VISUAL CLASSIFICATION OF SYNAPSES BASED ON A COMBINATION OF FEATURES

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Several methods of measuring features of the fine structure of synapses accurately on electron micrographs are now available. To calculate areas and volumes of terminals, and of the mitochondria and vesicles contained in them, and also to measure the thickness of pre- and postsynaptic membranes, the synaptic space, and the length of the active zones, the geometrical and stereological principles of Glagolev and Saltykov [2, 10, 12] are used. Determination of the absolute number of vesicles on average per terminal and per unit area of cross section of a terminal is now widely practiced [1, 8]. Active zones are classified visually on the basis of their configuration: Straight and positively and negatively curved active zones are distinguished [6]; according to the degree of abundance of the pre- and postsynaptic membranes after special staining of brain tissue [7]; according to the structure of the postsynaptic membrane [9]; according to relations between the pre- and postsynaptic membranes (symmetrical and asymmetrical active zones) [4, 5], and so on. Synaptic terminals also are classified according to the structure of the vesicles they contain [5, 11] or the distribution of vesicles over the area of cross section of the terminal [1].

A set of features of importance to synaptology has thus been formed in practice, but since these features differ in nature and dimensionality, different techniques are used to study them quantitatively.

In the investigation described below, in order to promote a more systematic and intensive study of large groups of synapses on the basis of a combination of morphological features, a visual classification is proposed for their comparison.

EXPERIMENTAL METHOD

The visual classification of groups of synapses based on a combination of features consists of the following stages: 1) compiling a list of features of synapse ultrastructure; 2) description of each feature according to visually determined classes; 3) recording the class numbers of all features of each synapse, according to the proposed scheme, from a large group of features and their statistical generalization.

Each feature is designated by classes according to its abundance (from minimal — 0 or 1 to maximal — 3 or 4).

1) Number of mitochondria in the terminal (0, 1, 2, ... — serial number of classes).

2) Area of cross section of the terminal (from 1 to 4 classes).

3) Length of the active zone (from 1 to 4 classes).

4) Thickness of the postsynaptic membrane (from 1 to 3 classes).

5) Degree of concentration of vesicles near the active zone (class 1 — vesicles distributed uniformly throughout the area of cross section of the terminal, no particular concentration; class 2 — some vesicles concentrated near the active zone; class 3 — all vesicles located near active zone).

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6) Fraction of area of cross section of the terminal occupied by vesicles (class 1 — up to 25%, class 2 — up to 50-70%, class 3 — up to 100%).

7) Configuration of the active zone (class 1 — straight active zone, class 2 — active zone with positive curvature, class 3 — with negative curvature. The classification is taken from [6]).

Each synapse is described by a line in the record on which the serial numbers of the classes are recorded in constant positions for each feature (Table 1). For convenience in work a group of synapses is divided into groups of 10. Each column in such a record contains the class serial numbers of one feature for 10 synapses. These 10 serial numbers together constitute a sample, for which the mean rating for the sample X_B is calculated. A random set of 10 synapses is thus characterized by seven mean point ratings (one for each feature). Having chosen several such groups of 10 synapses from each animal at a given point of the experiment, the investigator next constructs seven variance series, each of which obeys the law of normal distribution. Such a series is characterized by the mean value of the mean point ratings for the samples ($\overline{X}$) and its confidence interval (L). According to Strelkov's equation $L = \alpha R$, where α is the amplitude of the variance series and R a coefficient taken from Strelkov's table [3]. The confidence limits provide a basis for statistical comparison of homonymous values.

The method described above was used to study plasticity of synapses in the stratum radiatum of area CA-1 of the dorsal hippocampus in rats 24 h after the formation of a bilateral conditioned avoidance reflex (BCAR) by the usual method and after separate presentation of the corresponding photic and electrodermal stimuli.

Experiments were carried out on 15 noninbred male albino rats weighing 150-180 g. BCAR was formed in five animals (experiment), five rats were presented with separate stimuli (active control), and five rats were subjected to no stimulation whatever (passive control). The brain was removed immediately after decapitation, and each experimental rat was killed simultaneously with two controls.

After fixation for 15 min in 2.5% glutaraldehyde solution in phosphate buffer, brain slices cut with a razor from the region of the dorsal hippocampus were placed on the stage of an MBS-9 microscope. Pieces from area CA-1 were cut on the stage and processed in the usual way, with glutaraldehyde-osmium fixation. After dehydration with alcohols of increasing concentration the specimens were embedded in Araldite. Sections cut on the LKB-IV Ultratome were stained with uranyl acetate and lead citrate and examined in the JEM-100B electron microscope. Altogether 2250 synapses were studied (15 rats, 150 synapses from each rat). The time taken to describe 100 synapses was 1 h. All the work was done on negatives (magnification 10,000) by means of a negative viewer; the features used were chosen because they could be evaluated even in negatives containing certain defects.

EXPERIMENTAL RESULTS

In animals after conditioning, compared with the passive control, a statistically significant decrease was found in the length of the active zones of the synapses (1.96 ± 0.07 and 2.12 ± 0.056, respectively) and the mean area of cross section of the terminals (1.89 ± 0.06 and 2.05 ± 0.075), whereas the degree of concentration of vesicles near the active zone was increased (1.74 ± 0.035 and 1.48 ± 0.038).

In animals presented with separate stimuli (active control) a statistically significant increase compared with the passive control was found in the thickness of the postsynaptic membrane (2.65 ± 0.047 and 2.43 ± 0.07, respectively), and in the area of cross section of the terminal occupied by vesicles (2.53 ± 0.056 and 1.98 ± 0.056), whereas the degree of concentration of vesicles near the active zone was sharply reduced (1.18 ± 0.033, 1.43 ± 0.038). All values were expressed in mean point ratings (X±). Frequency analysis of the configuration of the active zones showed some increase in the number of active zones with positive curvature of their membranes in the experimental group and a decrease in the number of negatively curved active zones in the active control. However, statistical analysis showed that these changes were no more than a tendency. No change was found in the remaining features in the course of this experiment (see the list).

It follows from the above description that definite correlation exists between the physiological features used and quantitative changes in the fine structure of synapses of the stratum radiatum in area CA-1 of the rat hippocampus.

TABLE 1. Classification of a Group of Synapses Based on a Combination of Features of Ultrafine Structure

No. of synapses	No. of features						
	1	2	3	4	5	6	7
1	1	1	2	2	3	1	2
2	2	2	1	1	1	3	1
3	0	3	2	2	3	2	1
4	0	2	3	2	2	1	2
5	3	1	1	3	2	2	3
6	0	4	4	2	1	2	2
7	0	2	2	1	3	1	1
8	1	1	2	1	2	1	2
9	0	2	1	1	2	2	2
10	0	1	1	2	1	1	3
X_{B_1} X_{B_2} ... X_{B_n}	0,7	1,9	1,9	1,7	2,0	1,6	1,8
$\bar{X}$	0,3	1,9	1,8	2,4	1,7	1,9	1,6
L	0,02	0,07	0,06	0,05	0,03	0,05	0,05

Legend. Features (in points): 1) number of mitochondria in terminal, 2) area of cross section of terminal, 3) length of active zone, 4) thickness of postsynaptic membrane, 5) degree of concentration of vesicles near active zone, 6) area of cross section of terminal occupied by vesicles, 7) configuration of active zone. X_{B_1}, X_{B_2}, ...X_{B_n} — mean point ratings for each feature in 10 synapses; $\bar{X}$ — mean value of mean point ratings, L — confidence interval.

The suggested method of classification of a group of synapses thus shows that any morphological features of complex objects (in this case synapses), identified by a specialist, can be classified and described statistically. The number of features in the lists subjected to statistical comparison is unlimited, because the features are described by dimensionless numbers of points. The speed of work is high, for the specialist identifies and classifies features more and more quickly as he gains experience. The mathematical operations take minimal time because of Strelkov's table.

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