

Do the Granule Cells of the Olfactory Bulb of Teleosts have an Axon?¹

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With 4 Figures

Summary

We describe the presence of granule cells with an axon-like prolongation in the olfactory bulb of freshwater teleosts. These cells are present in the bulbs of fish belonging to order Cypriniforms and to order Salmoniforms. The granule cells with axon, or axon-like prolongation, are located in the Granule Cell Layer and usually show a single dendrite and a rounded or pyriform soma. We discuss the function and philogenetic implications of these neurons.

Introduction

The granule cells of the olfactory bulb have been studied in different classes of vertebrates (P. RAMÓN 1890, 1891, BLANES 1898, RAMÓN Y CAJAL 1911, HOLMGREN 1920, HERRICK 1924, HOFFMAN 1963, PRICE and POWELL 1970, ICHIKAWA 1976, MORI and SHEPHERD 1979, MORI and KISHI 1982, ALONSO et al. 1986, GARCÍA-VERDUGO et al. 1986, LÓPEZ-MASCARAQUE et al. 1986) as well as in diverse comparative studies (ALLISON 1953, ANDRES 1970, GARRIDO 1978).

Classically, these neurons are considered to be a homogeneous population but there are electrophysiological (MACLEOD and LOWE 1976, THOMNESEN 1978, SATOU et al. 1983) and immunocytochemical (BOGAN et al. 1982, DAVIS et al. 1982) data that point to zonal differences in the structure of the inner layers of the olfactory bulb and suggest a certain heterogeneity of the granule cells as a set.

In teleosts, this heterogeneity is clearly patent in the morphological studies; important differences can be seen that have allowed us to differentiate six granule cell subtypes according to their dendritic prolongations (ALONSO et al. 1986).

In the above-mentioned studies, the granule cells are consistently considered as neurons lacking in an axonic prolongation, comparable to the amacrine cells of the retina. However, observations reported at the turn of the century (JONSTON 1901, SHELDON 1912) describe the presence of granule cells, or assimilable cells, with an axon.

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Our group has recently established the neuronal types of the Granule Cell Layer in freshwater teleosts (Alonso 1987) in material impregnated with different variants of the Golgi technique. In the present work our aim is to offer new data concerning the presence of granule cells with an axon in the olfactory bulb.

Material and Methods

The study was carried out on 20 adult specimens of *Cyprinus carpio*, 20 adult specimens of *Tinca tinca* captured in the River Tormes (Salamanca, Spain) and another 25 adults of *Salmo gairdneri* (Fisheries "Salmantina", Salamanca). The fish were previously anaesthetized with MS-222 (Sandoz) at 0.03%. One group of each species was perfused with a suitable fixing solution by the intracardiac route after which the olfactory bulbs were removed. In the remaining fish, the olfactory bulbs were removed "in vivo" and rapidly submerged in the corresponding fixing solution.

Ten bulbs of each species were fixed in Carnoy and embedded in Paraplast. Thin 10 μm sections were cut along the transversal, longitudinal and sagittal planes. These sections were stained by the Nissl technique, at $\text{pH} = 4.6$ and were used as controls of the structure of the olfactory bulbs.

The remaining bulbs were processed according to the Golgi-Colonnier (COLONNIER 1964), Golgi-Meyer (MEYER 1982) and rapid-Golgi (REHN et al., 1986) techniques. They were included in agar-agar and sectioned according to our technique previously described (ALONSO et al. 1987) at varying thicknesses ranging from 100–150 μm .

Results

The Granule Cell Layer (GCL) in the species studied is the most developed stratum of the olfactory bulb. Granule cells, short axon cells, stellate cells, displaced mitral cells and different types of glial elements can be observed in it. The granule cells constitute the most numerous neuronal population and form an irregular network with their prolongations coursing in all directions.

We have observed granule cells with an axon-like prolongation both in the olfactory bulb of Cyprinid and Salmonid fish (Fig. 1–4). Their numbers are relatively low and in all preparations are less than 5% of the total number of granule cells observed. Among the different Orders studied, and with the techniques employed, a larger number of granule cells with axon were observed in the Salmonids than in the Cyprinids. They are consistently located in the GCL and no displaced granule cells (outside this stratum) were observed with such characteristics. Within this layer, these cells are normally situated in central and caudal positions.

The granule cells with axon usually show a single dendrite (Fig. 1 a and 1 d) and are morphologically comparable to type I granule cells, subtypes I a and I b. Less frequently, this axonic prolongation is observed in multipolar granule cells of subtype III b (Fig. 1 b).

The neurons have small rounded (Fig. 1 b and 2) or pyriform (Fig. 1 b) somata of 8–10 μm diameter. The dendrites are long with many branches, especially when coursing through the Plexiform Layer and the inner portion of the Glomerular

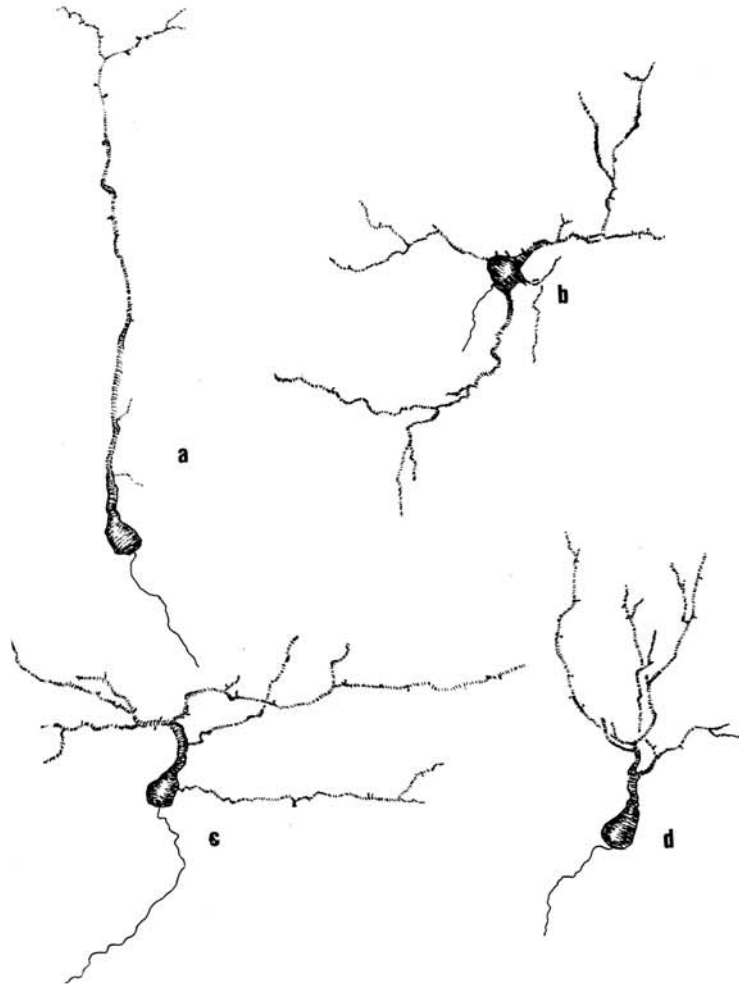


Fig. 1. Camera lucida drawings of granule cells with an axon-like prolongation. 1000 \times

- a) *Salmo gairdneri*. rapid-Golgi
- b) *Tinca tinca*. Golgi-Meyer
- c) *Tinca tinca*. Golgi-Meyer
- d) *Cyprinus carpio*. Golgi-Meyer

Layer (Fig. 1 b, 1 c, 1 d and 4). They have numerous spines, both sessile and pedunculated, situated on their soma and prolongations (Fig. 1). The spines are specially numerous on the terminal parts of the dendrites and are mainly of the pedunculated kind in this zone.

The axon-like prolongation always arise from the cell body and follows a long, fairly straight path (Fig. 4). The axon stems directly from the soma in a slightly thickened part like an axon hillock (Fig. 3) though thereafter its diameter remains constant. This characteristic clearly differentiates this prolongation from those of

the subtype II b granule cells. The axonal prolongations sometimes seem to show a branching though this finding is infrequent and not clearly demonstrated in our observations. No differentiation can be seen in the axonal surface and only in the last portions is it possible to observe some thickened zones which, however, do not have a varicose aspect. The axon courses towards the caudal portions of the olfactory bulb (Fig. 4) and in no case has the occurrence of a terminal arborization been observed.



Fig. 2–4. Granule cells with an axon-like prolongation (a)

Fig. 2. *Cyprinus carpio*. Golgi-Colonnier. 375 \times

Fig. 3. *Tinca tinca*. Golgi-Meyer. 375 \times (arrow: axon hillock)

Fig. 4. *Salmo gairdneri*. rapid-Golgi. 250 \times

Discussion

For many years (BLANES 1898, RAMÓN Y CAJAL 1911) the granule cells have been considered as the main modulating element of the olfactory bulb and their most noticeable characteristic has been the lack of an axon. However, references exist in the studies of JOHNSTON (1901) and SHELDON (1912) of cells that are apparently identifiable with granule cells and that have a prolongation which according

to the Golgi techniques has the characteristics of an axon. JOHNSTON (1901) referred to neurons that varied in size, were rounded or pyriform and whose axon seemed to be directed towards the telencephalic hemispheres as granule cells. SHELDON (1912) differentiated between the granule cells, fusiform cells and stellate cells, stating that the fusiform cells would project their axon through the olfactory tract towards the telencephalic hemispheres and that some of the stellate cells would also do the same but that "such could not be demonstrated with certainty for all". Moreover, SHELDON (1912) also described the so-called small granule cells, which would apparently function as association cells since in them it has not been possible to demonstrate the existence of an axon that projects through the tract. However, the recent description and typification of new neuronal types, such as perineuronal cells, short-axon cells and displaced granule cells, that were not considered by Johnston or Sheldon make it difficult to decide to what extent their granule cells and those studied by us can be considered as identical. It is probable that several neuronal types could be included under this general denomination. Our study confirms the existence of granule cells, a neuronal type clearly identified with both the light and electron microscope, with an axon-like prolongation.

Within the olfactory bulb, the population of granule cells with an axon could have a modulation effect if their axon do not leave the olfactory bulb; this function would be comparable to that of the short-axon cells. On the other hand, they could be considered as a further route to the mitral cells and the ruffed cells as pathways for projection to higher centers if their axon joins those of these other neurons and leaves the bulb through the olfactory tract.

The phylogenetic implications derived from the presence of such granule cells with axons are very interesting. For LÓPEZ-MASCARAQUE et al. (1986), the granule cells without an axon are developed during mammalian evolution. However, our results show that in the olfactory bulb of Teleosts most of the granule cells are anaxonal elements. KOSAKA and HAMA (1979) report that the granule cells of Teleosts may correspond to the periglomerular cell and the granule cell in the mammalian olfactory bulb. Nevertheless, there are other neuronal types as the perineuronal cells, the stellate cells, the ruffed cells and the displaced granule cells that, as a set, make it difficult to compare the cytoarchitecture of the olfactory bulb in higher and lower vertebrates and offer a scheme of bulbar organization in fish different to the "poorly developed" organization proposed by ALLISON (1953) and GARRIDO (1978). We prefer to consider that as in other aspects such as neuronal typology, modulation circuits, etc. . . the olfactory bulb of Teleosts and that of mammals have followed somewhat different evolutionary directions.

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