LAYER BY LAYER ANALYSIS OF SYNAPTIC ORGANIZATION IN THE OPTIC TECTUM OF THE FROG

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The distribution of axo-axonal and axo-dendritic synapses, nerve endings, and bodies of neurons by depth in the optic tectum of Rana temporaria L. was investigated under normal conditions and 6-9, 60, and 134 days after removal of the contralateral eye. Counting was carried out on long oriented sections examined in the electron microscope. In outer plexiform layer 9 the density of synapses was greatest near the surface of the tectum and decreased in the direction away from it; no inner sublayers with differing density of synapses could be distinguished. In the outer zone of layer 9 (to a depth of about 30 /m) many axo-axonal synapses were discovered. Endings of myelinated optic fibers of large diameter ("dark" terminal degeneration) were widely distributed in the same layer. The density of axo-dendritic synapses in deep plexiform layer 5 was similar to that in layer 9. Many nerve endings containing granular vesicles as well as pale synaptic vesicles were found in layer 5 and neighboring zones.

INTRODUCTION

The tectum mesencephali of the Anura is used as the test object in morphological and physiological studies devoted to the complex analysis of integrative brain mechanisms and the basis of visually controlled behavior. Many of the structural features of the tectum in frogs have been elucidated by light-microscopic studies both under normal conditions and after removal of the contralateral eye. Investigation of the ultrastructural organization began comparatively recently, and its analysis is still far from complete [4, 6, 15, 16]. This paper gives the results of a quantitative layer by layer study of the synaptic organization of the tectum in the frog Rana temporaria L. Some of the results have already been published [3, 6].

EXPERIMENTAL METHOD

Experiments were carried out on 11 large adult frogs. Seven normal animals and frogs 6-9, 60, and 134 days after removal of the contralateral eye (one frog at each time) were used. The frogs were kept at a temperature of about 20°C. The dorsal part of the tectum was fixed by Palade's method in 1% OsO₄ solution after preliminary irrigation of the brain surface with the fixative; it was then dehydrated in alcohols and embedded in Araldite, oriented relative to the block. Most investigations were carried out in the central region of the
dorsal part of the tectum, but in some cases the location of the specimen will be specially mentioned. Parasagittal sections up to 420 μ long (usual length 180–220 μ) were cut perpendicularly to the surface of the tectum and invariably included the outer limiting glial membrane; the sections were placed on supporting films, stained with lead citrate, and examined in the electron microscope.

The density of distribution of the structural components by depth in the tectum was studied by total counting on the screen of the electron microscope (magnification 10,000 ×) in series of successive fields of vision (each measuring 3.8 × 4.8 μ), forming continuous inspection strips. These strips were perpendicular to the outer surface of the tectum and were 15–20 μ apart. From three to six (usually five) such strips were examined for each specimen. The results of counting the density of distribution of the components were averaged and expressed as the number of structures per 100 μ² of section for each counting level (3.8–5.0 μ from the outer surface).

Identification of small axons and dendrites in the frog tectum is difficult [16]. Profiles containing tubules and not containing filaments, twisted in shape and with irregular contours, were classed as dendrites; pale dendrites were distinguished from unmyelinated axons by their darker matrix. Profiles with filaments or tubules (the latter were characteristic of this process), round in cross section but with a straight course and straight borders on longitudinal section, were classed as axons. By the use of these criteria it was impossible to identify chemical synapses formed by dendritic terminals [16]; all round or oval cross-sections containing a homogeneous population of (synaptic) vesicles were classed as axon terminals. *En passant* synapses, formed by typical thick dendrites, were seen. The terminals were not classified in more detail. Synapses of types infrequently found — somato-dendritic [2, 13], azo-somatic, and dendro-dendritic — were not counted. The most commonly used system for division of the tectum of the Anura into layers [9] was followed.

**EXPERIMENTAL RESULTS**

Distribution of Different Types of Synapses in Outer Plexiform Layer (Layer 9). The density of synapses in the tectum was greatest beneath the outer limiting membrane formed by the "feet" of ependymogliocytes, and decreased away from the brain surface (Fig. 1a, c). Axo-axonal synapses were located in the outer zone of layer 9. They were near the surface but hardly any were seen deeper than 50–60 μ, whereas the density of the axo-dendritic synapses decreased more gradually. The distribution of the cell bodies showed that the loose nuclear layer (layer 8) begins at a depth of about 220 μ (Fig. 1b); this agrees with the results of light-microscopic investigations.

In the rostral region of the tectum, in the zone at a depth of 0–30 μ from the surface, the density of axo-axonal synapses averaged 30/100 μ² and these synapses constituted 60% of the total number of synapses; in the caudal region the density of axo-axonal synapses was only half as high (14/100 μ²) and they accounted on average for 38% of all synapses (Fig. 1a, c). The mean density of axo-dendritic synapses in this zone (0–30 μ) was roughly the same in different parts of the tectum and varied in different animals between 18 and 26/100 μ².

The outer part of the neuropil in layer 9 is finely dispersed. Counting showed that the density of synapses of a particular type was similar at different points in the same region. Particularly high reproducibility of the results of counting was obtained for axo-axonal synapses (Fig. 1f), whereas the axo-dendritic synapses were less uniformly distributed, and at distances of 90 μ and more from the surface their density became very variable (Fig. 1e).

The localization and composition of the structural components revealed some special features when the tectum was studied after removal of the contralateral eye, an operation which leads to degeneration of the optic fibers. It was shown previously that at a temperature of 18–20°C, 6–9 days after the operation degeneration of the endings begins in the few but abundantly branching myelinated optic fibers of large diameter, and the degeneration as it develops is of the dark type. Degeneration of endings of myelinated fibers of smaller diameter takes place later. Myelinated fibers degenerate after their endings, and the process is complete by 100 days after the operation. Unmyelinated fibers preserve their structure, excitability, and synaptic transmission for 140 days or more. Degeneration of the endings of thin myelinated and unmyelinated fibers is of the pale type [4, 5].

Degeneration of the optic fibers was accompanied by a marked decrease in the absolute and relative number of axo-axonal synapses in the zone located at a depth of 0–30 μ, from 48% in the normal animal to 28% 60 days and 20% 134 days after the operation (Figs. 2e and 3a). Meanwhile the absolute density of axo-dendritic synapses not only did not fall during degeneration, but actually rose (from the normal average of 21/100 μ² to 23/100 μ² 60 days and to 29/100 μ² 134 days after the operation (Figs. 2e and 3a).