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THE LOCATION AND SIZE DISTRIBUTION OF NEURONS IN THE LAYERED CORTEX

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ABSTRACT

The neurons of the human cerebral cortex were planimetrically estimated with a semiautomatic system (Videoplan). One measuring operation provides many parameters and shape factors. A new symmetry factor can discriminate between granular and pyramidal neurons. The influence of class-width on the distribution is described in a system with 12 classes. With a computer one can construct artificial cytoarchitectonic images which select various cells with respect to their types, sizes and shapes. The cortical neuronal density profiles depend on the thickness of the strata. Thin strata show noisy and thick ones levelled data. A thickness of 30 to 40 μm for the strata is recommended for the cerebral cortex.

INTRODUCTION

By investigating the brain stereologically one has to consider its very complex structure. Consequently a simple random sampling cannot be performed in many parts of the brain. Two problems have been reported.

1. The estimation of cell populations which consist of very different neuron types. Their volumes vary by a factor of about 1000 times, from 200 to 200,000 μm^3. Their shapes are also very different. We find neurons of granular and

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pyramidal type. All neuronal processes were omitted and only the perikarya measured. The cytoarchitectonic Nissl-image is the basis for all quantitative estimations regarding number and size of the neuronal perikarya.

2. In most gray matters, the distribution of neurons and their shapes and size classes are highly inhomogeneous. Only small grisea of the brain can be fully evaluated. Many grays contain many thousands or even millions of neurons which cannot be exhaustively measured.

A special gray structure is the cerebral cortex which amounts to nearly 48% of the entire brain volume. The neurons of the cortex are arranged in layers lying parallel to the surface. Their spatial configuration opens a way for systematic evaluation (Haug, 1979; Haug et al., 1971).

PROCEDURES

The following results were obtained with a Videoplan and a sampling stage microscope whose image was displayed via a drawing mirror onto a digitizer board. This instrument allows the measurement of many parameters simultaneously. We differentiated between the origin, shape and size. The perikarya are shaped mostly like rotationally symmetric bodies having their axis perpendicular to the surface of the cortex. Most neurons have parallel rotational axes, assuming one measures only those parts of the cortex with an even surface. A further benefit is obtained by introducing the nucleolus as a marker for evaluation. This has two major advantages: First, the counting error will be minimized to the size of the nucleolus. Second, there is a high probability that the largest projection area lies inside the depth of section. The size distribution derived from these data are unfolded. The section thickness amounts to about 20 μm.

Our computer program makes it possible to estimate the coordinates of the center of gravity for each cell measured within a total field up to a square of 3,0 mm. We differentiate between the following cells: astroglia, oligoglia, granular, pyramidal and spindle neurons and cells that are difficult to identify.

The following parameters were estimated for each cell: the size of the projection area, the perimeter, the longest diameter and its angle to the pial surface as well as three shape factors. The latter are: the usual comparison with a sphere, an ellipsoidal factor and a factor regarding the deviation of the center of gravity from the middle of the largest diameter. We call this the symmetry factor. In order to use this information optimally, we transferred the data to our computer-system. With the help of these data we looked for the best differentiation between the various types of cells.

RESULTS

1. The shape factor comparing the perimeter and the area with a sphere is not very useful for our purpose, because it is of limited significance for discriminating between the different cell types (fig. 1a).

2. The symmetry factor is useful for a discrimination between granular and pyramidal cells (fig. 1b). The smaller granular cells yield a dense cloud of scatter points in the left part of the figure around the value one. The pyramidal cells with a large range of sizes have a mean symmetry factor slightly below 0.8. The density distribution decreases from the smaller to the larger sizes and has a high variation.

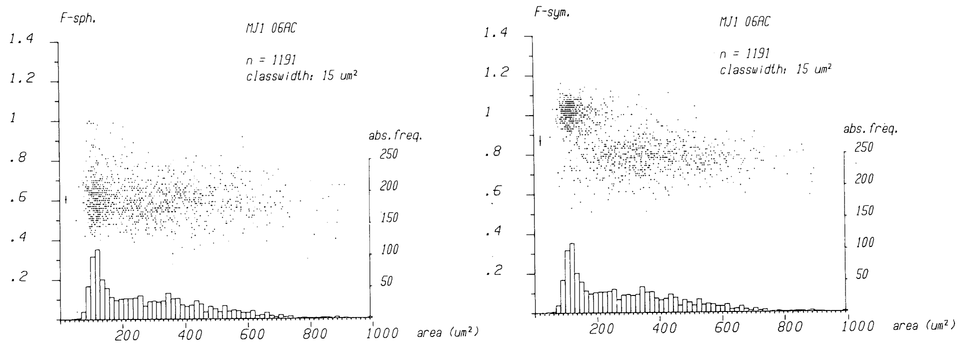

Fig. 1: Above scatter diagrams regarding the size of projection-area (abscissa) and the shape factors (ordinate) for spheres (fig. 1a) and symmetry (fig. 1b) of the human cortical area 6. Below the histograms of the frequencies in the neuronal size distribution.

3. Fig. 2 shows three different size distributions of the same sample. The primary values were arranged in three systems of different class-width. Each system was based on two different assumptions. The first assumption concerns an open system of size classification. The second shows a system having only twelve classes. This is useful for simple equipment with limited storage facilities. The twelfth class also contains neurons which belong to larger classes. Therefore this class is called the overflow-class.

Depending on the class-width the numbers of cells in the overflow class are very different. A small number in the overflow class is observed for a class-width of 30 and a high for one of 15 µm². On the other hand discrimination between the distribution in the lower classes is small for 30 and high for 15 µm². A deficit in the upper graphs might possibly be caused by two subpopulations of cells, which can be discriminated with the small minimum in the size-class in the graph with a 15 µm class-width. The weight of the overflow class within the entire sample depends on the size of occupation in this class. The consequences are:

1. An occupation of the overflow class of less than 1% has a negligible influence on the results. No correction is necessary.

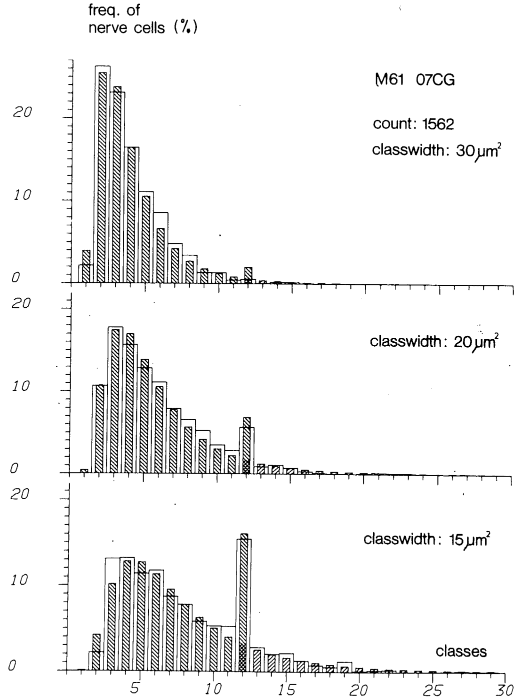

Fig. 2: The dependence of the shape of the unfolded size distribution on the width of classes from 1,562 neurons of human cortical area 7. The broad bars represent the actual values and the striped narrow ones, the best adapted logarithmic approximation; for an open system and for a system with 12 classes.

2. Occupation of the twelfth class of between 1 and 10% show an increasing influence. A correction should be performed. The mean actual area shifts from 1.0 to 1.5 times the mathematical class average. Regarding this method for correction see KÜHL et al. (1982).

3. An occupation rate of over 10% leads to some uncertainties, because the real size distribution is unknown. We suggest therefore using a size class width which avoids an occupation of class twelve of larger than 10%.

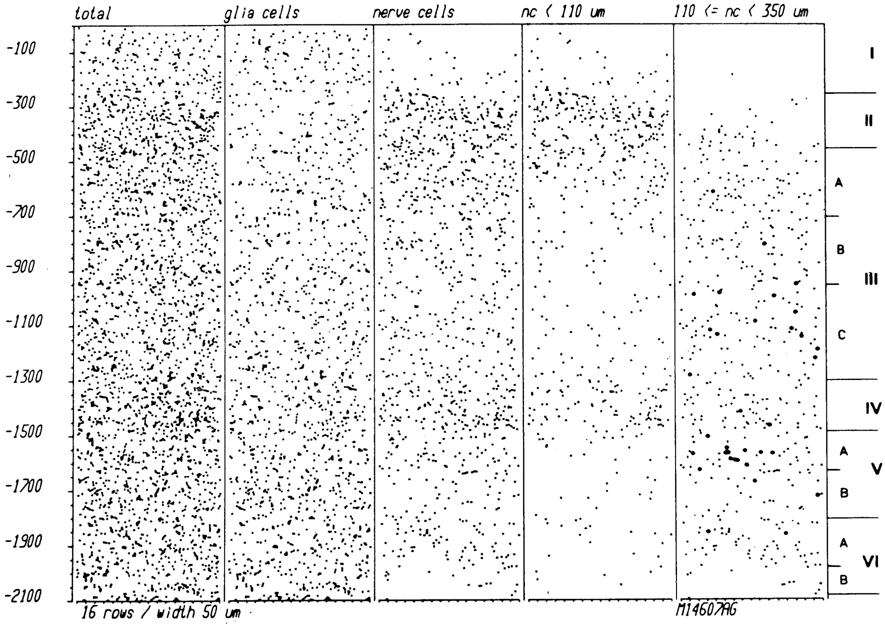

Fig. 3: Five scatter maps of cortical area 7 composed by the computer. The left ordinate gives the distance from the surface, the right the layering. nc <110 µm represents neurons having a projection area smaller than 110 µm². 110< = nc all larger neurons. The thick dots denote pyramidal cells larger than 350 µm².

With the center of gravity measurements one can construct distribution maps of the cells. Fig. 3 shows five such maps of the same region of cortical area 7. The left map depicts the location of all the cells. The layer arrangement can just be perceived. The arrangement of the glial cells is independent of the layering. The third map shows the density of distribution of all nerve cells. The layers II and IV can easily be detected. The fourth map contains only those neurons which are smaller than 110 μm^2, layers II and IV are nearly selectively represented. The last map demonstrates that layer II is free from larger cells. The medium sized cells have nearly the same density from layer III on until layer VI. Neurons larger than 350 μm^2 can only be seen in the deeper layer III and V.

The maps demonstrate that a computer can construct images which cannot be seen in the microscope. These artificial images may help in understanding specific cell arrangements. This example demonstrates a recommended graphical display of the results. A similar procedure to construct cytoarchitectonic images has been described by HOLLÄNDER et al. (1976).

The variation of density from the surface to the white matter within the layers of the cortex can be expressed by a profile. With the coordinates of single cell one can construct profiles for strata of different thickness.

Fig. 4 shows the density of profiles from the same cortex as in fig. 3. The thickness of the strata increases from 10 to 100 μm. The profile on the left with a stratum thickness of 10 μm shows an image with closely spaced sharp spikes which can be termed "noisy". The strong disturbance in the density arrangement has been lost in the second profile, but the influence of "noise" still remains. The third profile of 30 μm thickness demonstrates a course from the surface to the depth which evidently reproduces the actual density changes. The visual impression of the cytoarchitectonic image agrees with the smaller maxima and minima. The three profiles on the right show an increasing levelling of the smaller density minima and maxima outside of the layers II and IV.

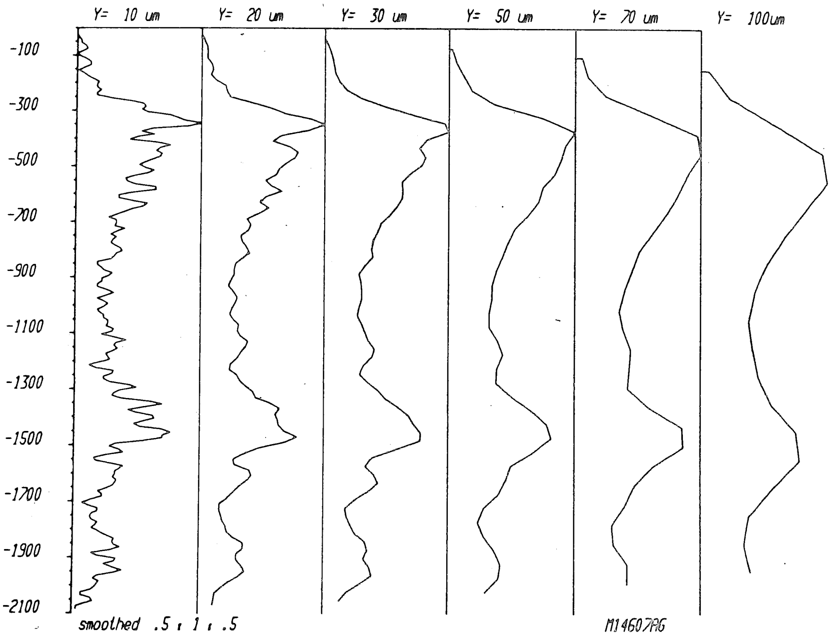

Fig. 4: Density profiles of the same cortex as in fig. 3 for different thicknesses (y = µm) of strata. All values are smoothed by halving the size of both neighbouring strata.

Fig. 4 demonstrates that the summation of values within a layered structure depends on the thickness of the strata and leads to profiles which show on the one hand noisy phenomena or a levelling of values in the layers. A small space exists between these boundary conditions. Its profile corresponds well with the actual density arrangement. In our example it agrees with a stratum of 30 to 40 µm thickness.

CONCLUSION

The possibility of measuring many parameters with one planimetrical operation is a great advantage in modern semi-automatic systems. We can conclude: A systematic analysis using the different parameters gives the opportunity of illuminating many features of one sample. These features are not only important for the significance of the results but also for their theoretical interpretation and for the development of new or the improvement of older procedures.

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