Numbers of specific types of neuron in layer IVab of cat striate cortex

(visual cortex/area 17/ γ -aminobutyric acid)

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Communicated by F. H. C. Crick, February 23, 1984

ABSTRACT Layer IVab of the visual cortex (area 17) of the cat contains about 51,400 neurons per mm³, including about 400–1200 per mm³ of each of three categories of neuron believed from previous work to represent discrete types. Each type forms about 0.5–1.5% of all the IVab neurons, which suggests that the total number of types in this layer might be much greater than previously supposed, perhaps as many as 50 or more. From their densities and estimates of their dendritic fields, we calculate that each type completely “covers” layer IVab in the tangential plane but only by a small factor (1.3–4.2).

The total number of neurons in the mammalian cerebral cortex seems astronomical when one is trying to grasp the mechanistic principles upon which the tissue is organized. Even estimates of the number of neurons per cubic millimeter seem, at 50,000 (1), intractably large. One would like to define a meaningful subset of cortical neurons whose number would be far smaller and of an order of magnitude about which we are accustomed to thinking mechanistically. In the cat retina, for example, although there are about 10⁵ ganglion cells (2), each neuron appears to belong to a fundamental type that is defined by its specific association of particular anatomical, physiological, and chemical features (3). Twenty-three distinct forms of ganglion cell have been described for cat retina (4); therefore, there may be 23 fundamental types. Each point on the retina is apparently “covered” by the dendritic fields of one to seven members of a particular cell type (e.g., see refs. 3 and 5). Thus, for the study of retinal circuitry one need only consider a patch of retina large enough to include at least one member of all 23 types of ganglion cell.

It is our broad hypothesis that neurons in layer IVab of cortical area 17 are also discrete in type and, therefore, that the layer can usefully be analyzed in a similar manner (6). We observed that a group of 32 adjacent neurons in a small patch of this layer, when partially reconstructed from electron micrographs of serial sections, could be classified into seven categories on the basis of their morphology, cytology, and synaptic patterns (7). This would be expected if the layer were a mosaic of diverse but discrete types. Subsequent study of γ -aminobutyric acid (GABA)-accumulating neurons in layer IVab supported this idea because each partially reconstructed GABA neuron could also be assigned to a discrete category on the basis of its morphology and synaptic pattern (8). We have also observed that layer IV neurons injected intracellularly with horseradish peroxidase are highly stylized in regard to soma shape and size, number of primary dendritic branches, pattern and extent of dendritic arborization, and dendritic spine density and morphology (6, 9). Eight distinct forms have been identified so far, and there

is no indication that anything like the full extent of the layer’s morphological diversity has been observed.

The receptive fields of simple and complex cells in layer IV have also been sorted into discrete categories based on the number of excitatory and inhibitory bands (10) and on their X-like or Y-like properties (11). So far 18 physiological categories have been identified (10–12). Whether these categories are fundamental or can be further subdivided is not yet clear. The hypothesis predicts that, as in the retina (e.g., see refs. 13 and 14) there will be correspondences between the fundamental physiological and morphological categories, but this has not yet been demonstrated.

In the present study, we selected three categories of IVab neuron believed from previous work (8) to represent discrete types and counted them in serial sections. Each proved to represent but a small fraction of layer IVab neurons, suggesting that in layer IVab there may be many types. We asked whether the dendritic fields of the neurons in each category overlap and so “cover” in the tangential plane of layer IVab. Our estimates suggest that each category does indeed cover and, as in the retina, only by a small factor.

METHODS

Tissue was obtained from two cats prepared so as to label the GABA-accumulating neurons within layer IVab. Each animal was anesthetized with pentobarbital and the region of area 17 corresponding to about the central 5° of vision was injected by micropipette with 2–20 μ Ci (0.2–1.0 μ l; 1 Ci = 37 GBq) of [³H]GABA. One hour after the isotope injection the animal was perfused with a phosphate-buffered mixture of glutaraldehyde and paraformaldehyde. Blocks from area 17 were osmicated, dehydrated, and embedded in Epon as described (8).

From each of three blocks near the sites of injection we cut a series of consecutive sections with a diamond knife. The first four sections in each series were “semithin” (approximately 1 μ m); these were followed alternately by two ultrathin sections (approximately 0.1 μ m), four semithin, and so on. The ultrathin sections provided information on the cytology and synaptology of specific neurons but, since they amounted to less than 5% of the total volume, were omitted from the calculations of tissue volume and cell density. The semithin sections were mounted on glass slides, coated with Kodak NTB3 emulsion, and exposed for 7 days at 4°C. They were developed in D-19 (Kodak) and stained with toluidine blue. The ultrathin sections were stained with uranyl acetate (30 min, 10% in methanol) and lead citrate (1 min, 0.1% in water).

Series I (cat A) was cut tangentially starting from the pia. The emergence of layer IVab, marked by Gennari’s line, was recognized from photographs of the block taken at intervals during sectioning (7) and at this point we began to collect

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Abbreviation: GABA, γ -aminobutyric acid.

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Table 1. Number of neurons per mm³ in layer IVab

Series	Ten-section sample	Volume of sample, $\mu\text{m}^3 \times 10^{-6}$	No. of nucleoli	Mean no. of sections per nucleolus	No. of neurons in sample	No. of neurons per mm ³ in layer IVab
I (tangential, cat A)	1	3.60	715	3.5	204	56,600
	2	4.67	696	2.7	258	55,246
	3	4.67	612	2.7	227	48,608
	4	5.06	635	2.5	254	50,198
	5	3.65	573	3.5	164	44,932
	Mean			3.0 $\pm$ 0.4		51,117
II (coronal, cat A)	1	1.69	304	2.7	113	66,864
	2	1.77	261	2.6	100	56,497
	3	2.09	236	2.2	107	51,196
	4	1.84	259	2.5	104	56,522
	5	1.69	269	2.7	100	59,172
	Mean			2.5 $\pm$ 0.2		58,050
III (coronal, cat B)	1	7.97	958	2.3	417	52,321
	2	7.32	966	2.5	386	52,732
	3	7.32	984	2.5	394	53,825
	4	7.32	1057	2.5	423	57,787
	5	6.55	1235	2.8	441	67,328
	Mean			2.5 $\pm$ 0.2		56,799

sections. Cutting continued for approximately 250 μm until the boundary between IVab and IVc was approached. We were confident that the last section in the series remained within IVab because some large stellate cells (which define the layer) could still be observed. The volume of layer IVab included in this series was about $1.04 \times 10^8 \mu\text{m}^3$. Series II (cat A) and III (cat B) were cut coronally. The borders of layer IVab were identified by the criteria described above and the other layers were trimmed away. The two coronal series contained, respectively, 64 and 60 thick sections and included $1.14 \times 10^7 \mu\text{m}^3$ and $4.25 \times 10^7 \mu\text{m}^3$ of layer IVab.

The element in each cell that could be counted most efficiently was the nucleolus which, being roughly 2–3 μm in diameter, frequently appeared in several consecutive sections. Our procedure, therefore, was to count all the nucleoli in ten consecutive sections and then correct for repetition of the same nucleolus in more than one section. Total number of neurons = (total number of nucleolar profiles)/(average number of sections in which a single nucleolus appears). To calculate the volume of tissue containing these neurons, we measured the areas of all the sections and estimated their average thickness. The latter was calculated as the mean diameter of the neuronal nucleoli divided by the mean number of sections in which a nucleolus appears.

RESULTS

In each of the three series, we estimated the average number of all neurons per mm³ in layer IVab by sampling the layer systematically, counting all the neurons in 10 adjacent sections, skipping 40 sections, counting in 10 more, and so on. The numbers for each series agreed fairly closely: series

I, 51,100/mm³; series II, 58,100/mm³; and series III, 56,800/mm³ (Table 1). Series I proved unsuitable for distinguishing the three categories of neuron to be described next because it was cut tangentially, making it hard to distinguish stellate neurons from pyramids whose apical dendrites had been cut away. Therefore, only series II and III were used for the remainder of the counting procedures.

Certain neurons in layer IVab accumulate exogenous [³H]GABA, producing in autoradiograms grain densities 2.5–25 times those of neighboring neurons (8). Because each semithin section in our series had been prepared as an autoradiogram, we were able to identify and enumerate all the GABA-accumulating neurons. The counts were 60 for series II and 78 for series III. This amounted to an average of 4798/mm³. GABA-accumulating neurons as a fraction of all neurons in layer IVab were for series II and III 9.3% and 7.6% (Table 2).

Counts of Neurons Considered to Be Discrete in Type. Three categories of previously identified neuron were counted. The first was a large stellate cell with dark cytoplasm and a high density of boutons containing flat or round vesicles ending upon its soma. This putative type, termed "class II," was identified by Davis and Sterling (7), using serial reconstruction, and shown by Hamos *et al.* (8) not to accumulate GABA. The second putative type, termed "GABA 1," was a large stellate neuron with a high density of boutons upon its soma and a strong accumulation of [³H]GABA (8). The third putative type, termed "GABA 3," was vertically fusiform with a deeply invaginated nucleus, a sparse distribution of somatic boutons, and a strong accumulation of [³H]GABA (8). Each neuron that might by size, shape, and labeling belong to one of these types was first located by light microscop-

Table 2. Numbers of specific types of neuron in layer IVab

Neuronal type	Series II				Series III				Series II and III	
	Volume sampled, $\mu\text{m}^3 \times 10^{-6}$	No. in sample	No./mm ³	% of total neurons	Volume sampled, $\mu\text{m}^3 \times 10^{-6}$	No. in sample	No./mm ³	% of total neurons	Mean no./mm ³	Mean % of total neurons
Class II	11.4	14	1228	2.0	32	21	656	1.1	942	1.6
Total GABA	11.4	60	5263	9.3	18	78	4333	7.6	4798	8.5
GABA 1	11.4	5	439	0.73	32	7	219	0.37	329	0.6
GABA 3	11.4	9	789	1.3	18	10	556	0.94	673	1.1

py in the semithin sections, and its identification was then confirmed by electron microscopy on the adjacent ultrathin sections.

Fourteen class II neurons were identified in series II and 21 in series III. This amounted to 942/mm³ (1.6% of all neurons). Five GABA 1 neurons were identified in series II and 7 in series III. This amounted to 329 cells per mm³ (0.6% of all IVab neurons). Nine GABA 3 neurons were identified in series II, and 10 in series III. This amounted to 673 cells per mm³ (1.1% of all IVab neurons). The GABA 1 and GABA 3 neurons accounted for only 20% of all the GABA-accumulating neurons. The remaining 80% must, therefore, belong to types GABA 2 and GABA 4 (8) and possibly to additional types not yet defined. Table 2 summarizes the results for all three types.

DISCUSSION

Beaulieu and Colonnier (1) recently estimated, using stereological methods, the number of neurons per mm³ in the binocular segment of the layer they term "IVA." Their designation corresponds closely to our layer IVab, and their estimate of about 51,400 neurons per mm³ is very close to the average of 55,900 neurons per mm³ obtained for our three series. Our figure is slightly higher, possibly because they corrected for shrinkage, while we did not. Given this difference and the differences in tissue preparation and counting procedures, however, the values are gratifyingly close. The proportion of neurons accumulating GABA in the present material, 8.2%, is within the range reported by Fitzpatrick *et al.* (15) for neurons in cat striate cortex immunoreactive for the GABA synthetic enzyme glutamic acid decarboxylase. This suggests that our method does not seriously underestimate the number of GABAergic neurons.

Perhaps our most important observation is that the categories of neuron believed to represent discrete types each form only a small fraction of all the neurons in layer IVab. None of the three types enumerated here were more than 1.6% of all IVab neurons. The other two or more types of GABA-accumulating neuron (8) accounted in aggregate for only an additional 6.6%. Conceivably, certain types of neuron not yet characterized are more numerous and account for a much larger share of the total. If not, the number of neuron types in layer IVab may be as great as 50 or more. This would be consistent with other recent anatomical and physiological evidence, cited in the introduction and reviewed elsewhere (6, 8), of neuronal diversity in layer IV. Similar calculations for vasoactive intestinal polypeptide (VIP) cells in rat cortex and Meynert cells in monkey cortex suggest the same conclusion (F. Crick, personal communication). This seems a startling jump in the number of types believed to be present (e.g., see refs. 16 and 17). However, retinal ganglion cells, formerly believed to be of but a few types, now appear to number 23, and it may not be beyond reason to think that the degree of neuronal diversity would be no less several stages higher in the visual system.

We considered whether the dendritic fields of neurons in each category would overlap in the tangential plane and, if so, by how much. The appropriate calculation is the same as for the "coverage factor" in retina [(cells per mm²) × area of dendritic field (ref. 5)] but needs to be distinguished because coverage in retina can refer directly to the visual field, whereas in cortex it simply refers to the tangential plane of the anatomical structure. Crick (personal communication) has introduced the term "tiling factor" for this calculation in cortex, and we adopt it here.

The exact dendritic diameters for these categories of neuron are unknown. However, the class II and GABA 1 neurons resemble large, smooth stellate neurons described by Hornung and Garey (18) and LeVay (19) whose dendritic

Table 3. Tiling (coverage) factors for neurons in layer IVab

Neuronal type	No. of cells per mm ² of cortical surface	Estimated dendritic area, mm ² × 10 ³	Tiling factor
Class II	236	17.7	4.2
GABA 1	82	17.7	1.5
GABA 3	168	7.85	1.3

fields are about 150 μm. The GABA 3 neuron, being vertically fusiform, would probably have a narrower field in the tangential plane, which we estimate at 100 μm. These values and the observed densities (Table 2) give tiling factors between 1.3 and 4.2 (Table 3). Thus, overlap between members of the same category appears to be fairly close to the minimum required for a complete tiling. It will be important to extend these observations to spiny neurons, which are thought to be numerous in layer IVab (19). If there are spiny types with much greater representation in the cortical mosaic than for types observed here, it will be of interest to learn whether they have correspondingly smaller dendritic fields or much larger tiling factors. One hesitates to extrapolate in such *terra incognita*. Nevertheless, the results might suggest an upper bound of about 10³ as the number of neurons whose interconnections need to be elucidated in a mechanistic analysis of cortical tissue. Perhaps the genome need not specify 10¹⁰ cortical neurons individually but rather circuits involving about 10³ neurons along with instructions for their orderly repetition.

We thank Dr. Ed Pugh and Dr. James Hamos for helpful discussions, Dr. Hamos for providing tissue for series III, John R. Megill for technical assistance, and Harriet Abriss for typing the manuscript. We are especially grateful to Dr. Francis Crick for his critical comments and for suggesting the tiling factor calculation. This work was supported by grants from the National Eye Institute (RO1 EY00828 and 5P30 EY01583).

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