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MORPHOMETRY OF SYNAPSES ON IDENTIFIED NEURONS IN THE VISUAL CORTEX

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ABSTRACT

To illustrate the potentiality of morphometry in the study of identified neurons, a Golgi-impregnated, gold-toned neuron was thin sectioned and partly reconstructed. With this procedure the size distribution of synaptic contacts and their distribution along the neuronal surface could be determined. Both symmetrical and asymmetrical synapses were found on the cell body and dendrites. The total number of synapses encountered was 367 and the mean length was 400 nm. The completely reconstructed part of the neuron received 262 synapses. Thirty percent of the synapses were symmetrical and the other 70 percent asymmetrical, giving a total receptive surface of about 9 percent. Analysis of the distance of individual synapses from the centre of the cell revealed an even distribution along the neuronal surface, without a significant abundance on proximal or distal parts.

INTRODUCTION

The visual cortex is a highly ordered, laminated structure. The different neuron types, with their characteristic dendritic and axonal arborizations, can be identified easily after impregnation with one of the Golgi procedures. Previous Golgi and ultrastructural studies could not give information about the synaptic organization of individual neurons. The reason for this is that routine Golgi impregnated material is not suitable for electron microscopy. In addition, three dimensional reconstruction from two dimensional ultrathin

sections, as usually applied in electron microscopy, is an immense task (Davis and Sterling,1979). Modification of one of the Golgi methods by Fairén et al.,1977, not only provided us with the opportunity to study the ultrastructure of identified neurons with all their ramifications, but also facilitated the quantitative analysis and reconstruction of these cells. The relevance of this quantitative approach is that several parameters, which influence neuronal activity can be estimated: 1) Distribution of synapses along the neuronal surface. Synapses on dendrites closer to the cell body are thought to have a stronger effect on the activity of the neuron than on distal parts (Rall,1964). 2) Size of synaptic contacts. Large synapses, sometimes perforated, are suspected to be more efficient in transmission than small synapses (Peters and Kaiserman-Abramof,1969 and Vrensen et al., 1980). 3) Distinction between asymmetrical (A) and symmetrical (S) synapses. It has been shown that symmetrical synapses, have an inhibitory effect in the visual cortex (Ribak,1978). This study was focussed on synaptic architecture and neuronal circuitry, which are essential to the understanding of the integration of impulses.

MATERIALS AND METHODS

The visual cortex of aldehyde perfused rabbits was processed by the Golgi-Rapid procedure. Subsequently sections of 100 μm were cut on a vibratome. Sections containing completely impregnated neurons were gold-toned according to Fairén et al.,1977. After dehydration and flat embedding in Epon, cells of interest were drawn, photographed, excised and remounted on a prepolymerized plastic block. Serial sections of 70 nm were cut, and ten consecutive sections were mounted on formvar coated copper grids before they were poststained with uranyl acetate and lead citrate. The middle section was selected, and all impregnated elements in that section were photographed at a final magnification of 3000x. Subsequently synapses on the impregnated neuronal profiles were photographed at a final magnification of 41000x.

The following measurements were made using a semiautomatic measuring device (Videoplan, Kontron):

1. Number of synapses on all neuronal profiles (N).
2. Length of synapses (contact zones) (L).
3. Total length of neuronal (dendritic, axonal and somatic)

membrane trace (B)
4. Distance of individual synapses to the centre of the soma (D).

From these basic measurements the following parameters were calculated: 1. Size distribution of synapses. 2. Distribution of the distance of the individual synapses to the centre of the cell body. 3. Receptive surface (RS), which can be calculated by means of the equation: $RS = \frac{N \times \bar{L}}{B} \times 100\%$ (Meek, 1981).

RESULTS

The neuron quantitatively described in this paper was identified as a multipolar non-pyramidal interneuron because of the fact that the dendrites were emerging from several sites on the soma and that the axon did not leave the cortex. Moreover, neurons of this kind lack the presence of a typical apical dendrite. This neuron, with an obliquely oriented soma and spineless dendrites, closely resembled the type 1 neurons described by Jones (1975). Dendrites tended to extent more horizontally than vertically, and its cell body was located in layer VI. To illustrate the sampling procedure, the Camera lucida drawing is projected in a cube (fig.1). As can be seen, all neuronal components within the vibratome slices will be

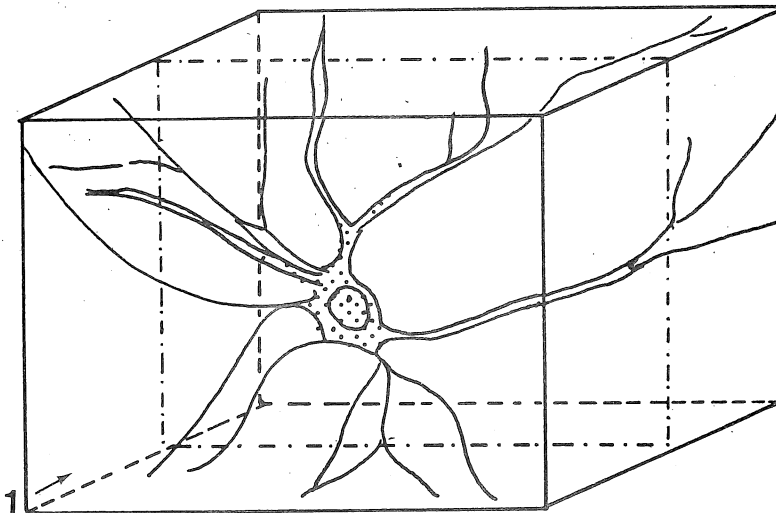

Fig.1: Camera lucida drawing of the analysed neuron

encountered, if sectioning is started at the most distal parts of the dendrites as indicated by the arrow. Serial sections are a prerequisite for an unbiased estimation of synaptic size, density and receptive surface respectively, just as for the distribution of contacts along the surface. Neuronal profiles were marked with gold particles and could therefore easily be distinguished from the surrounding neuropile (fig.2). This figure is a low power electron micrograph of a section through the centre of the neuron (see also rectangle drawn in interrupted lines in fig.1). Synapses were classified into symmetrical, having a thin rim of postsynaptic material and asymmetrical, having a more pronounced electron dense postsynaptic band of 30-40 nm (Colonnier,1968). Eigthy, of the 262 synapses counted

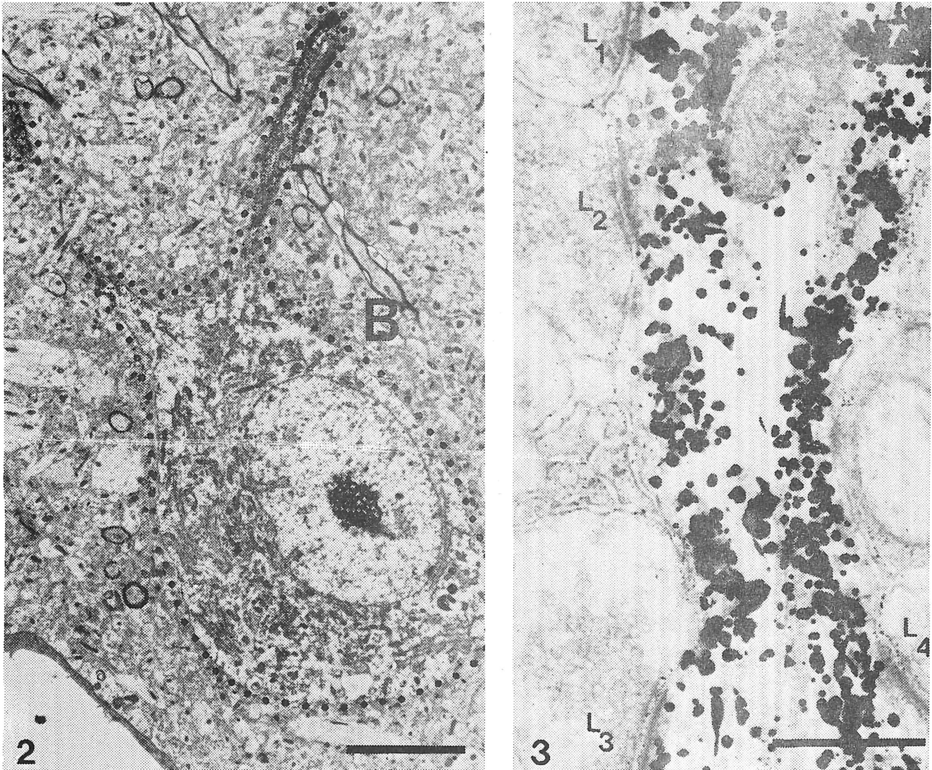

Fig.2 This EM picture shows impregnated parts, which can be distinguished from the surrounding neuropile. The boundary membrane trace is encircled with dots. Bar:5 μm

Fig.3 Gold particles are concentrated beneath the plasma membranes. Four synapses (L_1-L_4) make contact with this dendrite. Bar:0.5 μm

were symmetrical and were most frequent on initial dendrites. whereas the 182 asymmetricals were preferentially more distally located. Most synapses were simple, i.e. the active zone was not perforated or interrupted. In a random sample of synapses in the visual cortex, a mixed population of synapses, including simple and perforated, was observed (Vrensen et al.,1980). Fig.3 illustrates the irregular gold particles and 4 asymmetrical synapses (N = 4). All synapses on the cell analysed, either S or A, had a linear appearance. On account of this observation we assumed the synapses, on this cell, to be disc-like. In general interrupted synapses make up about 10 percent of the total number of synapses (Müller et al.,1981), and are mostly found on dendritic spines (Peters and Kaiserman-Abramof,1969). For the cell studied in this paper, the frequency was far less (1.7%).

The results of the synaptic length measurements are summarized in fig.4. The majority of the 367 synapses we found to be in the range of 340 to 460 nm (S.E.M. 6.5). They were larger than those measured in previous overall estimations (Vrensen and de Groot,1973). This difference may have been due to variation in the synaptic size of different neurons or to random variations in synaptic size as a function of location below pial surface (see e.g. Müller et al.,1981). Whichever of these alternatives holds true, this observation underlines the importance of the synaptic size measurements in individual neurons.

The measurements of the distance from single synapses

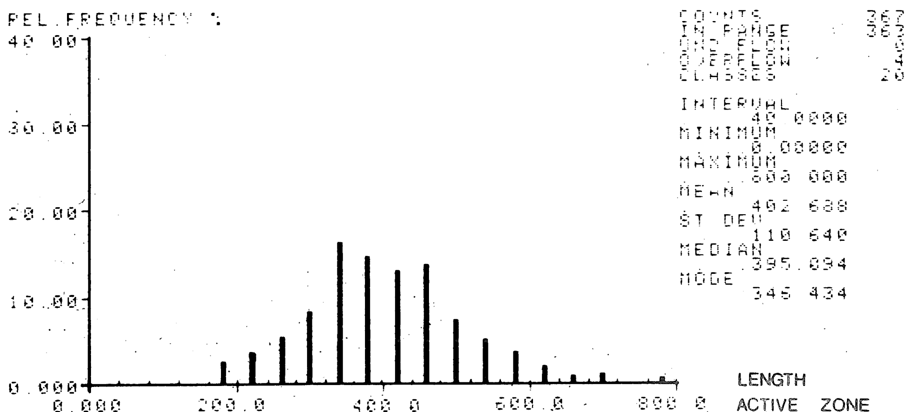

Fig.4 Frequency distribution of the length of synaptic active zones (n=367) in nm

to the centre of the cell body are summarized in fig.5. The ovoid cell body had dimensions of approximately 15x20 µm. Consequently, no contacts were found within a distance of about 7 µm. From 7 µm up to 40 µm, a more or less equal number of synapses was found. The decrease in frequency of synapses more distal than 40 µm, was due to the 105 synapses on dendrites far away from the cell body, which could not be traced back.

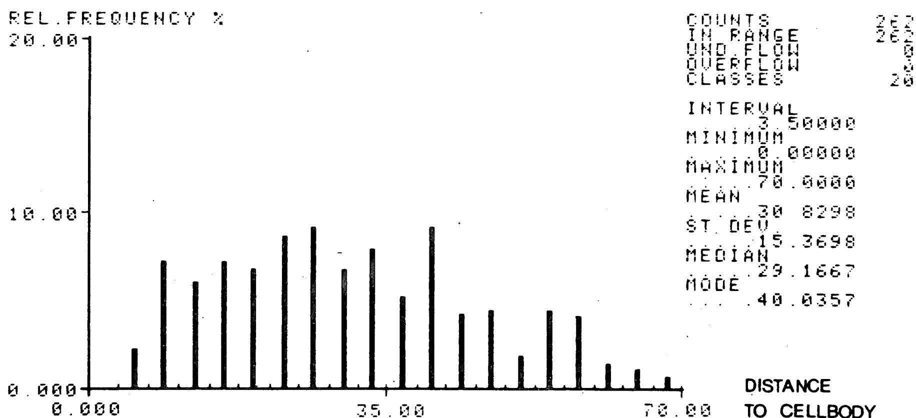

Fig.5 Frequency distribution of the distance of synapses from the cell body in µm (D)

As discussed above, all the synapses were considered as disc-like structures. With the formula given under materials and methods the receptive surface was calculated. A RS-value of 8.9 percent was found.

DISCUSSION

The method described here allows identification and characterization of Golgi impregnated neurons in light microscopic slices (100 µm) and subsequent examination of the same neurons in the electron microscope. This enables a careful quantitative analysis of synaptic architecture. This method was used to obtain the spatial distribution of synapses on an identified neuron. Simultaneously we could get an idea about size distribution and types of synapses as well as of the receptive surface.

A crucial point in this method is the sampling procedure. It seems obvious that selection of neurons from 100 µm

results in a biased sample, because smaller cells will be more likely to be observed to full extent than bigger cells (Cruz-Orive, personal communication). However, one has to realize that the main bias of the present method is inherent in the use of the capricious Golgi impregnation, which can be considered as one of the most unpredictable neuro-histological techniques, whereby less than 10 percent of all neurons are stained. There seems to be no preference for big or small cells. Both are thought to be impregnated to their full extent. In addition, the choice of cells for analysis is not dictated by the staining properties of the cell, but by the investigator who selects the cell of his choice out of many cells present. Regarding the bias, due to the fact that we use 100 µm vibratome slices, the following remarks can be made. It is certainly true for a big cell e.g. a pyramidal neuron in layer V with a more extensive dendritic tree, the chance of loosing distal dendritic segments is bigger than for small cells. However, pyramidal neurons have a specific dendritic orientation and the cutting procedure of the vibratome slices and selection can be such that the loss of distal dendritic segments will be small. In addition, small interneurons often exhibit dendritic branchings in all directions, so that the loss of distal segments might be about the same for small and big cells.

The interneuron described in this paper received far more A- than S- synapses, with a preponderance of the latter type on initial dendrites. The synapses found on this neuron were larger than those found in previous overall estimations (Vrensen and de Groot,1973). Besides the excitatory or inhibitory character of incoming signals onto a neuron, the integrated output is also dependent on the number, size, strength (efficiency) and localization of individual synapses. The RS-value of about nine percent, which at first glance seems to be rather low, has also been observed by Meek (1981) in the optic tectum of the goldfish. He found RS-values of between 1.6 and 7.2 percent and a well ordered synaptic input on identified neurons in different laminae.

From a functional point of view it is evident that the electrophysiological properties of neurons i.e. their firing threshold and firing rate, are partly determined by these morphological features.

At present, our knowledge of the integration of incoming impulses is too fragmentary to discuss this in detail. There are two fine structural approaches which in future will further contribute to the understanding of this integration process:

1. Physiological characterization of individual neurons followed by injection with HRP or a suitable dye, which would enable synaptic analysis along the lines given in this paper for Golgi impregnated material.
2. HRP or autoradiographic tracing of afferent fibers combined with Golgi impregnation of the individual neurons on which the afferents impinge.

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