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Mouse SmI Cortex: Qualitative and Quantitative Classification of Golgi-Impregnated Barrel Neurons*

(neuron classification/cortical organization/cluster analysis/computer microscope)

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ABSTRACT **The neurons in layer IV of the mouse somatosensory cortex are arranged in a remarkably consistent pattern of multicellular cytoarchitectonic units called "barrels." Each barrel is known to be related, in a one to one manner, to a contralateral whisker or vibrissa on the animal's face. In this study we have examined Golgi-impregnated neurons that comprise the "barrels." Several criteria, some being quantitative measures of dendritic arbors and somal sizes which were obtained with a computer-microscope system, suggest that all barrel neurons can be placed in two classes, the members of which are present in approximately equal numbers. The cells in the two classes can be further subdivided on the basis of the relationship of their processes to the barrels: 85% of them have processes restricted to a single barrel; 15% of them distribute their processes to two or more barrels. From these observations it is possible to suggest that a majority of neurons comprising the barrels would respond initially to movements of only one whisker while the remainder would respond to movements of two or more whiskers. In addition it has been shown that the quantitation of neuronal structure can provide a numerical basis for the classification of neurons in the central nervous system.**

The diverse forms of nerve cells have been of interest to students of the central nervous system who have long assumed that a neuron's morphology is related to its function and determined, in part, by its connections (1). In certain parts of the brain—most notably the cerebellum (2), olfactory bulb (3), and hippocampus (4)—these postulates have proven to be correct. However, the classification of neurons in the cerebral cortex has been particularly difficult and widely disputed (5–9), because the neurons are apparently of many types [Lorente de Nó (10), for example, identified as many as 57], which do not appear to be sharply segregated. With newer methods of functional (11) and morphological (12) analysis, it is becoming increasingly imperative that a comprehensive and objective scheme of classification of cortical cells be evolved.

The characteristic and consistent arrangement of layer IV neurons of the mouse somatosensory neocortex (SmI) into "barrels" affords an unusual opportunity to study the detailed morphology of specific groups of cortical neurons (13). The largest of these barrels is comprised of about 2000 neurons (14), and each barrel is known to receive its sensory input from a single sinus hair (vibrissa) on the contralateral face (15, 16). Over the past few years we have examined Golgi preparations with the long-term goal of trying to derive a "wiring diagram" for the barrels. In this report we describe some qualitative and quantitative characteristics of the neurons in the barrels. We have developed a comprehensive classification scheme, into which *all* impregnated neurons can be placed and which depends, in part, upon criteria that have been extracted from numerical data obtained with a computer-microscope system (17, 18).

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MATERIALS AND METHODS

Animals. The brains were taken from 20 Swiss Webster mice of both sexes which were between 60 and 180 days of age.

Histology. All brains were prepared by the Van der Loos (19) modification of the Golgi–Cox technique. This method has two distinct advantages: (*i*) it is compatible with counterstaining by the Nissl method, which permits the accurate localization of Golgi-impregnated neurons in relation to the barrels; and (*ii*) it is known to stain the barrel neurons randomly (20) so that it is possible to estimate the relative number of cortical neurons in a particular category. The methods of brain fixation, imbedding, sectioning, and staining are described elsewhere (14). Specimens for this study were kept in the Golgi–Cox fixative for 6–31 days and cut serially in coronal and tangential planes at thicknesses varying from 70 to 140 μm.

Barrel Field Reconstruction. The barrel fields of the tangentially cut brains were reconstructed by methods previously described (13).

Cell Selection. The qualitative observations are from a large number of the brains. The quantitative observations are based on sections from two hemispheres in which the barrel field was particularly well visualized; one impregnated for 6 days and cut at 80 μm and the other impregnated for 31 days and cut at 140 μm. In each of these sections *every* impregnated neuron (n = 142) associated with a total of 10 barrels was located with respect to the barrels and drawn with a Zeiss drawing attachment.

Data Collection. The relative frequency of each group of cells in the barrels was determined from the drawings (see Fig. 4). The cross-sectional areas of the somata were measured from camera lucida drawings with a small computer (18). The three-dimensional dendritic branch and end length data were obtained with a computer-microscope system (17). All cells were tracked under oil with a long working distance 100× objective. The latter quantitative measures were taken from 40 cells in the 140-μm section that had been impregnated for 31 days to assure completeness of impregnation and to minimize truncation effects.

Data Analysis. For each neuron the three-dimensional distances of all the dendritic branch points and ends from the soma were measured. The true lengths are easily extracted from automated plots of scaled length diagrams of the type

TABLE 1. *Summary of qualitative features of barrel neurons*

Neuron: Class, type, frequency (%)	Axon direction*	Dendritic specialization	Somal location	Distribution of dendrites
Ia (24)	Down	Spines	Hollow	Confined to a single barrel
Ib (18)	Down	Spines	Side	Confined to a single barrel
Ic (5)	Down	Spines	Septum	To two or more adjacent barrels
Id (2.5)	Down	Spines	Hollow	To two or more adjacent barrels
IIa (24)	Up	Smooth with beads	Hollow	Confined to a single barrel
IIb (18)	Up	Smooth with beads	Side	Confined to a single barrel
IIc (5)	Up	Smooth with beads	Septum	To two or more adjacent barrels
IId (2.5)	Up	Smooth with beads	Hollow	To two or more adjacent barrels

* Indicates the initial direction of the axon with respect to the surface of the brain *when* the axon is stained.

first introduced by Sholl (6). From these, an average branch and end distance were computed for each cell.

RESULTS

Description of Golgi-Impregnated Barrel Neurons. Some qualitative features of Golgi-impregnated barrel neurons are summarized in Table 1. For convenience we have chosen an arbitrary nomenclature for the barrel neurons. The *class* designations (I and II†) take into account their morphology in isolation, such as the initial course of the axon and the character of the dendrites (i.e., context-free criteria listed in columns 2 and 3 of Table 1). The *type* designations (a–d) take into account the relationships of the cells to the barrels, such as the somal location and the distribution of the dendrites (i.e., context-dependent criteria listed in columns 4 and 5 of Table 1). *All* of the impregnated neurons observed in layer IV could be categorized by these criteria.

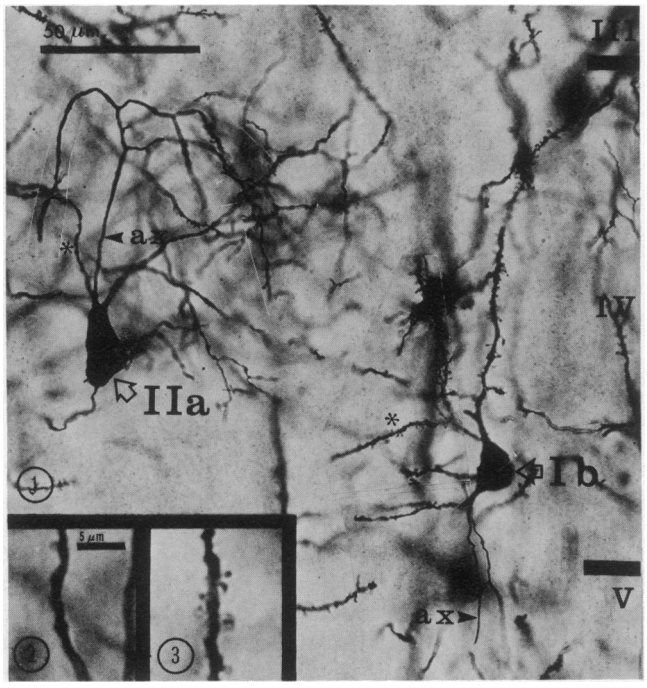

FIG. 1. Photomontage of a coronal section through the mouse barrel field to show Golgi appearance of two barrel neurons. The dendrites of both of these cells are confined to the same barrel. Neuron IIa has a large soma located in the barrel hollow, ascending axon (ax) which ramifies the barrel hollow, and smooth beaded dendrites (see Fig. 2). Neuron Ib has a smaller soma located in the barrel side, descending axon (ax), and spinous dendrites (see Fig. 3). Note the asymmetry of the dendritic arborizations of cell Ib. The *asterisks* mark the dendritic regions shown at higher magnification in Figs. 2 and 3. This preparation is counter-stained with a Nissl dye to accurately identify the cortical laminar boundaries indicated at the right, but the counter-stain has been optically filtered to better illustrate the Golgi-impregnated cells.

FIG. 2. Higher magnification of a dendrite of cell IIa in Fig. 1 to show the beads.

FIG. 3. Higher magnification of a dendrite of cell Ib in Fig. 1 to show the spines.

In sections normal to the pia it is possible to recognize the barrels in layer IV. The photomontage in Fig. 1 illustrates several characteristics of the barrel cells. The two neurons shown are readily distinguishable from each other on the basis of the initial direction of the axon with respect to the surface of the brain and the appearance of the dendrites. The cell labeled Ib has the properties of cells that we have defined as class I cells: their dendrites have numerous spines (Fig. 3) and their axons are directed initially toward the white matter. These cells were originally described by Lorente de Nó (10) as "células estrelladas" (stellate cells) and "pirámides-granos" (star pyramids). The cell labeled IIa has the properties of cells that we have defined as class II cells: their dendrites are smooth and beaded with occasional spines (Fig. 2), and their axons are directed initially toward the pia. Some of the cells of this class were identified by Lorente de Nó (10) as "células de axon corto glomerular" (short axon cells). The axons of class I cells are finer and less frequently fully impregnated than those of class II neurons.

To appreciate the position of somata of the neurons and the spatial distribution of their processes with respect to the barrels, it is necessary to examine tangential sections. With the computer-microscope it is possible to view reconstructions of the cells made from the tangential sections from different spatial angles (17) and in this way to correlate the appearance of cells seen in tangential preparations with those seen in other planes of section (compare Fig. 1 with Fig. 4).

In tangential preparations the cells in classes I and II can be further subdivided (Fig. 4). A majority of neurons belonging to classes I and II has dendritic fields that are restricted to a single barrel. On the basis of the location of the soma and the distribution of the dendrites, we have identified them as type a and type b. *Type a* cells have somata in the barrel

† The class designations are not meant to correspond to the nomenclature of Globus and Scheibel (7).

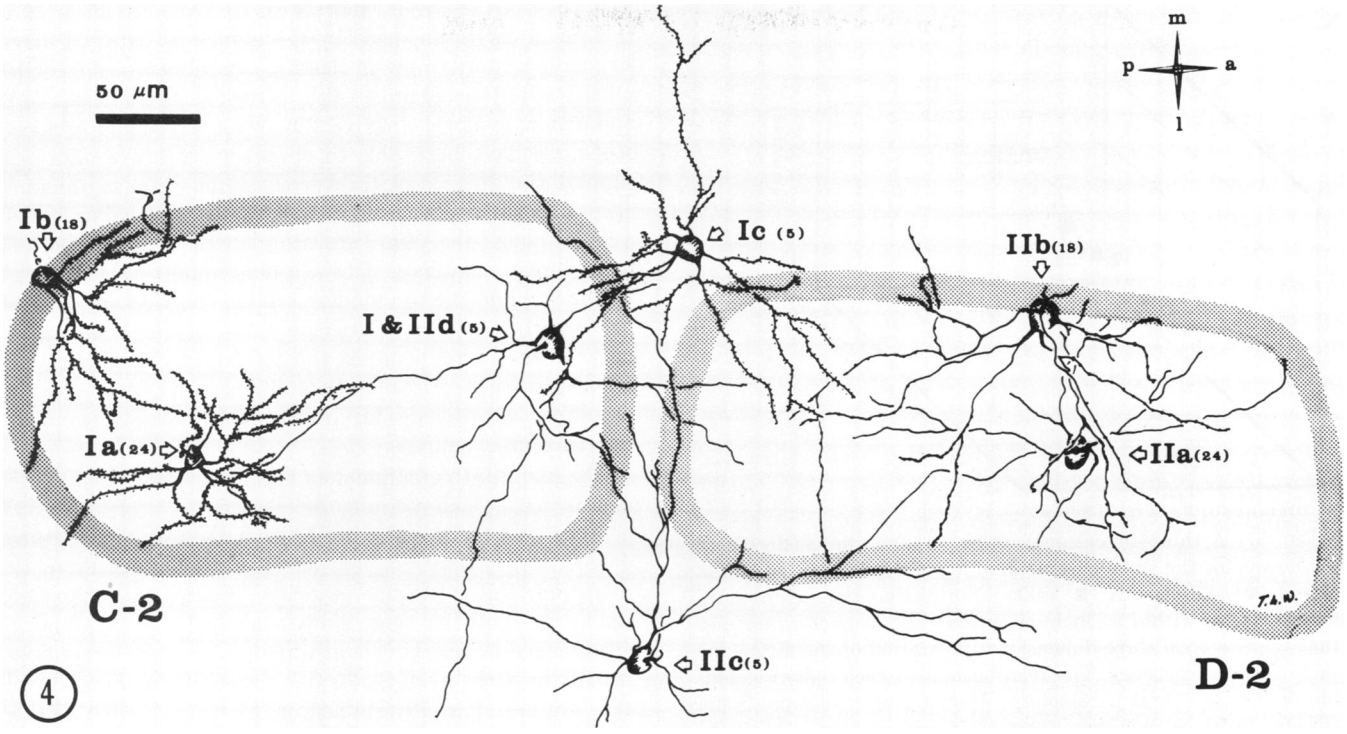

FIG. 4. Semi-schematic drawing of Golgi-impregnated barrel neurons to illustrate somal location and dendrite distribution as seen in tangential sections through SmI layer IV. The *stippling* indicates the sides of barrels C-2 and D-2 (for terminology, see ref. 14), which can be recognized with the Nissl counterstain. Axonal patterns are not shown. Note that the dendritic fields of cells Ia, Ib, IIa, and IIb are distributed within and to the barrels in which their somata lie. The somata of cells Ic and IIc lie outside the barrels, in the septa, and distribute their dendrites to two or more adjacent barrels. Cells Id and IId (for the sake of clarity only a IId cell is illustrated) have somata within a barrel but the dendritic processes are not restricted to a single barrel. Cells labeled I have spinous dendrites; cells labeled II have beaded dendrites. The relative frequencies (%) of each type of neuron are shown in parentheses by the cell labels. The orientation of the cells with respect to the brain is given at the upper right: m = medial; l = lateral; a = anterior; p = posterior.

hollow and radially distributed dendrites. *Type b* cells have somata in the barrel sides and eccentric dendritic fields. The axons of the type a and b cells, when fully impregnated, were observed to ramify within the barrel to which their dendrites were confined. The axons of Ia and Ib cells, which leave layer IV, have numerous ascending recurrent collaterals; the axons of IIa and IIb cells ramify by descending arcades (see Fig. 1). The axons of cells in both classes come into close relation to spiny dendrites that may belong to the class I cells or pyramids in other cortical layers. Two other types of barrel neurons can be recognized: their dendrites distribute to two or more adjacent barrels. The somata of the first of these, the type c cells, lie outside the barrels in the septa; the somata of the second, the type d cells, are within the barrel. We have not seen fully impregnated axons of the type c and d cells.

Relative Frequencies of Neurons Comprising the Barrels. Because the Golgi–Cox method impregnates the barrel neurons randomly (20), we have been able to estimate the frequencies of the categories of barrel neurons described above (see Table 1 and Fig. 4). When the cells are considered by *class* alone, they are present in equal numbers. Significantly, the members of these two classes are also equally distributed among the various *types*. About 85% of the layer IV neurons have their dendritic fields restricted to a single barrel (e.g., types a and b). Far fewer layer IV cells distribute their processes to more than one barrel, and the majority of these have their somata outside the barrels (e.g., type c). Since the number of neurons in one of the largest barrels (C-1) is known to be about 2000 (14), it is possible to estimate the absolute number of cortical neurons belonging to a particular category. That is, calculating from Table 1, there are about (±10%) 480 Ia, 360 IIb, 100 Ic, etc., neurons in layer IV associated with barrel C-1. This finding suggests that, if the functional characteristics of each cell category can be determined, precise modeling studies of the cerebral cortex can be done (21).

Quantitative Aspects of Golgi-Impregnated Barrel Neurons. Since the barrel cells were reconstructed with the computer-microscope, it was of interest to determine if the quantitative data obtained in this way would yield additional information about the barrel-related neurons of layer IV and support our classification. More succinctly, could these cells be placed successfully into the above defined classification solely on the basis of the numerical data? For this, we chose three context-free features of the cell‡: (*i*) the cross-sectional areas of the cell somata; (*ii*) the average three-dimensional distance to each dendritic branch from the cell soma measured *along* the cell process; and (*iii*) the average three-dimensional distance from the somata to each of the ends of the dendrite, again as measured *along* the cell process.

‡ It is possible to extract both context-free and context-dependent parameters from the quantitative data. We have found the context-free parameters to be reliable indices of cell category. Theoretically, this appears to relate to the biology of these neurons (see *Discussion*). From a practical standpoint, the context-free parameters used circumvent the problems associated with an incomplete demonstration of a cell's form.

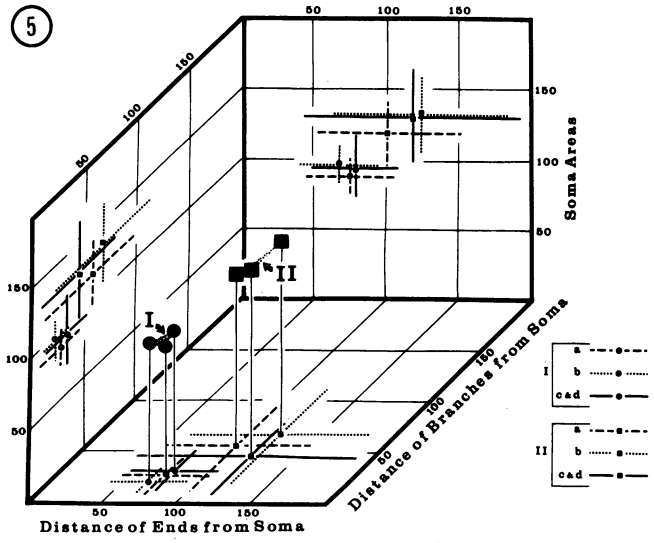

FIG. 5. A three-dimensional graph to show the clustering of the means for the three parameters for the different types of the class I (●) and class II (■) cells. Notice that the mean values for cell types within a single class are close to each other and that there is good three-dimensional separation of the means for each of the two classes. The relationships of each pairwise combination of the three parameters—Distance of Ends from Soma (in μm) on the x axis, Soma Areas (in μm^2) on the y axis, and Distance of Branches (or Branch Points) from Soma (in μm) on the z axis—are shown on the x-y, x-z, and y-z planes. In each plot the solid symbols (● and ■) represent the mean values and the lines represent the standard deviation about the mean for each cell type within a neuronal class according to the key at the lower right of the figure. Cell types c and d, which are numerically few (see Table 1), have been combined to increase their statistical significance.

To visualize the relationships between all combinations of the pairs of parameters, we constructed three two-dimensional graphs. From these graphs, there is an obvious tendency for the cells to be separated according to the above defined classes I and II, but there is considerable overlap in the distributions (Fig. 5). The relationships between all three parameters can be seen in a three-dimensional graph, which further enhances the discrimination between the two cell classes. If the arithmetic means are computed over the three parameters for cells in categories Ia-IIc and d and plotted, they form two tight clusters corresponding to the class I and II designations (see details in Fig. 5). In fact, if a linear plane is established as an arbitrary decision boundary between the mean positions, it is possible to assign 85% of the cells to class I or II. The misclassified cells were found to be truncated most severely by the microtome§.

DISCUSSION

We have described the Golgi appearance of the layer IV neurons that comprise the barrels, estimated the relative frequency of these cells, and shown that the classification of these neurons is supported by the numerical data. Of necessity most past attempts to classify cortical neurons depended upon qualitative features, such as axonal distribution (5, 6) and dendritic morphology (7, 22). With the computer-microscope, we have been able to obtain quantitative data that support the division of the barrel neurons into two distinct classes. Both classes would commonly be regarded as stellate cells, although the term "stellate" is still controversial, and for this reason we have chosen a noncommittal terminology. We have found the techniques of data collection and analysis particularly useful in clarifying some of the relationships between the barrel neurons, and we expect that they can be applied to other cortical layers and areas with profit, possibly providing a rationale for determining broader taxonomic relationships of neurons.

The quantitative parameters obtained with the computer-microscope, which permit the classification of the barrel neurons with numerical criteria, are measures independent of dendritic specializations and axonal distribution. From this some practical consequences follow. First, in normal material, cells could be successfully classified even if dendritic appendages are poorly demonstrated, if the axon has not been stained or if the axon is lost due to sectioning. Second, cells that are only partially stained or fragmented, such as those which have been injected with an intracellular marker (11), might be classified using only a few cellular features. This means that, where observations on individual neurons are limited by experimental conditions (11) and/or technical difficulty (12), the likelihood of correspondence to cells seen in normal material can be known.

A concept, which is derived from the field of image-processing (23), has been of considerable value in the present work, that is, the identification of neuronal characteristics as context-free and context-dependent. The division of neuronal characteristics in this way may provide some insight into the development and function of the barrel neurons. All of the neurons can be divided into two classes on the basis of qualitative and numerical context-free parameters. This suggests that all of the barrel cells could be derived from as few as two pools of neuroblasts resulting in characteristic somal, dendritic, and axonal morphologies which are probably interrelated and genetically specified. But all of the neurons of *both* classes exhibit very specific context-dependent morphologies: a precise relationship of processes to the barrels. This could be taken to mean that the neurons of both classes are responsive to developmental instructions that are extrinsic to the neurons, such as spatially segregated thalamocortical afferents (24), for which there is precedent elsewhere in the nervous system (25). It is this developmental stimulus which is presumably altered when the vibrissae are damaged in early postnatal life (16).

The description of the barrel neurons that we have given suggests certain predictions concerning the functional properties of the cells which are likely to reflect the context-free and context-dependent features of these cells. It might be assumed that these neurons would exhibit characteristic class specific (context-free) physiological properties. With "ideal" electrodes, one could expect to find these cells with equal probability. But perhaps of greater interest are the type specific responses (context-dependent), which might be elicited in the barrel neurons by vibrissal stimulation. On the

§ We are aware that there are more sophisticated mathematical techniques for establishing optimal boundaries and in forming clusters (27) and are investigating these methods. The "objective" reclassification of these cells is not statistically rigorous, since the "training set" was used as the "test set." However, the result suggests that these methods will be useful aids for classification of unknown cells.

basis of our observations, we expect that 85% of the cells (types a and b) would respond initially to movements of *only one* whisker, while the remaining 15% (types c and d) would respond to movements of *at least two* whiskers. In this regard, the type c and d cells could be thought of as good candidates for the first step in the cortical integration of activity from more than one vibrissa.

The further development of the computer-microscope system can add to our knowledge of the organization of the mouse SmI neocortex and resolve some questions relating to the intrinsic connectivity of the barrels. Two additional capabilities, which are designed to collect more complete data, are currently in the final stages of development. One will facilitate tracking of neuronal processes through adjacent serial sections. The other will permit many different cells to be displayed three-dimensionally and rotated together. With this information, more sophisticated analytical procedures can be used to search several cells for apparent points of contact between any two cells and can determine the three-dimensional distances from the respective cell somata of these possible contacts.

The perfection of the classification and clustering techniques can be developed along several lines. One is to continue searching for cell parameters that may provide even more accurate measures of cell differences. A second is to develop analytical decision procedures, like those which have been described (26, 27), that may enable automatic, accurate, rapid, and objective neuronal classifications to be established with a minimal number of parameters or incomplete data.

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