TWO TYPES OF MITRAL CELLS IN THE TELEOSTEAN OLFACTORY BULB

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SUMMARY
The olfactory bulb of the tench Tinca tinca is studied using a variant of the Golgi technique. Two types of mitral cells are distinguished according to the morphology of their somata, the number and extension of their dendritic arborizations, the arising place of the axons, and their location in medial or lateral portions of the olfactory bulb. The phylogenetic importance of these results is discussed.

KEY WORDS: mitral cell, olfactory bulb, teleost, Golgi technique

INTRODUCTION
The neural organization of the teleostean olfactory bulb has developed in directions somewhat different from that of the mammal (1,2). In higher vertebrates, the second order neurons of the olfactory pathway are the mitral and tufted cells. In teleosts, tufted cells are not observed and a new type of neuron, the ruffled cell, only present in the olfactory bulb of bony fish, could also project to the telencephalic hemispheres (2,3).

In most mammals, the mitral cells constitute a narrow single-cell layer; they show primary and secondary dendrites, or dendrites with and without a tuft, and project through the lateral olfactory tract to different areas of the basal brain (4,5). On the other hand, the teleostean mitral cells are disposed at different levels in the
olfactory bulb, not forming a thin, sheet-like layer (4,5) and they are described as not showing secondary dendrites (6,7). Physiological studies suggest that the medial and lateral parts of the olfactory bulb of cyprinoids can work relatively independent one from each other (8) projecting through different pathways, the medial and lateral subdivisions of the olfactory tract, to different telencephalic areas (9). Such topographical relation, reported from physiological studies, is also supported by the confined dendritic fields of the teleostean mitral cells (10). The structure of the mammalian olfactory bulb is well established, with its neuronal elements distributed in layers and clearly identified. However, our knowledge of the structural organization of the teleost olfactory bulb is only fragmentary (11). Thus, in order to study the morphological variations of the teleostean mitral cells at different locations, and comparing them with previous data on these neurons (7,10-12), we have carried out a Golgi study in the olfactory bulb of the tench, Tinca tinca.

MATERIALS AND METHODS

Twenty tench, Tinca tinca (L.), weighting from 75 to 235 g were used. They were anaesthetized with MS-222 (Sandoz) at 0.03%, placed in a plastic holder and transcardially perfused with 100 ml of 0.63% saline containing 5 U.I./ml of heparin, followed by 300 ml of a mixture containing 2% paraformaldehyde and 2% glutaraldehyde in 0.12M phosphate buffer (pH 7.3). Then, the olfactory bulbs were removed and stored in an osmium dichromate solution (1g osmium tetroxide and 12 g potassium dichromate in 500 ml distilled water) for 3 days and, thereafter, for 2 days in 0.75% silver nitrate. The blocks were cut by using a Vibratome (Campden Instruments).

When the sections were not impregnated, they were subjected to a second impregnation, consisting in a modification (13) of the section Golgi procedure (14). Briefly, some ten sections were sandwiched between layers of Parafiln and the pile resulting was covered with 5% agar to form a single "tissue block" of rejoined sections. These blocks were immersed in a freshly prepared osmium dichromate solution. The sections were examined under the microscope, photographed, and drawn using a Zeiss drawing tube.

RESULTS

The mitral cells of the tench olfactory bulb are disposed at different levels in both intermediate strata of the bulbar lamination, the glomerular and plexiform layers. Rarely, they can be
Fig. 1: Type I mitral cell. 1150x.
Fig. 2: Type II mitral cell. 1150x.
Fig. 3: Type II mitral cell with dendrites out of the main dendritic field. 1150x.
found in the olfactory nerve fibre and the granule cell layers. They present very different morphologies according to the shape of their somata, the number and extension of their dendritic fields, and the arising-place of the axons. In this way, two types of mitral cells are easily differentiated:

Type I: the fusiform and triangular cell bodies are the most frequent in this type. They show two or more dendritic trunks extending frequently parallel to the surface of the bulb and arborizing in different tufts which can be very far from each other (Fig. 1). The axons arise in general from one dendritic trunk near the cell body, and with lower frequency, from more distal portions of the dendrites or from the soma. This type predominates in the medial portions of the olfactory bulb, and all the neurons located in the inner strata of the olfactory bulb, the plexiform and the granule cell layers, belong to it.

Type II: The second type shows a round or ovoid cell body from which stem one to four dendrites which arborize in only one dendritic field (Fig. 2). Moreover, some small dendritic branches can also be found, some independent from the main dendritic field and not forming a tuft (Fig. 3). The axon arises from the soma, generally in the opposite pole to the stemming of the dendritic trunks. This type is mainly located in the lateral portions of the olfactory nerve fiber layer and the glomerular layer. We have not observed it in the plexiform or granule cell layer and rarely in the medial portions of the external layers.

DISCUSSION

Our observations corroborate the majority of the results obtained by other authors in the cyprinoid olfactory bulb (6,7,10,11). However, we have found some differences. Thus, the teleostean mitral cells do not constitute an homogeneous population as they have always been considered; and they are not the largest neurons in the teleostean olfactory bulb (7) since we have observed ganglionar cells of the terminal nerve with a larger size.

Using different morphological methods, as the Golgi technique (6,7,11) or intracellular injections with HRP (10), it has not been reported the characterization of both types proposed by us. It can be
due to the fact that these authors do not differentiate between medial and lateral portions of the bulb (6,7,11) or, that their HRP injections (10) are too deep to find a significant number of type II mitral cells. The same reason can also explain why these authors have not found ruffed cells that we have observed in the same species using the Golgi technique (2).

The importance of the typology proposed is not only that it correlates very well with previous anatomical and physiological descriptions, but it has an evident significance for the comprehension of the phylogenetic evolution of the olfactory bulb. Thus, the type I of mitral cells is clearly particular of teleosts and similar cells have not been found in the olfactory bulb of higher vertebrates. However, our type II of mitral cells shows morphological characteristics closer to the mammalian mitral cells. Thus, the type II cells, located mainly in the lateral portions of the bulb, present only one dendritic arborization and the axon arises from the soma. Neurons in such zone of the bulb are observed to project through the lateral olfactory tract. These characteristics coincide with the mammalian mitral cells which project through the unique second olfactory pathway present in higher vertebrates, the lateral olfactory tract. Their morphology, as has been described (4, 5), coincides basically with our type II mitral cells. The short dendrites observed in type II cells, not related apparently with the dendritic arborization field, could be homologous to the secondary dendrites of mammals. The mitral cells located in the medial and lateral portions of the olfactory bulb of cyprinoids are known to project through the medial and lateral subdivisions of the olfactory tract. These subdivisions are specifically related with different behaviours such as feeding and sexual behaviour (15). It can suppose an assignation of our typology to specific functions. However, it remains unclear, since these behavioural studies use transections of the medial and lateral olfactory tracts, being the terminal nerve also cut. Therefore, the elicited responses could be also due to the transection of the terminal nerve.

The next step to study the synaptic connections and possible ultrastructural differences between the types I and II could be the combined Golgi-EM technique.
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REFERENCES