TWO TYPES OF MITRAL CELLS IN THE TELEOSTEAN OLFACTORY BULB

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SUMMARY
 The olfactory bulb of the tench <u>Tinca tinca</u> 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 ruffed 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, <u>Tinca</u> <u>tinca</u>.

MATERIALS AND METHODS
    Twenty tench,   <u>Tinca</u> <u>tinca</u> (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 Parafilm and the pile resulting was covered with 5%
agar   to   form a single "tissue block" of   rejoined   sections.   These
blocks   were   inmersed   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
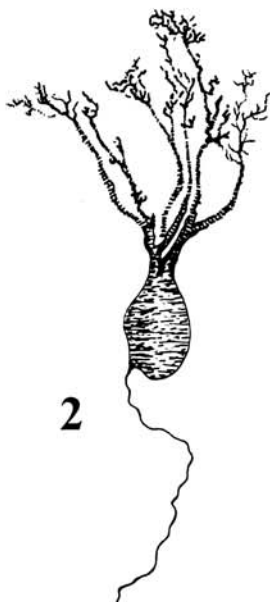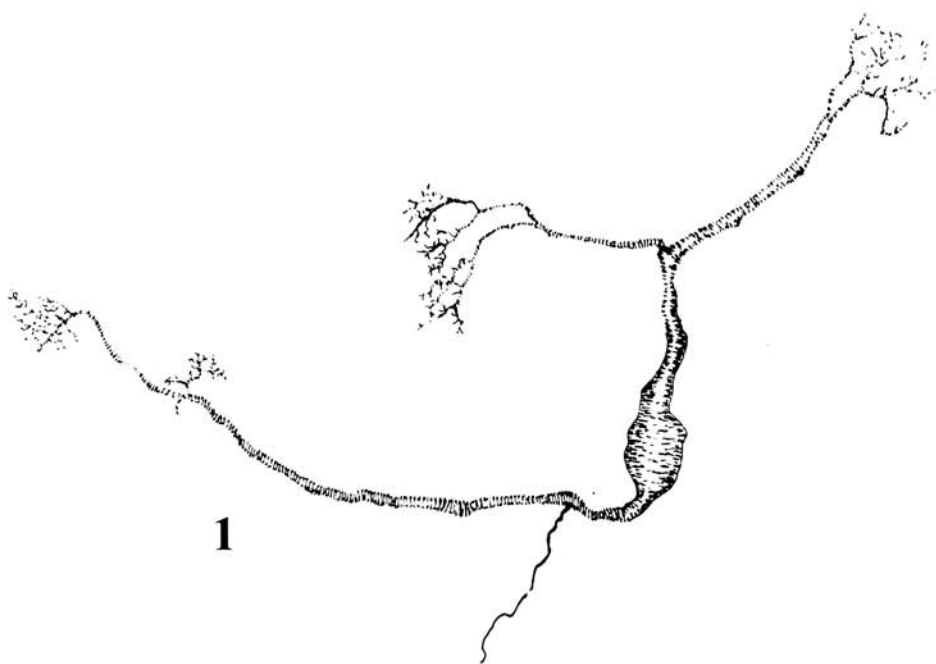
Fig. 1: Type I mitral cell. 1150x.
Fig. 2: Type II mitral cell. 1150x.
Fig. 3: Type II mitral cell with dendrites out of the main dendritic
        field. 1150x.

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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