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DISC-LIKE AND COMPLEX-SHAPED SYNAPSES: NUMBER, SIZE AND DENSE PROJECTIONS. A CRITICAL NOTE.

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ABSTRACT

Attempts were made to derive a numerical relation between two differently shaped synapse populations in the hippocampus of an ageing rat. In addition, the size of their pre-synaptic surface areas and their corresponding number of dense projections were estimated. Stereological methods, applicable to arbitrarily shaped particles, are recommended to investigate these entities. The methods make use of an optimal combination of serial and random section measurements. The findings indicate that the size of the pre-synaptic surface area and the number of pre-synaptic dense projections are highly correlated. Moreover, it appears that these dense projections are regularly arranged and that they most likely follow the pattern suggested by the "tile model". Consequently, this model may be useful to calculate the number of dense projections on an individual synapse of arbitrary shape; undoubtedly an interesting parameter in neurobiology.

The goal of the present paper was to discuss the possibilities and limitations of the different techniques involved, rather than to describe in detail the proposed stereology.

Keywords: Complex-shaped, dense projections, perforated synapses, section-thickness, serial sectioning, stereology.

INTRODUCTION

When investigating hippocampal synapses in ageing rats, it was found that a portion of the synapses clearly deviates from the disc-like shape generally accepted in the literature. These synapses are different in a way that: (i) they have "perforations", i.e. part of the pre-synaptic surface area is devoid of dense projections; (ii) on the average, they are larger than the classical disc-like synapses; (iii) they have an irregular shape. Although no definite proof exists, there is some indirect evidence in the literature that changes in size and shape of synapses are related to changes in synaptic functioning (see e.g.: Greenough et al., 1978; Vrensen and Nunes Cardozo, 1981; Fields and Ellisman, 1985). Whatever the case, the fact that such synapses occur and that they may change numerically, means that these synapses cannot be ignored from a biological point of view. To detect such shifts in synaptic populations it is imperative that reliable quantitative methods are available. In the present paper a number of stereological methods is proposed which generally can be applied on any type of synapse regardless of its shape. This

paper describes: (i) the problems of distinguishing between disc-like and complex-shaped (perforated) synapses, and the consequences for deriving their relative numerical densities; (ii) the estimation of the size of the pre-synaptic surface areas of both types of synapses and the number of dense projections. Both (i) and (ii) are obtained using an optimal combination of serial section and random section measurements.

The stereological methods recommended in this paper were proposed by Cruz Orive (1985) and were tested on a large number of synapses in rats. Some of these evaluations have been reported (De Groot and Bierman, 1983; De Groot, 1985) and a joint paper including a complete description of all the methods and results is planned.

IDENTIFICATION OF SYNAPSES AND ESTIMATION OF THEIR NUMERICAL DENSITY

The first problem encountered is the recognition of perforated synapses. Since the diameter of the synaptic disc is generally larger than the thickness of ultrathin (50 nm) sections, the synapse will likely be transversely cut by sectioning. In a random ultrathin section of conventional OsO4-treated tissue, a transversely cut perforated synapse can only be recognized as such when the cut runs through the perforation. When the tissue is selectively stained for synapses with ethanolic phosphotungstic acid (E-PTA) the perforation may go unrecognized in a random 50 nm thick section, even when the perforation is in the plane of sectioning, because the unit-membrane itself is not stained by E-PTA. In semithin (500 nm) E-PTA-treated sections some of the synapses are completely incorporated within the section and can, therefore, be identified. The latter sections can be used to derive a numerical relation between different synapse populations, but only when dealing with synapses of equal size and shape. If one type of synapse is larger than an other it stands a greater chance to be cut by sectioning and consequently stands a smaller chance of being completely incorporated within the section. Thus, random semithin E-PTA sections cannot be used to derive relative numerical densities between synapse populations of different sizes. It appears that a correct distinction between larger complex-shaped (perforated) synapses and smaller disc-like synapses can only be made in ultrathin serial sections (De Groot and Bierman, 1983).

Up to a few years ago synapses were generally accepted to be disc-like structures. The conventional "random section" methods for estimating the numerical density of synapses are all based on this "disc-like" shape assumption. Application of such methods leads to misleading results when the shape of the synapse deviates from the assumed shape (Verwer and De Groot, 1982). Both the "serial section" technique of Cruz Orive (1980) and the "disector" method of Sterio (1984) are available for the estimation of the numerical density of synapses of arbitrary shape. Apart from the shape assumptions, both methods yield unbiased estimates of N_V irrespective of overprojection due to section thickness and of truncation due to lost caps and lack of resolution. However, to be absolutely sure that one looks at a synapse that might be perforated, tracking of the whole synapse in adjacent sections is necessary. Thus, serial sectioning is required; the choice between the two latter methods is arbitrary since in this case they both require the same amount of work.

Serial sectioning is a laborious, time-consuming and costly approach, involving the sampling procedure, ultramicrotomy, electron microscopy, photography and filing of the material. Therefore, a procedure was developed suitable for routine application. The time needed for tissue-sampling, ultramicrotomy and electron microscopy is reduced con-

siderably by particular "shaping" of the tissue-block for ultrathin sectioning and orientated collection of the sections on special grids. Using a combination of photography and videography, particles which require different magnifications (e.g.: synapses and synaptic substructures) can be measured on the same "low power" micrographs (For details, see De Groot, 1985).

SYNAPTIC SURFACE AREA AND CORRESPONDING NUMBER OF DENSE PROJECTIONS

In general, the perforated synapse is larger and the accumulation of the presynaptic dense projections deviates from the regular triangular arrangement that exists in the classical disc-like synapse. Since these dense projections probably play an important role in the process of transmitter release (see e.g. Akert and Pfenniger, 1969; Akert et al., 1971; Akert, 1973; Vrensen et al., 1980), it is relevant to extract quantitative parameters for their density and their distribution on the presynaptic membrane.

An unbiased estimate for the mean surface area of arbitrarily shaped particles is obtained from unbiased estimates of the surface per volume S_V and the number per volume N_V ratios. The proposed method includes a correction for capping and overprojection of the S_V ratio.

Two models are proposed to calculate the number of dense projection per pre-synaptic surface area of arbitrary connectivity. In the first model, the "conic" model, the dense projections are regarded as cone-like structures sitting on the presynaptic surface. An unbiased estimate of the number of dense projections per synapse $\bar{n}_0$ can easily be obtained from the unbiased N_V ratios of both the dense projections and the synapses. The second model, the "tile" model, in addition attempts to answer the question whether the dense projections are randomly distributed on the pre-synaptic surface area or whether they are arranged according to a regular pattern. In the latter case, a dense projection and its surrounding hexagonal area is hypothesized to be a basic unit, involved in guiding the vesicular release of transmitter. The corner-points of the hexagonal area are probably occupied by vesicle attachment sites as suggested in freeze-etch preparations. This hypothesis forms the basis for the "tile" model (see Cruz-Orive, 1985, Fig. 4). In this model an unbounded lattice of fundamental regions (e.g.: the hexagons), each containing a fundamental domain (e.g.: a dense projection) is covered with a plane mobile figure of arbitrary connectivity (e.g.: the pre-synaptic surface area). Using classical methods of integral geometry the expected number of fundamental domains completely inside the mobile figure is calculated. Hence, the number of dense projections is calculated.

A parameter of primary importance in the stereological approaches, mentioned in this paper, is the section-thickness t . Various techniques for the determination of the thickness of ultrathin sections were applied and evaluated (De Groot, in preparation). From these evaluations the methods of "electron-scattering", "Small-fold", and "interference-microscopy" may all lead to reliable estimates of t . In contrast, analysis of the section-thickness of transversely cut re-embedded sections - i.e.: the "re-embedding" method - appears to be unreliable.

RESULTS AND CONCLUSIONS

As an example, the above mentioned procedures were applied to synapses of the hippocampal CA3 area of a 30 month old rat and the results are shown in table I.

Table 1. Quantification of synapses in the hippocampal CA3 area (str. pyramidale)*** in a 30 month-old female rat

synapse type	*est* N_V	*est Es*	conic model *est* En_0	tile model *est* En_0
non-perforated	0.941	0.041	7.34	7.40
perforated	0.185	0.140	23.20	22.20
perforated*		0.124*		
perforated**		0.016**		

* *Es* of "active zone", i.e. total surface area minus the area of the perforation
** *Es* of "perforation"
*** Cells and large neurites are excluded from the measurements.

est N_V , estimated mean number of synapses per unit volume (μm^{-3})

est Es , estimated mean size of pre-synaptic surface area (μm^2)

est En_0 , estimated mean number of dense projections per pre-synaptic surface area

Notice that in this example, approximately 16.5% of the synapses is perforated and that the perforated synapses are indeed larger than the non-perforated ones. There appears to be a correlation between the size of the synapses and the number of dense projections and also a striking similarity exists between the results obtained with the conic model and those obtained with the tile model.

The latter findings indicate that the dense projections are regularly arranged and that they most likely follow the pattern suggested in the tile model. This, in turn, would imply that the tile model can serve to calculate the number of dense projections on an individual synapse of arbitrary shape, undoubtedly an interesting parameter in neurobiology.

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