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INHIBITORY SYNAPSES ON THE PERIKARYONS OF MITRAL, TUFTED AND GRANULAR CELLS OF THE RAT OLFACTORY BULB

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Introduction

GRAY (1962) first suggested the possiblity of distinguishing the excitatory or inhibitory natures of neural connections on the basis of the ultrastructural characteristics of the synapses. The combined aldehyde-osmium fixation method (UCHIZONO, 1965) soon became one of the routine electron-microscopic preparatory procedures, and provided a new possibility for the distinction on the basis of the difference of the vesicle forms of the presynaptic element. After further work, doubt arose as to the application of the above possibilities, but according to present knowledge certain central and peripheral nervous system synapses can be well charaterized fine-structurally.

This paper wishes to draw attention to synapses observed on the soma of the neurones of the rat olfactory bulb; the inhibitory nature of these is suggested on the basis of fine-structural criteria and earlier physiological observations.

Materials and Methods

14 fully developed (4—6 months old) albino rats were used in the study. The animals were anaesthetized with Nembutal and perfused for 30 minutes. The olfactory bulb was removed, segmented with orientation, and submitted to immersion fixation in a solution prepared according to KARNOVSKY (1956). This was followed by post-fixation for 30 minutes in a buffered 1% OsO_4 solution (MILLONIG, 1962), dehydration in the usual manner, and embedding in araldite (Durcupan, Fluka). Sections were prepared on a Tesla ultrotome. Semithin (0.5—0.7 μ) samples were prepared simultaneously with the thin sections, and the electron-microscope studies were checked continually with the help of these. Photographs were taken on Tesla BS 242 D and JEOL 100 B electron-microscopes.

Results

The fine-structural characteristics of the structure of the rat olfactory bulb have aroused the interest of many research workers. One of the relevant papers (ANDRES, 1965) presents a detailed analysis of the structure of olfactory bulb, the neurones and glia cells of its layers, and some of its synapses. It is known from the above work too that on proceeding inwards one encounters in the olfactory bulb a layer of fila olfactoria, and the glomerular, outer plexiform,

mitral, inner plexiform and granular layers. Our work deals with the synaptic relations of the mitral, tufted and granular cells, and with the three characteristic neurones of these layers, with particular regard to the synapses on the soma.

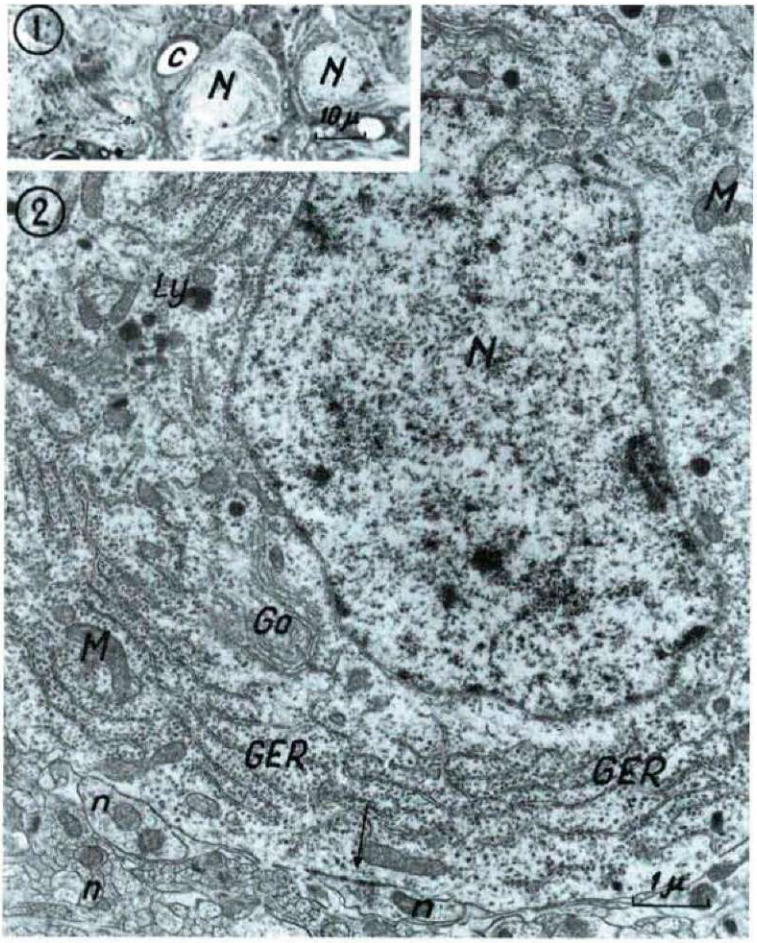
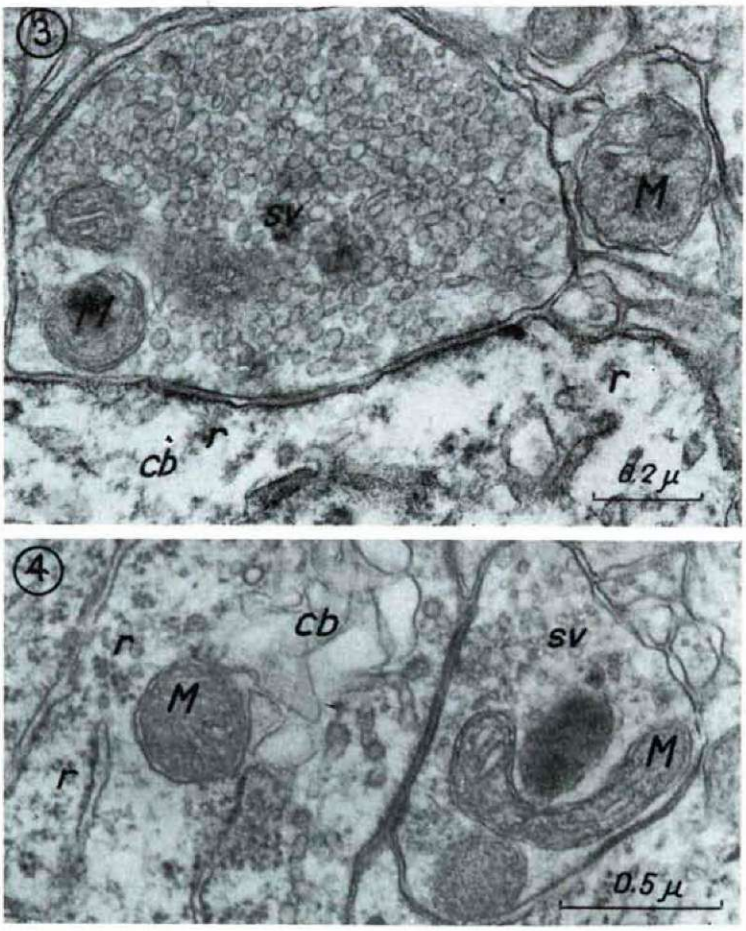


Fig. 1. Mitral cells on semi-thin section (toluidine blue staining). N = nucleus, c = capillary.
Fig. 2. Electron-microscope picture of mitral cell. N = nucleus, Ly = lysosome, M = mitochondrium, GER = granulated endoplasmic reticulum, Go = Golgi apparatus, n = nerve fibre. The arrow indicates the synapse.

The mitral cells (Figs. 1–2) are arranged in a single row of cells; their axons are grouped in the lateral olfactory tract; branching in the region of the glomerulus olfactorius, their main dendrites receive the excitatory impulses of the fila olfactoria, while their secondary dendrites form numerous synapses with the processes of the granular cells in the str. plexiforme externum. The perikaryon is richly provided with cytoplasmatic organelles (Fig. 2), and nerve

fibres frequently lie close enough to its enormous external surface to be suitable for the formation of synaptic connections (Figs. 2–4). In the connection of the perikaryon and its environment one can observe a synapse from the perikaryon towards the nerve fibre (Gray-II type), and also connections of the nerve fibres, presumably polarized in the direction of the perikaryon; the two can sometimes be observed side by side in the form of a reciprocal synapse. We shall deal here only with the synaptic systems polarized towards the soma. These synapses (Figs. 2–4) are characterized by the fact that the aldehyde-osmium fixing method used reveals ovoid vesicles in them. The density of the vesicles is variable: they sometimes fill the whole of the fibre (Fig. 3), while at other times they can be observed only sporadically (Figs. 2 and 4). Round and ovoid vesicles appear among them in various proportions. The exact spheroidal-ovoid ratio can be determined only with a goniometer. Neverthelles,



Figs. 3—4. Grey-II. type synapses on soma of mitral cells. M = mitochondrium, sv = synaptic vesicles, cb = mitral perikaryon, r = ribosomes.

the size of the ovoid vesicles is always less than that of the round vesicles, and independently of the form they can be identified with certainty in the given plane.

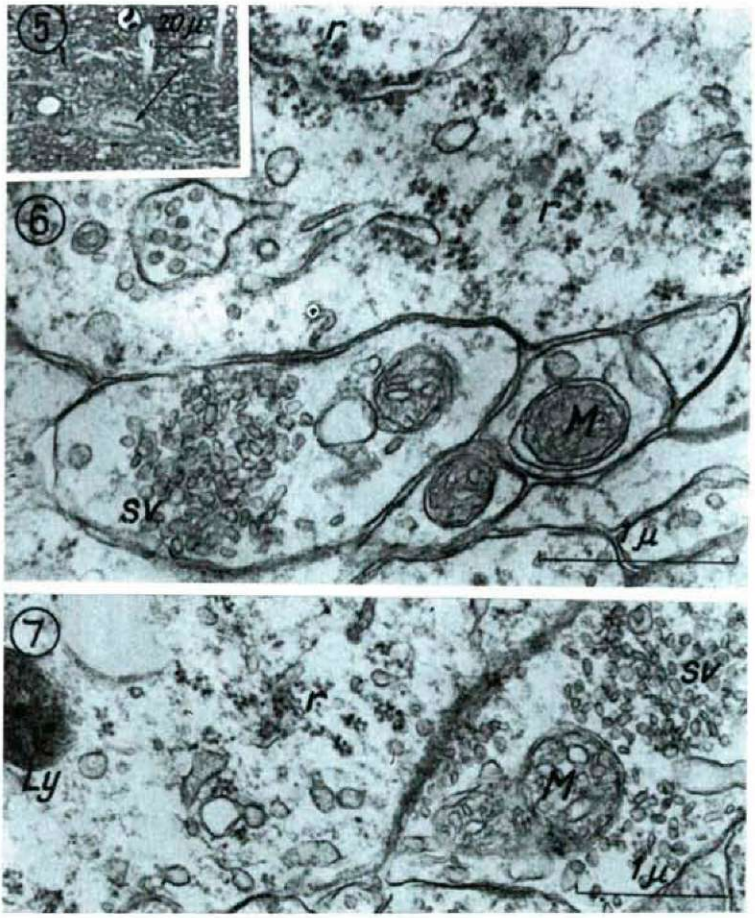


Fig. 5. Tufted cell on semi-thin section. The arrow indicates the nucleus of the tufted cell. Figs. 6—7. Synapses on body of tufted cells. M = mitochondrium, sv = synaptic vesicles, Ly = lysosome, r = ribosomes.

The tufted cells (Fig. 5) are found in the region of the str. plexiforme externum, and are often difficult to distinguish from other cell types. The light- and electron-microscope studies carried out in parallel provide a good possibility for the elemination of this source of errer. The cytoplasm/nucleus ratio for the tufted cell is less than that for the mitral cells, and the size of the cells too is variable. Many nerve fibres reach at the perikaryon, similarly to that of the mitral cells, and here too synapses polarized towards the perikaryon are frequently found (Figs. 6–7), and in places may be considered as reciprocal. The vesicle density of the nerve fibres too is similar to that of the fibres connected

to the mitral cells discussed above, but at the same time a greater number of the vesicles exhibit a flattened form. The position of the vesicles varies: they can be observed rarely in the vicinity of the synaptic thickening (Fig. 7), but they never come into such an intimate connection with the presynaptic membrane as usual in the Gray-I type synapses.

The granular cells are small neurones arranged densely beside each other; it appears from a light-microscopic photograph of a semi-thin section (Fig. 8) as if the cell nuclei were in contact with each other. It turns out from electron-microscope photographs that the cell nuclei (Figs. 9–12) are surrounded by only a very thin cytoplasmic border, the thickness of which is sometimes less than 0.2 μ. In this thin cytoplasm only very few organelles can be seen. Nor is

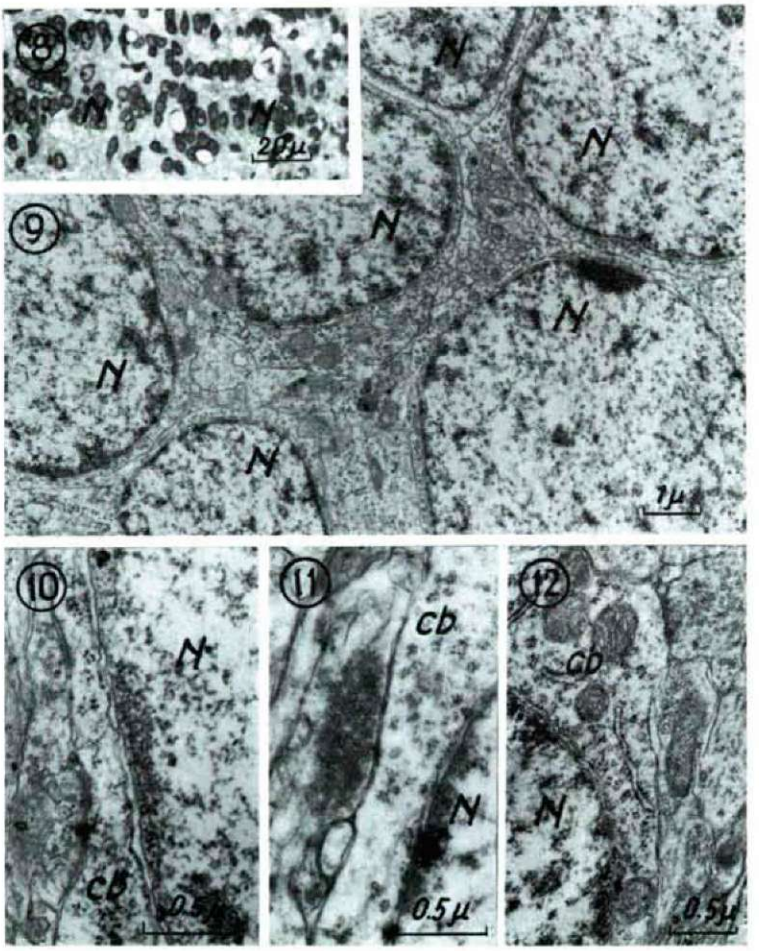


Fig. 8. Granular cells on section stained with toluidine blue.
Fig. 9. Electron-microscope picture of granular cells.
Fig. 10—12. Synapses on perikaryon of granular cells. N = nucleus, cb = granular perikaryon.

there an abundance of granular cells in the synapses. The observed synapses were always found to be unidirectional, that is to be polarized from the nerve fibre towards the cytoplasm of the granular cell. The vesicle density was variable, while the pre- and postsynaptic membranes were thickened to the same extents. Based on their size and form, the vesicles can be classified in the ovoid category.

Discussion

As mentioned in the introduction, the aim of this work was to characterize the synapses observed on the soma of some neurones of the rat olfactory bulb, on the basis of morphological criteria and earlier physiological studies.

Of the different fine-structural criteria of the synapses, attention was drawn by GRAY (1962) primarily to the different natures of the synaptic thickening, when he found that the synapse types reported three years previously (GRAY, 1959) in the cat spinal cord could be correlated with the excitatory or inhibitory natures of these same synapses. This assumption was supported by the results of ECCLES (1964). The characteristics of the membrane specialization were also observed by COLONNIER (1968). The introduction and application of the aldehyde-osmium fixation method permitted a generally consistent distinction between the forms of the synaptic vesicles (UCHIZONO, 1965, 1966; BODIAN, 1966; WALBERG, 1966). Nevertheless, a distinction based on the different forms of the vesicles can not be the only basis for the structural classification of a synapse: a number of papers have drawn attention to this. Thus, relations have been found between vesicle form and age (LARRAMENDI et al., 1967), and between form and osmolality conditions (BODIAN, 1970; VALDIVIA, 1971); as regards certain synapses, however, the relations between ovoid vesicle and inhibition, and spherical vesicle and excitation is dubious (PAPPAS, 1966; PAPPAS and BENNETT, 1966; MUGNAINI, 1970). These examples emphasize the importance of taking into account not only the vesicle form, but also the type of synaptic thickening; as proved by recently published correlations, the two characteristics together can lead to a correct classification with a fair degree of certainty.

PRICE (1968) characterizes the reciprocal dendrodendritic synapses of the rat olfactory bulb in agreement with electrophysiological measurements, and characterizes the excitatory synapsing region by the joint occurrence of asymmetric thickening and round vesicles, and the inhibitory synapsing region by symmetric synaptic mebrane thickening and flattened synaptic vesicles. After aldehyde-osmium fixation, WESTRUM (1969) generally found flattened synaptic vesicles in the Gray-II type synapses of the rat prepyriform cortex. In an earlier publication (HALÁSZ and CSILLIK, 1969), we too reported a similarly good correlation for the rat cerebellum region, while PINCHING (1970) found a connection between the excitatory and inhibitory synapses and their structures in the synapses of the glomerular layer of the rat olfactory bulb. In support of the previous correlations, we have also found the grouping of the vesicles to be characteristic of excitatory and inhibitory, in the two types of synapses (HALÁSZ, in press). According to our observations, which are supported by the present work, the ovoid vesicle form and symmetric synaptic thickening are

not accompanied by the characteristic grouping of the synaptic vesicles beside the presynaptic membrane, as observed in the Gray-I type synapses.

A number of papers discuss the synaptic relations of the mitral, tufted and granular cells of the olfactory bulb at the light- and electron-microscope levels. According to CAJAL (1911), the tufted cell axon may terminate on the perikaryons of the mitral cells. The observation of a reciprocal synapse in the same region is reported by HIRATA (1964) and ANDRES (1965); PRICE and POWELL (1970) also observed synapses on the soma of the mitral cell. According to WILLEY (1969) all of the synapses to be found on the mitral cell are reciprocal; similar conditions can be concluded from our earlier studies (HALÁSZ, in press), but of these only the nerve fibre → mitral cell polarization is dealt with in the present work. In addition to the excitatory impulses picked up by the main dendrite on the mitral cells, an inhibition too is displayed; but the means of transmission of this inhibition to the mitral cell is debatable. The existence of the inhibition is proved with regard to the granular dendrite → mitral secondary dendrite in the reciprocal dendrondritic synapses of the str. plexiforme externum (SHEPHERD, 1963). it is probable that at the same time the mitral cell receives an inhibition on the perikaryon too. The synapsing nerve fibres found here by us can be characterized by the ovoid vesicles, the symmetric synaptic membrane thickening and the scattered arrangement of the vesicles. Based on the above observations and the results provided by the physiology, we consider these nerve fibres to be inhibitory. This nerve fibre may originate from an interneurone, whether that be the granular cell or one of the short-axon cells (PRICE and POWELL, 1970).

The synaptic relations of the tufted cells are less well elucidated. Some hold the view that the tufted cell is to be interpreted as a secondary neurone similarly to the mitral cell, on the grounds that it may be excited in a similar way to the mitral cell (NICOLL, 1972). An inhibitory effect has also been demonstrated on their secondary dendrites (RALL et al., 1966; WESTECKER, 1970. According to other conclusions, the tufted cell is a modified periglomerular cell, and thus an interneurone, as supported by the studies of HINDS (1970). This latter assumption does not exclude the possibility that the soma may receive inhibitory impulses, as occurs in the case of other interneurones in the glomerular layer of the olfactory bulb (PINCHING, 1970). At the same time, the structural characteristics seem clear-cut. On the above basis, the observed synapses are classified as of an inhibitory nature.

The positions of the fibres of the granular cells were determined by CAJAL (1911). Accounts of the soma synapses have also been published by HIRATA (1964) and ANDRES (1965). HIRATA (1964) referred to the possibility that this connection might be inhibitory. It does not emerge from the OsO_4-fixed material of ANDRES (1965) which conception may be supported on the basis of the visecle form of the synapse in question. In the present work it is considered that the characteristics of the inhibitory synapses are disclosed on the fibre synapsing with the granular perikaryon. The fibre may be a part of an interneuronal system similar to that found by PINCHING (1970) in the glomerular layer of the olfactory bulb. Electrophysiological studies are called for to clarify the further characteristics of these neuronal cycles, as has already been done with regard to the primary neurones of the olfactory bulb.

Summary

The synaptological relations of the mitral, tufted and granular cells of the rat olfactory bulb are dealt with. Based on the synaptic thickening, and the form and position of the vesicles, it is found that the nerve fibres synapsing with the perikaryons of the above cells may be of an inhibitory nature. This assumption is discussed in connection with the relevant electrophysiological results.

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