ACTA STEREOL 1982; 279-287
STEREOL 82 SHEFFIELD

TRACING STRUCTURES IN SERIAL SECTIONS BY COMPUTERIZED SUPERIMPOSITION

Siegfried Eins and Joseph Lessmann

Department of Anatomy, University of Göttingen, Kreuzbergring 36,
D 3400 Göttingen (Federal Republic of Germany)

ABSTRACT

A straight-forward computer program is described to pick up selected morphological data from serial sections using a semiautomatic image analysis system. The program is able to plot a surface map of, e.g. vessels completely penetrating all the cortical laminae. The local vessel densities may be discussed as being correlated with neurogenetic processes.

INTRODUCTION

Three-dimensional structures may have extensions in two directions in the same order of magnitude as section thickness but may be much longer in the third direction. Their structural properties (e.g. branchings) may not be observed within a single section. Electron microscopy of tubules and blood vessels or fibres in histology may serve as examples in life sciences. To analyze these structures in space it may be useful to have at hand a simple method, which is more powerful than visual observation, but less complicated than a true 3-dimensional reconstruction of objects from serial sections.

Appropriate coordinates of interesting structures are picked up using a semiautomatic image analysis device via a camera lucida attached to the microscope or from micrographs. The image information obtained may be displayed on the graphic screen of a microcomputer, edited, stored and superimpsoed on other sections.

This kind of study is useful to determine, e.g. the linear density of branchings, the absolute length of fibrous structures or the exact position of structural elements in space. As an application we will show the map of rats' cortical surface demonstrating the position of those large penetrating vessels which have their terminal bifurcation only in the very deep cortical layer or even in the white matter.

MATERIALS AND METHODS

Histology: 9 adult rats are fixed by perfusion with 5 % formaldehyde. The removed brains are sliced as a complete frontal series of alternating 40 and 120 µm sections. Only the thicker sections are used in this study after silver impregnation (Lessmann, in prep.). No parts of the large vessels are lost by leaving out the Nissl-stained 40 µm sections.

Image analysis: Images (camera lucida or photographs) are digitized on a MOP AM 02 (Kontron) semiautomatic image analyzer. The obtained coordinates of interesting section structures are transferred to a microcomputer and shown on the graphic display unit (HP 9845, 56 kbyte memory, Hewlett-Packard). This image information is stored on tape cassettes, one section per file.

In our application the visible segments of large radially running vessels contained in a given section are picked up. At the same time the pial profile of each brain section starting from the median fissure and terminating at the fissura rhinalis on both hemispheres is drawn. This contour is used for manual alignment of serial sections, as well as for calculating the pial profile length between a vessel entrance point and median fissure.

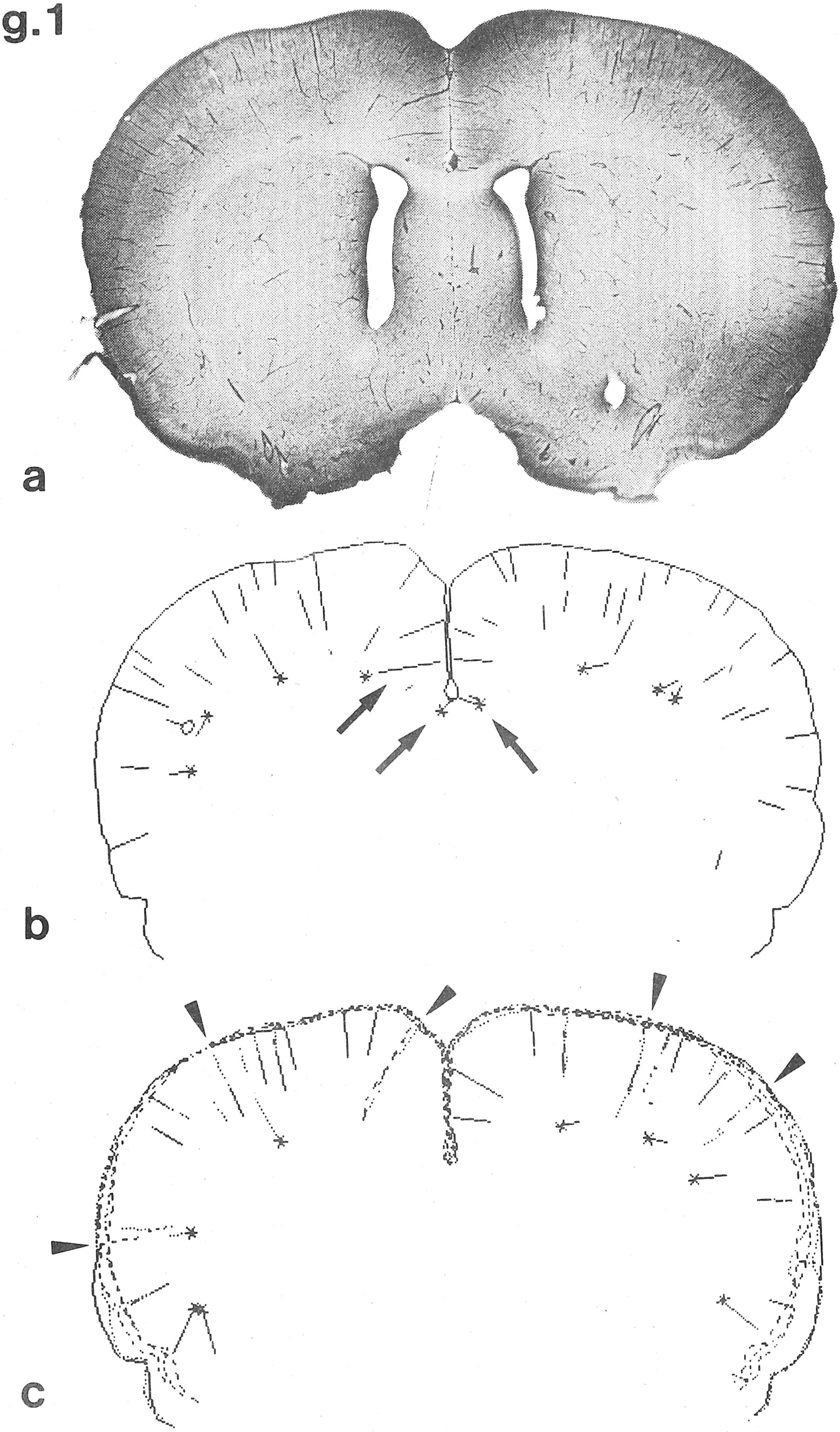
RESULTS

The presented computer program is written for tracing arborized "1-dimensional" objects in 3-dimensional space using serial sections. It is assumed that neither recording of the exact course of the elongated structure nor presence and position of intermediate branchings are of interest. Relevant structural descriptors are spacially defined starting and end points. Additionally, not a single element of a structure but all equivalent components of a whole organ must be recognized which are in agreement with the above mentioned starting and terminating criteria.

Depending on the density of the observed structure classifying and scratching routines should be available to keep the graphics' display free from too much image data which might disturb further analysis. Classification of a structure is only possible if it is completely displayed between the above defined starting and end points by superimposition on several adjacent sections. During this analysis it becomes clear which earlier stored parts of an element are without any significance for further reconstruction and may be eliminated. The degree of operator involvement in the editing and interactive process on the screen depends on the complexity of decisions to be made in a given application.

We used this program for generating a flattened pial surface map containing the entrance points of all long non-capillary blood vessels which completely penetrate the rat's cerebral cortex. This class of vessels is characterized by a final bifurcation into smaller horizontally running vessels only in the deepest cortical layer (lamina VI) or even inside the white matter. As a rule, the overall vessel course including pial entrance and terminal branching is not visible in a single section plane. Therefore, tracing by superimposition of a varying number of sections has to be applied to get all contributing parts (Fig. 1).
Every step of superimposition of the actual

Fig.1



section on the images of earlier stored sections is used to extract the relevant information for the map and to clear the included section images from redundant image parts. For a given superimposed display the program serially addresses all pial entrance points by a graphical pointer. If the operator decides that the vessel is completely seen, a label (+ in Fig. 2) is drawn into the map. Its position is given by the plane of vessel entrance and the calculated length of the pial profile between the entrance point and the median fissure. This way, the map is successively updated by a retrospective (vessels originating in earlier stored sections) analysis. At the same time, the program automatically searches for the matching parts of this vessel segment in the other planes of the overlay. They are all eliminated from the corresponding section images before restoring the actualized section data into its specific file. To avoid an overloading of superimposed displays

Fig. 1: (a) Micrograph of one serial section (its position in the series is indicated by an arrow head in Fig. 2). Segments of large penetrating vessels and the cortex/white matter borderline may be easiyl recognized. Magnification 7 x.
(b) Computerized display of the section shown above containing the pial profile and parts of penetrating vessels contained in this slice. Terminating vessel labels indicate final branching in the deepest cortical layer (*) or in upper cortical laminae (o). The labels were given as codes during coordinate measurements.
(c) Superimposition of five adjacent sections including that shown in (a) and (b). Vessels completely seen directly in one section (→ , in (b)) or by superimposition are already removed. The residual image shows vessels becoming more and more completed with progressive analysis (►).

the scratching routine is also applied for those earlier stored vessel parts which are recognized as being of no significance in further steps of

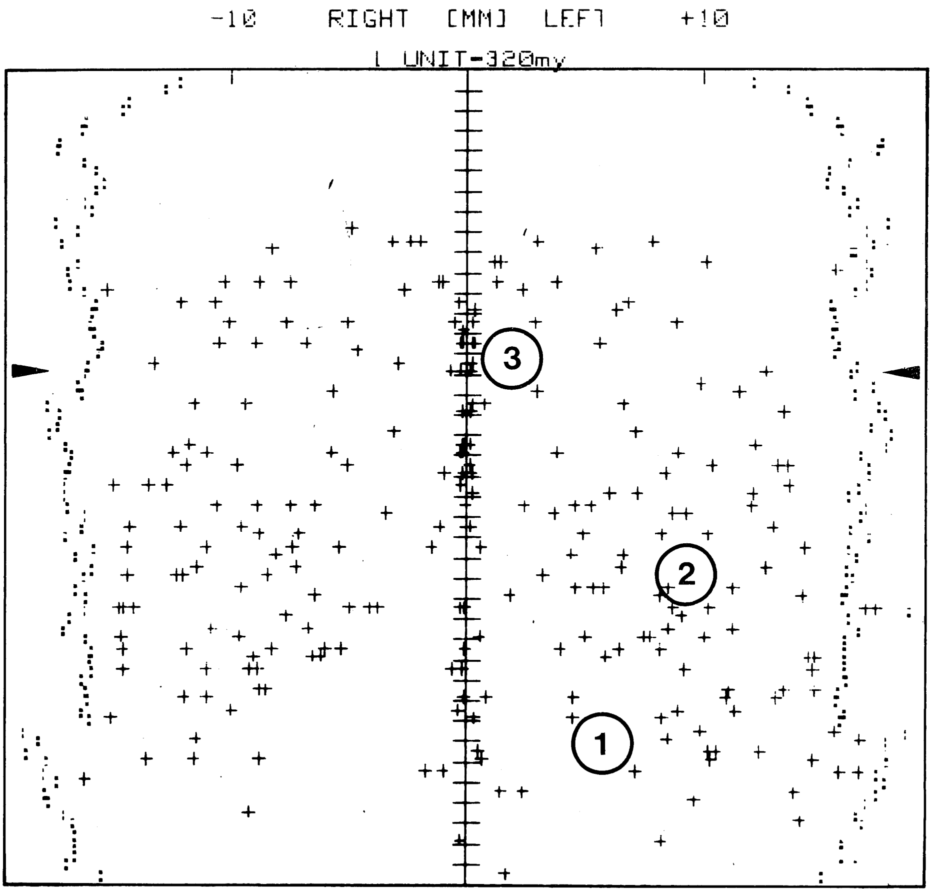


Fig. 2: Map of the flattened pial surface of one rat brain showing the position of long penetrating vessels (+). The dotted boundary represents the fissura rhinalis which was used as a basal limit of vessel mapping. The vertical axis (frontal on top) marks the median fissure. The arrow heads indicate the level of sectioning for Figs. 1a and b. Location in the left hemisphere of visual (1), auditory (2) and cingular (3) cortices is roughly added by hand.

analysis (e.g. vessels not reaching the deepest cortical layer, double observation of the same vessel in adjacent sections, non-interesting branched vessel segments, parts of vessels originating basally from the fissura rhinalis).

DISCUSSION

In neuroanatomy serial sectioning and computerized methods have been used to reconstruct fibre tracts (Zimmermann et al. 1980) or to analyze on a more cellular level the branching tree of neuronal processes (Sobel et al. 1980). Our approach is limited to recognize morphologically defined points linked by a certain structure. Therefore, much simpler computer equipment is needed. But such comfortable features as, e.g. tilting the reconstructed object, removal of hidden parts etc. are not available. Furthermore, from the analyzed tissue component only selected morphological information is used to solve a special problem, not to give a complete reconstruction in space. Mapping may be regarded as one possible application.

Organization of the central nervous system is frequently discussed on the basis of morphologically and functionally distinct areal subdivisions which may be distinguished on the cortical surface (Krieg 1946, Zilles et al. 1980, Wolff et al. 1981). This areal pattern is supposed to be related to vessel architecture for reasons which are explained in more detail by Wolff et al. (1981). This relation becomes more and more interesting because it is assumed that different vessel classes represent "time markers" for the regional history of a certain cortical area (Eulner 1980). Local vessel density, therefore, reflects the heterogeneous cortical development and is correlated to neuron migration, lamina formation and other neurogenetic processes (Wolff et al. 1981).

To have a reference to the already existing and widely used maps of the rat cortex (Krieg 1946, Zilles et al. 1980) a vessel map should also be drawn on the cortical surface, although the longest penetrating vessels manifest themselves in or beneath lamina VI. This was the reason for tracing them back to their pial origin. But the maps as shown in Fig. 2 may not be directly compared with the mentioned areal maps because of the different views used: Krieg's map (1946) is a top view on the rat cortex, whereas Zilles et al. (1980) published the cytoarchitectonics in sections of different position and orientation. A flattened map, certainly, has the advantage that it allows us to look into folds (region 3 in Fig. 2).

The reliability of correct tracing and, therefore, completeness of the map is very high in the central part of the series where, generally, less than six sections have to be superimposed to cover the whole vessel. However, the frontal cap region is less easy to survey because the vessels run more or less perpendicularly to the section plane and have to be followed through as many as 15 serial sections. (The most frontal upper part of the maps (Fig. 2) must, therefore, be reexamined using the opposite direction. By going from the middle towards the cap the number of vessel segments which have to be considered is restricted to those recognized a priori as belonging to the longest ones).

To give a preliminary interpretation of Fig. 2 one surely may differentiate, e.g. between visual (young), auditory and cingular (both old) parts of the cortex showing low or high levels, respectively, of vessel densities (number of crosses in the corresponding area of the map).

Supported by DFG grants SFB 33 E3 and Wo 279/3-2.

REFERENCES

Eulner S. Beziehungen zwischen Wachstum und Vaskularisation der Großhirnrinde. Eine morphometrische Studie an der Albinoratte. Thesis, Göttingen, 1980.

Krieg WJS. Connections of the cerebral cortex: I. The albino rat: A. Topography of the cortical areas. J Compar Neurol 1946; 84: 221-75.

Sobel I, Levinthal C, Macagno ER. Special techniques for the automatic computer reconstruction of neuronal structures. Ann Rev Biophys Bioeng 1980; 9: 347-62.

Wolff JR, Holzgraefe M, Eulner S, Záborszky L. Modular organization of rat neocortex: vascularization, growth and connectivity. In: Szentágothai J, Hamori J, Palkovits M, eds. Adv. Physiol. Sci., Regulatory Functions of the CNS Subsystems. 1981; 2: 79-82.

Zilles K, Zilles B, Schleicher A. A quantitative approach to cytoarchitectonics. VI. The areal pattern of the cortex of the albino rat. Anat Embryol 1980; 159: 335-60.

Zimmermann IR, Karnes JL, O'Brien PC, Dyck PJ. Imaging system for nerve and fibre tract morphometry: components, approaches, performance, and results. J Neuropath exp Neurol 1980; 39: 409-19.