Developing Cognitive Models by Stereological Measurements of Cerebral Cortex

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ABSTRACT
We aim to develop a working diagnostic and research method for measuring morphologically the main function of brain, the intelligence, under microscope in histological slides using the approach of multifactorial systemic estimating. The first two steps: the choice of the brain system-forming factor and preparing of methods for stereologic measurements are presented in this article.

Keywords
Brain, measuring of intelligence, quantitative functional morphology, systemic multifactorial analysis, stereology.

1. INTRODUCTION
A new paradigmatic methodical approach, created and named by us “Quantitative Functional Morphology" (QFM), [3,9], makes it possible to measure virtually the potential maximum value of the main function of an organ or tissue (biological safety margin) on histological sections or on electron microscopic images of tissue biopsies under light or electron microscopy. The methods of the QMF approach are developed in detail for the cardiac muscle [2, 3, 4, 7, 9, 10, 11, 12]. Soon after their development, it became clear that the basic principles of the QFM are also applicable to a number of organs and tissues [5, 6, 8, 13]. From this number of organs the brain fell out for a long time. We initially thought that the brain can’t become the object of QFM system analysis because of the complexity of its organization and multitude of functions.

2. DESIGN OF DEVELOPING METHOD
System-forming factor
We took the first serious step forward in the direction to develop a method for system integrative morphological multifactorial study of the brain. As a system-forming factor for the brain, in particular for the cerebral cortex, the magnitude of virtual electric potential was chosen which could be generated and propagated in a unit volume of the cerebral cortex per unit of time.

Getting quantitative morphological data
To calculate this hypothetical electrical potential, it is necessary to have values of a numerous stereological parameters of brain histostructures. The accuracy of measurement of these parameters, the possibility of their exact reproduction in repeated measurement are important points and are one of ten main fundamental principles of the QFM. It is necessary to measure in cerebral cortex, the stereometric parameters of numerical density (Nvi), volume density (Vvi) and surface density (Svi) of a number of structures: neurons, glial cells, intercellular space without cells, brain capillaries. Later the quantitative and qualitative characteristics of synapses inside the cortex must be considered. The measurement of these cortex parameters of the brain of different animal species has its own peculiarities. Our research is mainly carried out on rats, also on posthumous human brain. The details of the measurement of stereometric parameters of rat brain are considered in this article.

Where and how to measure?
To measure the above mentioned parameters in rat brain, it was found expedient for the beginning of the study to perform measurements in the same easily identifiable area of cerebral cortex. This cerebral cortex area, lying on the projection of the largest part of the hippocampus was chosen for measuring. This part of cortex contains the areas V2MM (secondary visual cortex, mediocadial area), V2ML, (secondary visual cortex, mediolateral area), V1 (primary visual cortex), (Fig. 1).

To find out and cut a layer from this part of the cortex is easy for the macro preparation as well as it is easy to recognize it on histological sections.

Most structures of the cerebral cortex are oriented (capillaries, pyramidal cells, axons, dendrites) in three-dimensional space, which should be taken into account in choice of cutting mode of the tissue and in choice of measurement methods. It is necessary to obtain 2 mm thick tissue layer from the rat cerebral cortex. The sagittal longitudinal axis of the incision surface must extend perpendicular from the surface of the cerebral cortex to its lower boundary, to the white matter. Measurements should be performed on histological sections of standard thickness – 4μm, which, with an adequate stained preparation, provide a clear two-dimensional picture of the rat brain.

Slide staining methods
Methods of histological sections staining must be standardized. At the beginning we have chosen the following staining methods: hematoxylin-eosin, Nissl method, PAS with prestaining of sections by hematoxylin. The last method was modified [9], and the pattern received by combining staining, is more suitable for the needs of stereological studies.

To carry out stereological measurements, was found appropriate to use the integrative method developed earlier [9], which is a combination of point counting and linear scanning methods. It is based on a linear method for determining the structural volume composition, the specific surface of oriented structures with directed secants [1,14].

For this purpose, a measuring grid of rectangular shape with five longitudinal and ten transverse ribs was made. Two longitudinal ribs and three transverse ribs of the measuring grid are equipped with finer 5 divisions on the sides of squares. These grids can be small for insertion into the eyepiece of a binocular microscope, or they can be projected onto pictures on the desktop, or they can be projected on a computer monitor over a cortex pattern.

Calculating of stereological parameters
The volume density (Vv) was calculated by the formula \( Vv = n / N \), where \( n \) is the number of nodal points of the grid that came to the structure under study, \( N \) is the total number of nodal points of the square grid that passed through the
vertical band-area of cerebral cortex undergone to measure. The surface density of the structures was calculated from the formula \(S_{vi} = \frac{2N_i}{L_t}\), where \(L_t\) is the total length of the control lines of the square grid that passed through vertical band-area of cerebral cortex undergone to measure, \(N_i\) is the number of intersections of \(L_t\) control lines with the contours of the surface of each studied structure.

3. RESULTS
The development of the method was carried out on histological sections with a thickness of 4μm of the brain of white, mature male rats. The above presented method was used to receive the mean values of stereological parameters of the brain cortex of five white outbred male rats weighing 220-230 grams.

The values of the stereometric parameters were, for neurons-pericarions: \(V_{vn} = 0.17\text{mm}^3/\text{mm}^3\), \(S_{vn} = 67\text{mm}^2/\text{mm}^3\), for neuropile: \(V_{vnp} = 0.70\text{mm}^3/\text{mm}^3\), \(S_{vnp} = 82\text{mm}^2/\text{mm}^3\).

4. CONCLUSION
These values will be included in the systemic model-equation to measure the main function of the cerebral cortex: the value of virtual electric potential generated and propagated by 1mm3 brain cortex in one second. The model-equation needs also some coefficients. Their determination is the task of the forthcoming study. About the dilemma: manual or computerized automated image analysis method must be used for stereologic measurements. The answer for this stage of study is: computed methods for image automated analyses will be chosen or specially developed after that, when all or most details of stereometric measures will be developed and checked with the manual method.

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REFERENCES