of vibrissa movements, nose twitches were difficult to observe against the background of nearly continuous movements of the head associated with vocalizations. By the fourth day bursts of nose twitching coincided with brief periods of respiration slightly more rapid that the resting rate (polypnea). This relationship was more obvious in successively older animals. Increases in rate, duration and incidence of nose twitching and polypnea paralleled the increases noted in vibrissae movements.

5. Locomotion

Locomotion was considered to be a complex behavior pattern whose description depended on several factors, but predominantly two, the posture of the animal and muscular strength. Four descriptive stages were recognized: thrash (upright posture not maintained); sprawl (upright posture usually maintained, but little or no weight borne by the legs); crawl (upright posture maintained, some weight borne by the legs, but with the belly in contact with the substrate); walk (upright posture maintained,

weight borne completely by the legs with the belly not in contact with the substrate).

Thrashing was exhibited by first and second day animals, sprawling by third through tenth day animals, crawling by sixth through eleventh day animals, and walking by eleventh day and older animals. Considerable overlap in these classifications is apparent (Figure 7), suggesting that the criteria on which the categories were based were perhaps not the most reliable indicators of the stage of development of locomotion.

6. Grooming

Self-grooming in the adult usually consists of rapid wiping, brushing, or scratching with the forepaws of an orderly sequence of body parts, generally beginning with the muzzle and proceeding caudally to the mystacial vibrissae, the face in general, the pinnae, variable parts of the rear body and finally the tail (from the base to the tip). Variations of this pattern were frequently observed, e.g. scratching with the hind foot or an incomplete sequence. Grooming behavior seen in the context of exploration of a novel situation, however, appeared less variable, the notable qualification being that the grooming sequence often did not include the rear body and tail.

Changes occurring during the development of grooming were of two general types: the extent of the body groomed, and the posture assumed during grooming. These two types of change may in fact be related, since it would be difficult for a mouse to groom its head without being supported by its hind legs alone.

Grooming behavior was evidenced in first through eighth day animals by wiping at the nose in response to a noxious stimulus. On the eighth day grooming of the muzzle by one animal was accomplished on all fours, rather than lying on its side, with its weight being borne by the feet and forearms (the forepaws being used for wiping the muzzle). Muzzle grooming predominated through the thirteenth day, although some twelfth day animals also groomed their vibrissae. By the twelfth day grooming was done with the weight borne solely by the legs. The grooming posture, however, was not upright as in adults, rather the animals were hunched over with the forepaws just barely held above the substrate. On subsequent days, the extent of the body groomed proceeded caudally (Figure 7) and the grooming posture became more upright. Eighteenth day animals demonstrated complete grooming sequences in fully upright postures.

7. Comparison with *Mus*

The descriptions of the development of the behaviors presented above for *P. leucopus* are valid also for those *Mus* observed. Two points of difference should be noted, however: *Mus* demonstrated sprawling (locomotion) on the first day, although crawling appeared no sooner than in *P. leucopus*. *Mus* also demonstrated approach responses to gentle unilateral deflection of mystacial vibrissae on the sixth day, whereas *P. leucopus* did not exhibit this response until the ninth day. Observations of the single litter of *M. musculus* are also presented in Figure 7, and are indicated by triangles.

D. Peripheral lesions (*P. leucopus* and *M. musculus*)

1. Skin

In some instances early vibrissal lesions resulted in no obvious disruptions of the vibrissal field that were histologically observable at low (X25) magnifications (Figure 8A). External examinations of the skin in some of these cases revealed the absence or malformation of vibrissae (i.e. curled or bent hairs). When disruptions were observed, they were characterized by marked changes in the size of individual follicles (including complete absence) and/or changes in the arrangement of follicles (Figure 8B). These disruptions were assessed by comparison of the lesioned and non-lesioned sides.

Oliver[34] has demonstrated in the rat that a lesioned mystacial vibrissa follicle will regenerate unless the dermal one-third of follicle is destroyed. Since damage in this study was inflicted in the epidermal-dermal direction it was necessary to destroy essentially the entire follicle to prevent its regeneration. The observed disruptions resulting from externally effected lesions attest to the improbability of destroying the entire follicle in this manner.

2. Neocortex

Disruptions in the contralateral barrel fields of some of the animals subjected to early vibrissal lesions were noted (Figure 9A-D). When compared to the ipsilateral barrel fields they consisted of changes in size and shape (including complete absence) of individual barrels and changes in the arrangement of

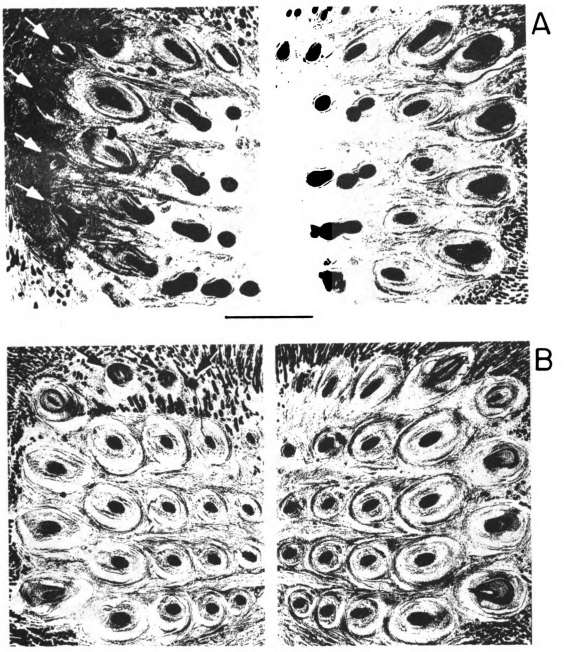

Figure 8. Histological appearance of disruptions of the cutaneous pattern of lesioned mystacial follicles.

A. 12th day M. musculus; vibrissae $\mathcal{N}$ - $\top$ lesioned (fifth day) right side. Photomicrographs of skin of the right (R) and left (L) sides of the snout. $\mathcal{N}$ - $\top$ are present on both sides, but reduced in size (arrows) on the right side. Thionin, 70 μm; scale bar is 1 mm.

B. 10th day P. leucopus; vibrissae A1-4 lesioned (first day) right side. Photomicrographs of skin of the right(R) and left (L) sides of the snout. A1-3 are present on both sides, but reduced in size (arrows) on the right side. A4 is absent on the right side. Thionin, 70 μm; scale bar is 1 mm.

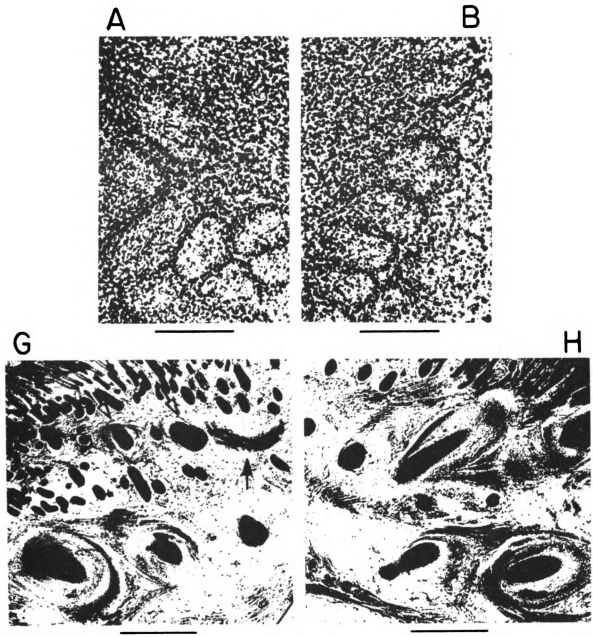

Figure 9. Comparison of peripheral arrangement of mystacial vibrissa follicles and the cortical arrangement of barrels: first day lesion. 13th day M. musculus; vibrissae A1-4 lesioned (first day) on the right side.

A. Photomicrograph of disrupted barrel pattern in the cerebral cortex (left side). Tangential section, 25 μm, thionin; scale bar is 200 μm.

B. Photomicrograph of the same region of the cortical barrel field as shown in A but in the opposite (right hemisphere). Tangential section, 25 μm, thionin; scale bar is 200 μm.

G. Photomicrograph of disrupted follicle pattern in the skin (right side). Vibrissa follicles A1-4 (arrows) are smaller; A4 is strangely shaped. Thionin 75 μm; scale bar is 0.5 mm.

H. Photomicrograph of the A and B rows of follicles on the left side. Thionin, 75 μm; scale bar is 0.5 mm.

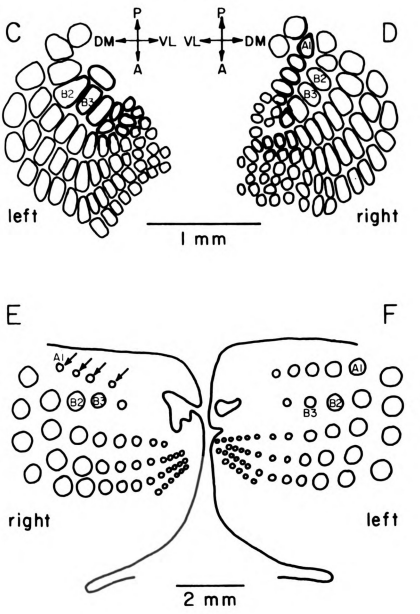

Figure 9 (continued).

C. Reconstruction of part of the left barrel field. Portions of barrels seen in A are drawn with heavier lines. The absence of barrels is apparent along the vintrolateral border of the barrel field.

D. Reconstruction of part of the right barrel field. No disruptions in the pattern of barrels are present.

E. Reconstruction of follicle pattern on the lesioned (right side). Abnormally small follicles are indicated by arrows.

F. Reconstruction of follicle pattern on the left side.

Barrels and vibrissae are tentatively numbered correspondingly to facilitate orientation.

barrels: barrels were usually larger (unless absent); barrel shapes were less regular; the barrel arrangement became less ordered.

Disruptions were observed in animals lesioned on the first postnatal day, specifically in those who also demonstrated marked changes (not necessarily total absence) in follicle size or arrangement (Figure 9E-H). Disruptions were not noted in the fields of any of the animals lesioned on the fifth day (Figure 10), or in the fields of those animals whose vibrissae were removed daily (Figure 11).

3. Peripheral-central correlations

Disruptions of the normal barrel pattern which were confined to the medial edge of the posteromedial division of the barrel field (Figure 12B) were found in animals who suffered disruption of the follicular arrangement (Figure 12A) in the most ventral row(s) of mystacial vibrissae (i.e. rows D and E). Cortical disruptions confined to the lateral edge of the posteromedial division (Figure 12D) were found in animals with lesions of the most dorsal row (A) of mystacial vibrissae (Figure 12C). Disruptions in the posterior half of this same division were found in animals with lesions in the posterior half of the mystacial group.

Fortified by these results, the large barrels of the posteromedial division were tentatively labelled in the same manner as were the mystacial vibrissae (Figure 13). When the barrels were so labelled, the "addresses" of the disruptions conformed precisely to the

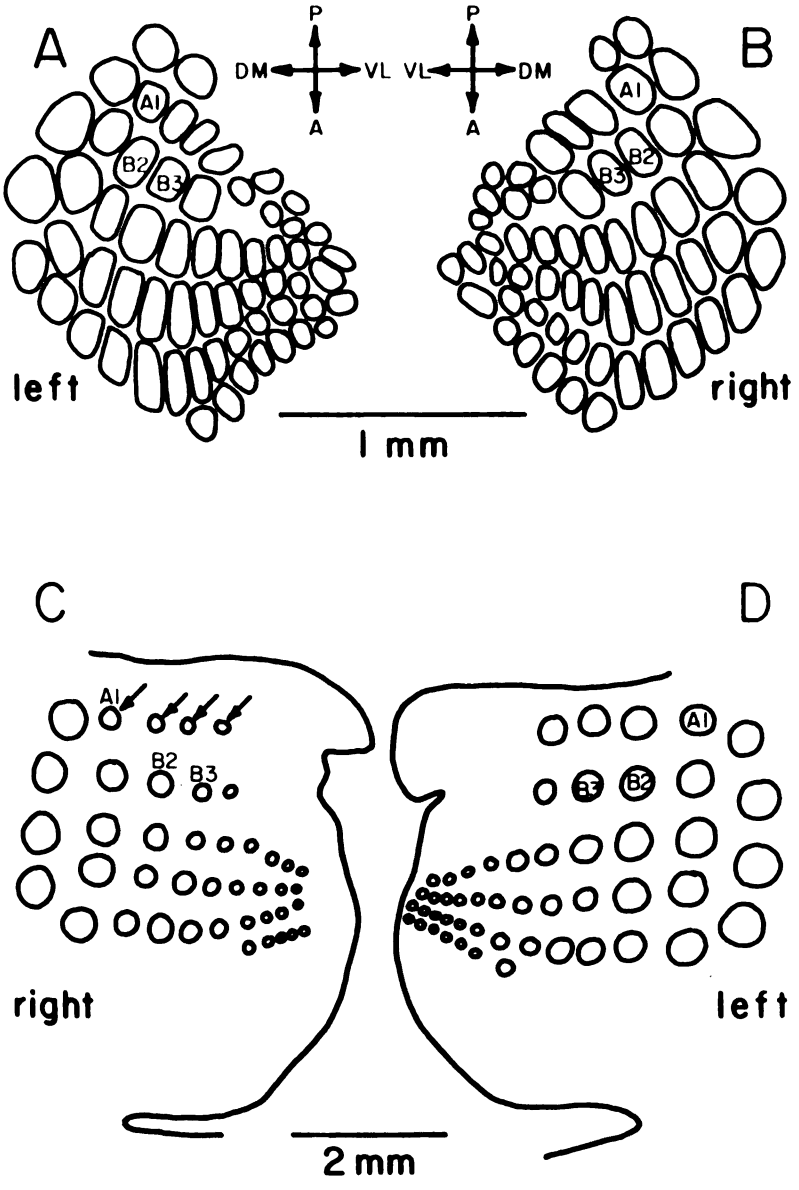

Figure 10. Comparison of peripheral arrangement of mystacial vibrissa follicles and the cortical arrangement of barrels: fifth day lesion. 12th day M. musculus; vibrissae A1-4 lesioned (fifth day) right side.

A. Reconstruction of part of the left barrel field showing no disruption in barrel arrangement.

B. Reconstruction of part of the right barrel field showing no disruption in barrel arrangement.

C. Reconstruction of right follicle pattern. Abnormally small follicles are indicated by arrows.

D. Reconstruction of left follicle pattern.

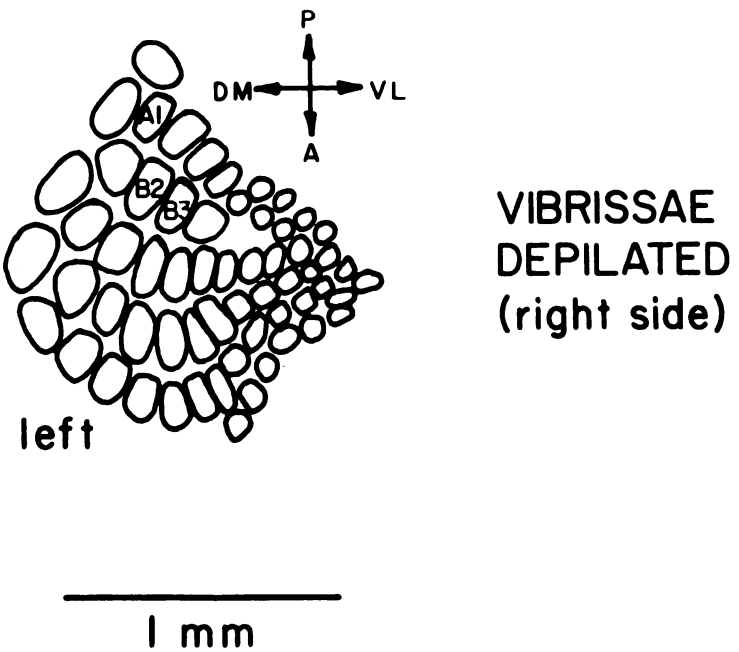

Figure 11. The arrangement of barrels after vibrissa depilation. Reconstruction of part of the left barrel field of a Mus in which the mystacial vibrissae were unilaterally depilated (right side) daily from the first through seventh days and sacrificed on the tenth day. On the eighth ahd succeeding days the mystacial vibrissae and fur of the snout were bilaterally absent, except for the muzzle, presumably having been nibbled off by the mother. Other animals of the same litter were likewise partially denuded from the eighth day on. No disruptions in the arrangement of barrels are present.

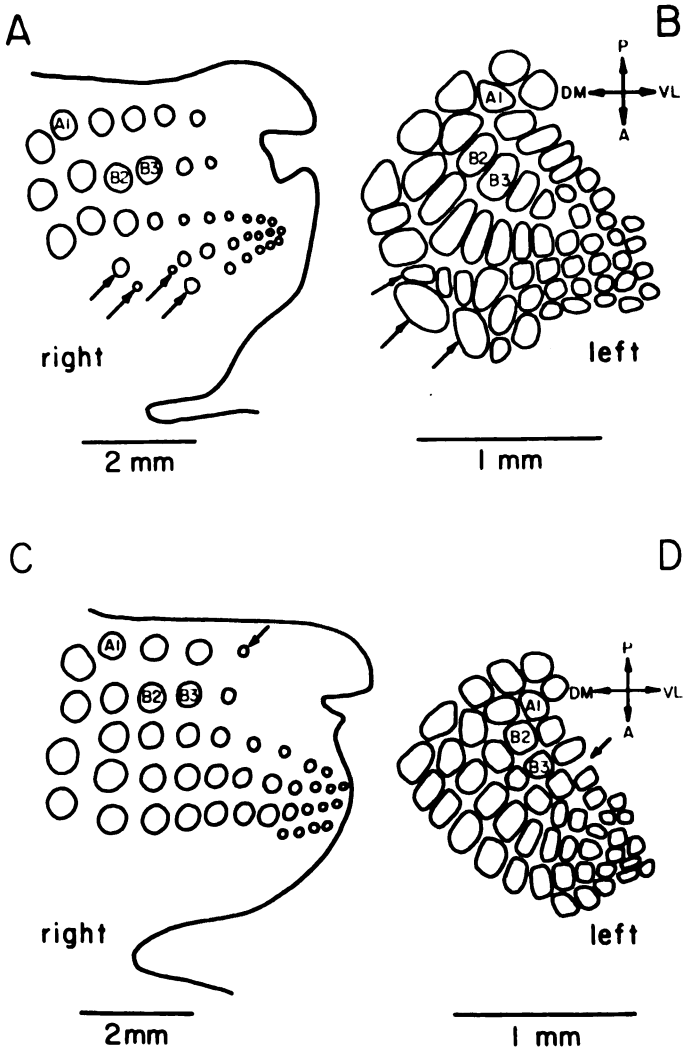

Figure 12. Correlation of the locations of damaged follicles and barrel field disruptions. A and B: 14th day M. musculus; vibrissae E1-4 lesioned (first day) right side. C and D: 16th day P. leucopus; vibrissae A1-4 lesioned (first day) right side.

A. Reconstruction of the right follicle pattern. Disruption of the number, size, and arrangement of vibrissa follicles appears in the ventral rows of mystacial vibrissae as indicated by arrows.

B. Reconstruction of part of the left barrel field. Disruption of the number, size, and arrangement of posteromedial barrels appears along the dorsomedial boundary of this division, as indicated by arrows. Note especially the large size and peculiar shapes and orientations of barrels in this region.

C. Reconstruction of the right follicle pattern. Abnormally small vibrissa appears in the most dorsal row of vibrissae, as indicated by the arrow.

D. Reconstruction of part of the left barrel field. A gap (arrow) appears between barrels along the ventrolateral boundary of the posteromedial division of the barrel field.

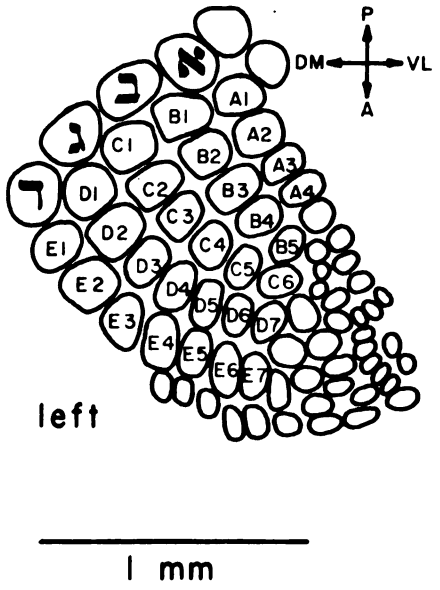

Figure 13. Assignment of notation of barrels. The notation is precisely that used for identifying mystacial vibrissae, and is applied according to the orientation suggested by the barrel arrangement and by the locations of disruptions produced by focal lesions of vibrissa follicles.

"addresses" of the lesioned follicles (Figure 14).

The reduced sizes of lesioned follicles were not consistently related to the sizes of their corresponding barrels: When a follicle was absent, so was its barrel; but when a follicle was reduced in size, its barrel was either missing, reduced, or enlarged. From the histological appearance of lesioned follicles, the sizes of the corresponding barrels could not be predicted. The disrupted pattern of follicles, however, was reflected consistently in the barrel pattern. Barrel patterns, on the other hand, were sometimes disrupted with no corresponding disruption of the follicle pattern. Such disruptions in barrel pattern occurred near primary, lesion-related disruptions and appeared to be related only indirectly to the peripheral lesions.

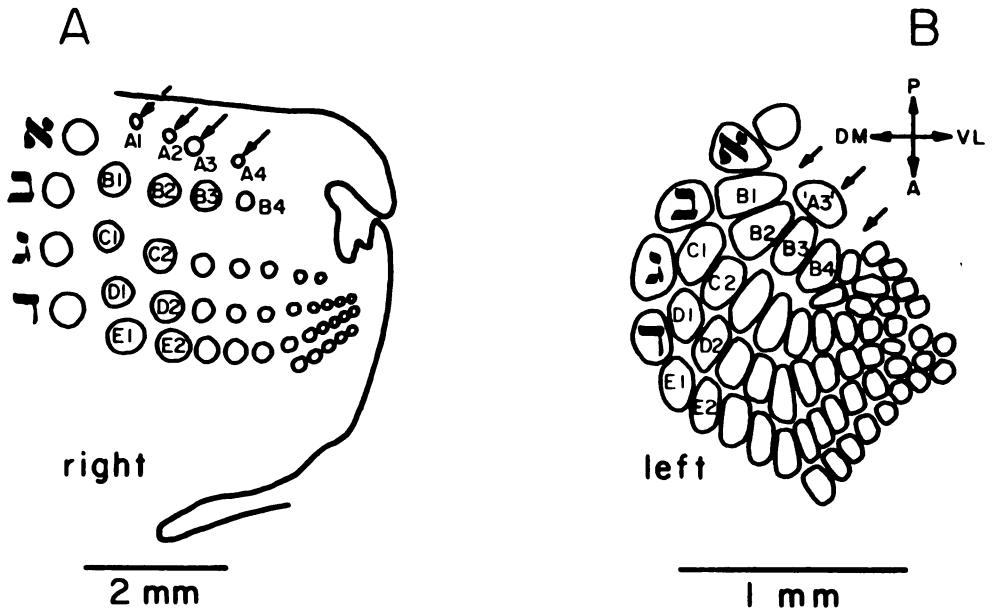

Figure 14. Specific correlation of vibrissa lesions and barrel field disruptions. 13th day M. musculus; vibrissae A1-4 lesioned (first day) right side.

A. Follicle pattern reproduced from Figure 9G with the follicles identified as in Figure 4B.

B. Barrel pattern reproduced from Figure 9C with the barrels identified as in Figure 13. A single barrel is present in the A row between two gaps in the barrel pattern. The remaining A row barrel is tentatively labelled as A3 according to its relative position. Note the large size and unusual orientation of this barrel and the unusual orientations of surrounding barrels.

DISCUSSION

A. Development of barrels and other aspects of cortical ontogeny

Cortical barrels first appear in Peromyscus on the fifth postnatal day and appear well-defined structurally by the eleventh day. It would be valuable to place this morphological development in the context of the schedule of maturation of individual neurons which comprise barrels. Unfortunately such information does not exist either for Peromyscus or for Mus. A number of studies of developing rat cortex do suggest at least the temporal sequence and approximate schedule of cortical development, which with great reservation, can be compared to the results of this present study.

Neurons of cortical layer IV of the rat are "born" on about the seventeenth gestational day (or about 5 days before birth) as revealed by H^3-thymidine labelling and autoradiographic analysis[4,21]. This compares with the origin on the thirteenth gestational day (about 6 days before birth) of layer IV neurons of the mouse[1]. At birth these neuroblasts have already migrated to the cortex, but cannot be recognized as a distinct cortical layer until about the fourth or fifth postnatal day[4,21], i.e. after the neuroblasts destined for the more superficial layers (I, II, III) have migrated through layer IV and assumed their assigned places[1,21].

Caley and Maxwell[6] first noted rudimentary synapses in the rat's parietal neocortex (layer not identified) on the fifth postnatal day; after the tenth postnatal day they observed many

mature synapses. Cytological differentiation and maturation of neuroblasts, marked by increases in the number and complexity of organelles, appearance of synapses, disappearance of extracellular spaces, growth of processes and neuropil, proceeds along a gradient from the inner to the outer cortical layers. By the twenty-first postnatal day the pattern of cortical fine structure is present. Kobayashi, et al.[26] concluded that mouse cortex is essentially cytologically mature by the seventeenth postnatal day.

Deza and Eidelberg[9] investigated the development of various types of cortical electrical activity in rats lightly anesthetized with tribromoethanol. They recorded (intracellularly) no "spontaneous" action potentials before the fourth postnatal day, although such activity was invariably present in the diencephalon from birth on. Spontaneous cortical EEG activity was first recorded on the sixth postnatal day, began to increase rapidly on the fifteenth postnatal day and, achieved adult characteristics by the twentieth postnatal day.

Histochemical changes in the activities of certain oxidative enzymes within the neocortex of the rat were studied by Kuhlman and Lowry[27], Friede[14], and Labedsky and Lierse[28]. Kuhlman and Lowry[27] noted that the activity of glutamic dehydrogenase increases markedly in all cortical layers between birth and the tenth postnatal day and subsequently changes little. The activity of malic dehydrogenase, however, increases in the outer layers and decreases in the inner layers after the tenth day. They also

noted similar changes (but of smaller magnitude) in the activity of lactic dehydrogenase. The activity of glucose-6-phosphate dehydrogenase remains almost unchanged. Friede[14] described a continual increase in the activity of succinic dehydrogenase after birth. He also described the changes in laminar distribution within the cortex over time, observing that differences in distribution of enzyme activity in different areas of the neocortex are present from the seventh day on. By his assessment the adult pattern of distribution of activity is obtained by the sixteenth day. The changing distribution of the activity of this same enzyme was studied by Labedski and Lierse[28] in various regions of the rat's brain. Their observations of the cortex compare well with those of Friede with the additional point that the greatest increase in activity occurs between the tenth and twentieth postnatal days. Moreover, small "nests" of relatively intense succinic dehydrogenase activity are found in layer IV at least as early as the tenth day. The appearance of these nests is remarkably similar to that of barrels in the negative image of Nissl stained coronal sections through the barrel field. Similar distributions of enzyme activity were found in guinea pig cortex by Friede[15].

Thus it appears, if the qualitative comparison between rats and mice be allowed, that the morphogenesis of barrels begins at about the time that ultrastructurally identifiable synapses first appear, dendrites of cortical neurons begin to branch, and

electrical activity can first be recorded. Likewise, the morphogenesis is essentially complete before ultrastructural, electrophysiological, and biochemical maturity has been attained by cortical neurons.

D. Development of behavior

The schedules of development of the various behavior patterns described in this study are consistent with the observations of earlier studies of Peromyscus, especially P. leucopus[29]. They also agree with the general schema presented by Gottlieb[16] for the ontogenetic sequence of sensory function in birds and mammals. More importantly, the observations of the components of sniffing behavior made in the course of this study are quite similar to those reported by Welker[52] on the development of sniffing behavior in rats.

Polypnea (rapid, shallow respiration) may have been present at birth in both Mus and Peromyscus, however, it was not definitely observed until the third day. Welker[52] identified this component in the rat on the first day from analysis of high speed movies. Nose twitching in response to an olfactory stimulus was seen in some Peromyscus on the second day and in all Peromyscus and the one Mus observed on the third day; Welker did not observe distinct repetitive movements of the rat's nose until the seventh day. Repetitive movements (retractions) of vibrissae of Mus and Peromyscus were first seen on the fifth day while in the rat such movements were first seen on the fourth day[52]. Essentially mature patterns

of sniffing were observed in Peromyscus on the twelfth day, in Mus on the fourteenth day, and in the rat on about the fifteenth[5] and sixteenth[52] days.

It is interesting, and perhaps suggestive, that rudimentary whisking of the mystacial vibrissae first occurs on the same day that barrels are first in evidence. Such an observation must be considered with caution, however, since other cortical and sub-cortical structures are still maturing at this same time. That the third through the sixth day constitutes an important developmental period for Mus is reflected by the fact that most of the reflex responses observed by Fox[13] underwent significant changes during this period. While very few of these reflexes were studied in Peromyscus, the vibrissa placing reflex appears during this period (fourth day) in both Peromyscus and Mus[13]. In general the reflexogenic periods described by Fox[13] for Mus are also applicable to Peromyscus leucopus.

C. Peripheral lesions

The results obtained from the peripherally lesioned mice bear on two related important general topics: the importance of an intact periphery for normal development of central neurons; the accomplishment of recepto- and effectotopic organization of central neurons. Indeed, these two topics may be inextricably related.

Peripheral lesions of sensory receptor surfaces (e.g. limb removal[3,22,23,37] enucleation[8], cochlear and vestibular extirpation[30]) in mammals, birds and amphibians are known to affect central neurons both

qualitatively (cellular structure) and quantitatively (number of cells), the exact effects depending on the time and severity of the lesion. Reports of these effects, however, have been largely restricted to the fate of neurons in direct synaptic contact with the lesioned or deafferented neurons. Transneuronal effects on cortical neurons have been reported but have been restricted to rather subtle cytological changes, e.g. changes in the number of dendritic spines, occurring in adult animals as a result of sensory deprivation or enrichment[10,17,18,47].

The present results indicate that drastic as well as subtle transneuronal effects in cortex may result from alteration of receptors. It is not clear whether lesioning vibrissa follicles in newborn mice results in cortical hypoplasia, reduced neuropil, neither, or both. What is clear is that the normal arrangement of layer IV neurons can be grossly disturbed by small, discrete peripheral lesions. That the distorted arrangement of cortical cells conforms to the distorted arrangement of vibrissa follicles suggests that the periphery exerts an organizational as well as a trophic influence on central neurons. Since mice lesioned on the fifth postnatal day, when barrels are already present in rudimentary form, do not show distorted arrangements of cortical cells, it follows that the disarrangement of barrels is a result of interference with the normal process(es) of barrel morphogenesis. Stated another way, the arrangement of barrels is imposed by the arrangement of vibrissa follicles. But how the normal arrangement

of vibrissa follicles is established, aside from attributing the pattern to "genetic determination", is completely unknown.

Precisely what peripheral damage resulted from cauterization of follicles has not been determined. The damage was in some instances less than intended, leaving enough follicle for regeneration to occur[34]. In others the extent of the lesion was obviously greater than intended, affecting neighboring vibrissae. In newborn mice (the time of the early lesions) vibrissa follicles are innervated, but the mature innervation pattern and characteristic receptor endings do not yet appear for a number of days[38]. It seems likely that, whatever the damage, the lesions did affect the delicate sensory nerve fibers innervating the follicles. Thus it is not possible to determine at this point what is the necessary and sufficient damage for causing the cortical effects observed. It must also be considered that, since in depilated animals the hairs grew back a little bit each day between depilations, depilation was not an adequate control in that it did not completely prevent "normal" stimulation of the follicular receptors. Since subtle changes do occur in visual cortex, resulting from visual deprivation in young animals with anatomically intact visual systems[47], it is probable that the tactile equivalent of visual deprivation (hair removal) would similarly result in subtle changes in individual neurons which could only have been demonstrated by other techniques (e.g. ultrastructural of Golgi analysis).

D. Functional relationship between barrels and vibrissa follicles

The identification of particular cortical barrels with particular vibrissae (Figure 13) was first suggested by Woolsey and Van der Loos[59] on the basis of barrel size, barrel arrangement and location of the barrel field and was reiterated by Welker[51] on the basis of micromapping of the rat's somatic sensory cortex.

The results of peripheral lesions reported here show conclusively that a functional (morphogenetic) relationship does indeed exist between individual vibrissa follicles and individual barrels. Such a conclusion could not be made from the micromapping results of Welker[51] since her methods of analysis required section of the brain parallel to the direction of the electrode penetrations of the cortex (i.e. perpendicularly to the cortical surface): Individual barrels cannot be identified from such sections[59].

In a pilot study carried out in conjunction with this resent study, microelectrode recordings were made in the somatic sensory cortex of one adult Peromyscus leucopus, in the same general manner as by Welker[51]. One electrode penetration was accomplished in the region of representation of the large mystacial vibrissae. Histological analysis of this experimental brain was performed according to the methods described above for demonstrating the barrel pattern in normal brains. Serial reconstruction of the barrel field was also accomplished as for the other barrel fields of this study. The electrode penetration was identified as a thionin-stained blood-filled hole penetrating the cortex approximately perpendicularly. This electrode hole was distinguished from thionin-stained blood-filled blood vessels by the absence of vessel walls around the perimeter of the hole. The hole was located in the

wall between barrels C4 and C5 (Figure 15A). Two distinct responses were recorded: more superficially to deflection of vibrissa C4 and beneath that to deflection of vibrissa C5 (Figure 15B).

The weight of evidence thus indicates that each large barrel corresponds functionally to a single mystacial vibrissa follicle as hypothesized by Woolsey and Van der Loos[59]. Several questions remain, however, concerning the response properties of cells within a single barrel and the location of the representations of the various facial structures not determined by Welker[51], e.g. common fur hairs between vibrissae, the rhinarium, the smaller vibrissae on the snout and upper lip, and the vibrissae on the mandible. With regard to the functional organization of cells within a single barrel, it is interesting that a vibrissa follicle itself is approximately barrel-shaped.

E. Functional parcellation of the barrel field

The relationship between large mystacial vibrissae and the large postermedial barrels, i.e. those of the PMBSF, is firmly established. The functional relationships of the large number of smaller, anterolateral, peninsular, and insular barrels remain in question. It is likely that each small barrel corresponds to a single vibrissa follicle, particularly in view of the close correspondance between the number of vibrissae and the number of barrels counted by Welker[51].

From scrutiny of the distribution of vibrissa follicles on the skin and the arrangement of barrels the following functional

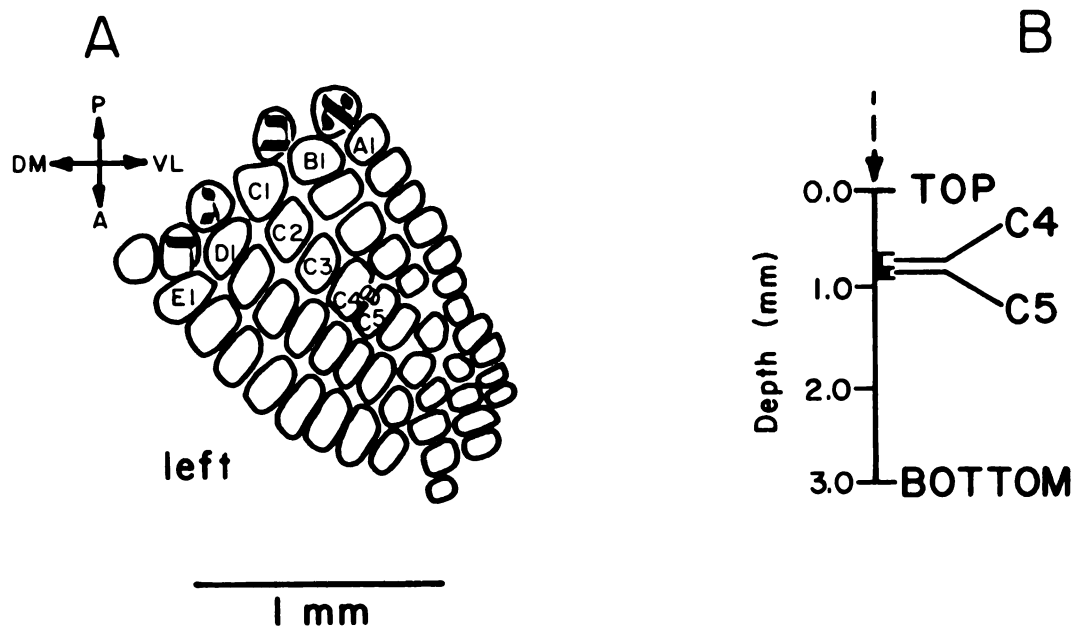

Figure 15. Micromapping data and localization of a single electrode penetration within the barrel field of P. leucopus.

A. Reconstruction of the barrel field from tangential sections. A thionin-stained blood-filled hole resembling a blood vessel but lacking any wall was found penetrating the cellular wall between barrels C4 and C5. This hole in more superficial sections was seen along the C4 side of the wall and in deeper sections was seen along the C5 side of the wall.

B. Depth location of electrical responses to peripheral cutaneous stimulation, "TOP" indicates the cortical surface; "BOTTOM" indicates the deepest point of penetration of the microelectrode. The upper response was obtained in response to deflection of mystacial vibrissa C4; the lower response was obtained in response to deflection of mystacial vibrissa C5.

parcellation is proposed (Figure 16): the posteromedial division is the locus of the representations of the mystacial vibrissae, the supraorbital vibrissae, the postorbital vibrissae, and the rhinal vibrissae; the anterolateral division is the locus of the representations of the labial, submental, and interramal vibrissae (including the vibrissae on the furry buccal pad as labials); the peninsular division is the locus of the representation of the posterior labial vibrissae; the insular division is the locus of the representation of a circular patch of vibrissae at the juncture of the upper and lower lips, the orangular vibrissae (at the angle of the mouth). What appears to be a supernumerary barrel is sometimes found next to the T barrel (Figure 15 and see reference 59: their figure 14 , reconstruction 11R). This barrel may be more consistently present than published barrel field reconstructions would suggest. It was discovered convincingly twice in the course of this study and there were no corresponding supernumerary follicles. This barrel may correspond to the single postorbital vibrissa follicle.

The representations of non-vibrissal structures probably are to be found in their somatotopically proper places: that of the fur hairs between the mystacial and labial vibrissae in the medial midway notch devoid of barrels; that of the rhinarium in a small barrel-less region bounded by B row, C row, and rhinal barrels; that of the fur hairs between the vibrissae in the septa between barrels. This latter suggestion is based in part on the observation that the walls which occur between barrels of neighboring rows are wider than those which occur between barrels in the same row, a situation which reflects precisely the presence of many more

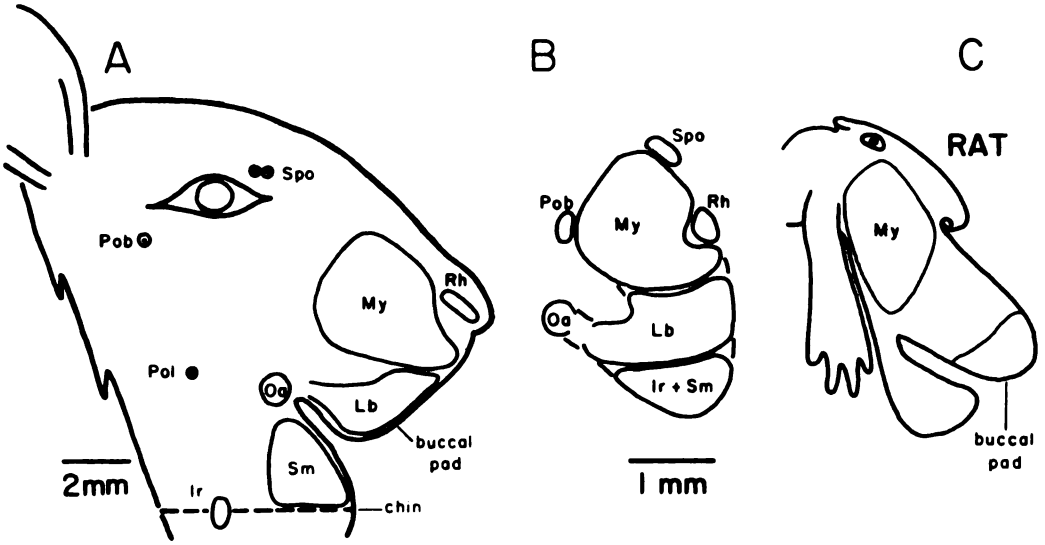

Figure 16. A functional parcellation of the muroid barrel field: hypothesized correspondence between groups of vibrissae and groups of cortical barrels.

A. The location of individual vibrissa follicles and groups of follicles in the skin of P. leucopus (cf. Figure 4A). An orangular group of vibrissae (Oa) is located at the angle of the mouth.

B. The hypothesized parcellation of the barrel field. Note especially the nearly geometric similarity between the barrel field parcellation and the actual locations of vibrissae.

C. The somatotopic arrangement of projections to the rat's cortex from cutaneous mechanoreceptors, redrawn after Welker[51]. Note the correspondence between the somatotopic "map" and the barrel field parcellations.

common hair follicles between than within rows of vibrissa follicles.

F. Morphogenesis of barrels

Judging by the sequential appearance of barrels during their early development, the sides and hollows are formed simultaneously by the same process: rapidly expanding neuropil forms the hollows and at the same time compresses the cell bodies out of the region of the hollow into the sides. This hypothesis is also suggested by the shapes of the large barrels.

Each side of the nearly rectangular large barrels (as seen in tangential section) is closely apposed to the adjacent sides of the neighboring barrels, much as if the two sides had been compressed together by forces emanating from the centers of the hollows. In order for rectangular barrels to be formed, the foci of the forces compressing the sides would have to be arranged in a rectangular grid (as the large mystacial vibrissa follicles are). If the foci were arranged in a hexagonal grid (as the small labial vibrissa follicles approximately are), the resulting barrels would be hexagonally or roughly circularly shaped (as the small barrels of the anterolateral division of the barrel field are).

Lorente de No[31] described in the rat the elements of what are now called barrels. He also described cortical afferent fibers with profuse terminal aborizations that are associated with barrels. Scheibel and Scheibel[42] described the grouping of axons

of ventrobasal thalamic neurons into small bundles which appear to remain intact all the way to the deep layers of cortex. They also reported their impression that these bundles are formed of axons from a number of neighboring thalamocortical cells, so that "information from cells of similar somatotopic signature may run forward in segregated bundles and, once arrived at cortical receptive layer IV, penetrate terminal arbors in similarly adjacent positions" (reference 42, p. 33). Waite[50] has reported that the representations of vibrissae in the rat's ventrobasal thalamus are localized to discrete clumps of neighboring cells. The axons from cells within a single clump might well comprise a bundle or bundles of fibers as suggested by Scheibel and Scheibel[42].

Barrels might then be formed in the following manner. The endings of bundles of thalamocortical axons begin to arborize profusely within layer IV on about the fifth postnatal day into a roughly spherical or cylindrical bush of neuropil, thereby pushing "large" objects like cells and blood vessels away from the focus of arborization (blood vessels are found almost exclusively within or along barrel walls of the adult). Since this process occurs simultaneously at several neighboring points, the cells of layer IV are pushed away from the focus of arborization until they encounter a wall of cells retreating from the pressure of arborization at a neighboring focus. Barrels, with hollows consisting of terminal arborizations of thalamocortical axons and sides consisting of compressed cortical neurons, are then completely

formed. The presence of septa is attributable to the groups of unstained apical dendrites of layer V pyramidal cells described by Peters and Walsh[35] which are sandwiched between the apposed sides of neighboring barrels (as observed in the course of this study--they also occur within the hollows). The wider septa between rows may result from the unstained terminal arborizations of axons of thalamocortical neurons related to the common fur hairs between rows of vibrissae.

The foregoing hypothesis of barrel morphogenesis can be summarized by stating that patterns of cortical sensory organization are directly dependent on the patterns of thalamic sensory organization. This approach was taken by Welker[53] in seeking to explain the development of the specialized gyral pattern associated with the somatic sensory neocortex of the raccoon [54]. He tested this hypothesis by severing the thalamocortical fibers in infant raccoons less than a week old, i.e. before most of the sulcuses had formed. Nevertheless, the normal gyral pattern and cytoarchitecture was attained, but the entire operated hemisphere was slightly smaller than the unoperated hemisphere. What the functional state of the raccoon cortex was at the time of lesioning is not known but the somatic sensory neocortex of newborn cats, which, judging by sulcus development, is slightly more developed at birth than that of raccoons, is unquestionably functional[41]. Therefore the possibility remains that earlier lesions might result in the absence or disturbance of the normal gyral pattern.

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