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A Golgi Study of the Granule Cells in the Olfactory Bulb of *Cyprinus carpio* L. and *Barbus meridionalis* Risso

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With 11 Figures and 1 Drawing

Summary

The olfactory bulb of *Cyprinus carpio* and *Barbus meridionalis* was studied by the Nissl and Golgi-Colonnier techniques. The most abundant neurons of this organ were found to be the granule cells which constitute a heterogeneous population classified by the authors into three types: monopolar, bipolar and multipolar, each with its own subtypes. Relationships were found between the number of dendrites, the form of the neuronal body and the deep or superficial situation within the granule cell layer.

Using the Nissl technique we distinguish dark and light granule cells according to the intensity of their staining.

Introduction

Among the different kinds of neurons in the olfactory bulb, of major interest, owing to their abundance, are the so-called granule cells. Originally believed to be glial cells, Blanes (1898) and Cajal (1911) demonstrated that they were neurons.

They have been studied in fish (Holmgren 1920, Ichikawa 1976, Garrido 1978, Velasco 1980), amphibians (Herrick 1924, Hoffman 1963), reptiles (P. Ramón 1891, Mori and Shepherd 1979), birds (P. Ramón 1890) and mammals (Van Gehuchten et Martin 1891, Blanes 1898, Cajal 1911, Allison 1953, Price and Powell 1970, Mori and Kishi 1982). This kind of neuron has classically been considered as forming a homogeneous population within the olfactory bulb. However, electrophysiological (Hara and Gorbman 1967, Mac Leod and Lowe 1976, Thomnesen 1978, Satou et al. 1983, Huvé et al. 1983), immunocytochemical (Bogan et al. 1982, Davis et al. 1982) and behavioural (Stacey and Kyle 1983) studies have revealed zone differences within the structure of the internal layers of the olfactory bulb and point to a certain heterogeneity in the granule cells as a whole.

The present study was designed to classify the granule cells of the olfactory bulb of *Cyprinus carpio* and *Barbus meridionalis* and to describe their morphology and prolongations as well as their localization in the layer which receives the same name – the granule cell layer.

Material and Methods

The study was carried out on 25 adult specimens of *Cyprinus carpio* and another 25 adults of *Barbus meridionalis* captured in the River Tormes (Salamanca, Spain) with a length range of 16–30 cm. and a weigth range of 100–325 g. 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 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 $pH = 4.6$ and were used as controls of the structure of the olfactory bulbs.

The remaining bulbs were processed according to the Golgi-Colonnier (Colonnier 1964) technique, embedded in celloidin and cut along the same planes as above at varying thicknesses ranging from 80–120 μm.

Results

The olfactory bulb of *Cyprinus carpio* and *Barbus meridionalis* is composed of a series of layers which from the outermost to the innermorst are known as:

1. Olfactory nerve fibre layer.
2. Glomerular layer.
3. Plexiform and mitral cell layer.
4. Granule cell layer.

The limits of the granule cell layer with the plexiform and mitral cell layer are not clearly delineated; rather there is a gradient in which the number of neuronal bodies decreases and the number of prolongations increases.

The granule cells constitute the most numerous neuronal population of the olfactory bulb and are the major component of the so-called granule cell layer. This latter also contains short axons cells which are larger than the granule cells and displays fairly ramified dendrites and an axonic prolongation which clearly differentiates them from the cells examined in this study. The granule cells are sometimes found in small groups of up to 4 or 5 intimately adjacent cells. The granule cells are very small neurons whose neuronal body is almost exclusively occupied by a rounded nucleus, limited by a thin band of cytoplasm, with a single nucleolus which is clearly visible according to the Nissl technique. By the same technique it was also possible to appreciate differences in affinity for the dye, leading us to distinguish between light and dark cells.

According to the Golgi-Colonnier technique, the neuronal bodies display different morphologies and a variable number of prolongations, none of them axonic, which stem from the cells body. These dendrites usually extend towards the outer layers, though they do not consistently adopt a radial disposition.

In view of these characteristics, it was possible to carry out the following classification:

Type I. Monopolar granule cells.

a) Cells in which the dendrite branches near the soma (Fig. 1).
b) Cells in which the dendrite branches at a distance from the soma (Fig. 2).

Type II. Bipolar granule cells.

a) Cells with two dendrites of similar characteristics (Fig. 3).
b) Cells with one dendrite better developed and larger in diameter than the other (Fig. 4).

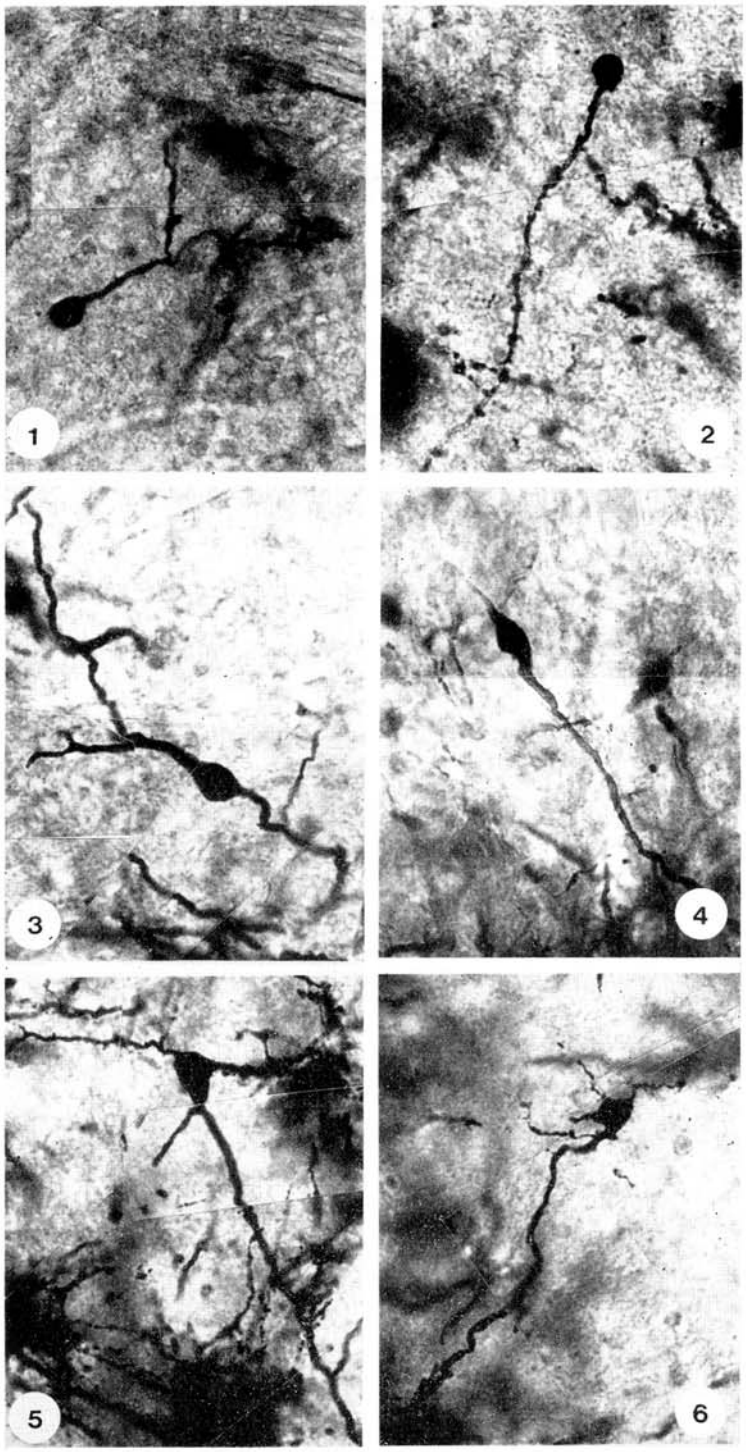
Type III. Multipolar granule cells.

a) Cells in which all the dendrites have similar characteristics (Fig. 5)
b) Cells which have one dendrite better developed and larger than the others (Fig. 6)

The monopolar granule cells (Type I) exhibit pyriform neuronal bodies and a thick dendrite which branches repeatedly. These are found mainly in the outermost zones of the granule cell layer. In those cells subclassified as subtype Ia, the length of the dendrite is relatively short and, therefore, its branching occurs closer to the neuronal body. In subtype Ib, the dendrite runs greater distances and its first branching occurs farther away from the soma than in the previous case. When a monopolar granule cell is found in internal zones of the granule cell layer, it always belong to subtype Ib.

Of the bipolar granule cells (type II), subtype IIa corresponds in general to fusiform neurons with two prolongations stemming from opposite ends of the neuronal body. The soma may either be located in external zones of the granule cell layer, in which case the dendrites follow a course parallel to the surface of the bulb, or they may be located more to the interior, in which case their prolongations adopt a radial configuration. Very rarely, the dendrites stem from the neuronal body to form an acute angle and the soma displays a rounded aspect. Both in *Cyprinus carpio* and *Barbus meridionalis*, in the best impregnated preparations, it is sometimes possible to observe, though always in the internal members of this subtype IIa, a spherical or ovoid bulge in the internal portion of one of their dendrites (Fig. 7). Spines may be observed in this zone (Fig. 8). Subtype IIb is normally located in internal regions of the granule cell layer. They are neuronal bodies of pyriform or irregular fusiform aspect in which the longest and thickest dendrite exits from the thinnest portion of the soma.

The last group in this classification is composed of the multipolar granule cells (type III). Subtype IIIa corresponds to neurons of irregular or star-shaped soma giving rise to dendrites of similar characteristics in numbers usually greater than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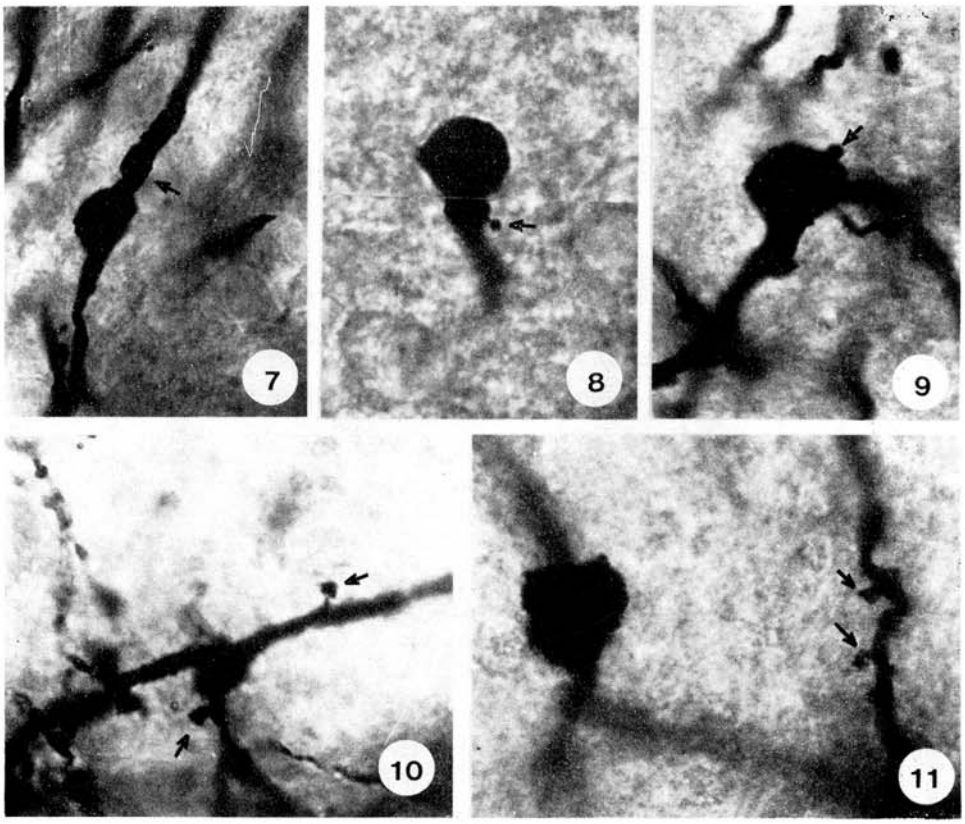


Fig. 7. Ovoid bulge (arrow) in one prolongation of a granule cell, subtype II a. Golgi-Colonnier, 1000×

Fig. 8. Pedunculated spine (arrow) in the ovoid bulge close to the soma. Golgi-Colonnier, 1000×

Fig. 9. Sessile spine (arrow) in the neuronal body of a granule cell. Golgi-Colonnier, 1000×

Fig. 10. Pedunculated spine with a round head (arrow). Golgi-Colonnier, 1000×

Fig. 11. Pedunculated spines with a polyhaedral head (arrows). Golgi-Colonnier, 1000×

Fig. 1. Granule cell, subtype I a. Golgi-Colonnier, 400×

Fig. 2. Granule cell, subtype I b. Golgi-Colonnier, 400×

Fig. 3. Granule cell, subtype II a. Golgi-Colonnier, 400×

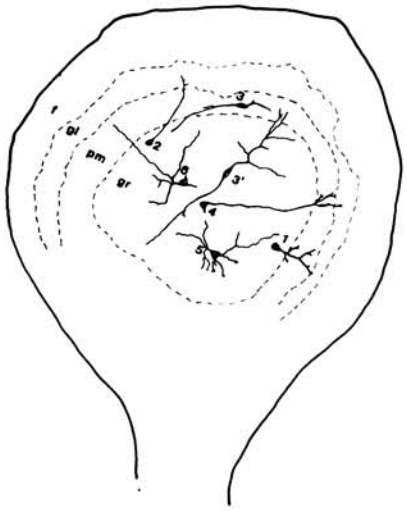
Fig. 4. Granule cell, subtype II b. Golgi-Colonnier, 400×

Fig. 5. Granule cell, subtype III a. Golgi-Colonnier, 400×

Fig. 6. Granule cell, subtype III b. Golgi-Colonnier, 400×

four, though when they have three, or at the most, four dendrites, the morphology of the soma tends to adopt a pyriform or triangular aspect. This subtype is the scarcest of those mentioned. Subtype IIIb with a consistent round or star-shaped soma, displays a long straight dendrite which extends towards the periphery and others, shorter and thinner, often filiform, which extend within the innermost region of the olfactory bulb.

In all the subtypes mentioned above the presence of spines was observed; these were variable in morphology and number and were seen to be disperse both in the soma (Fig. 9) and in most of the dendrites (Fig. 10 and 11). They are seen to be either sessile or pedunculated. Of this latter type, three kinds could be distinguished: the most abundant displays a spherical head and a peduncle with a length of between 2–4 fold the diameter of the head (Fig. 8 and 10); the second type also displays a round head but the stem is much longer; finally there are spines with a polyhaedral or pyramidal head (Fig. 11). The second of these, with



Drawing: Summary diagram of the disposition of granule cells in the olfactory bulb of *Cyprinus carpio* and *Barbus meridionalis*.

1: Subtype I a
2: subtype I b
3: subtype II a, externally situated
3': subtype II a, internally situated
4: subtype II b
5: subtype III a
6: subtype III b

f: olfactory nerve fibre layer
gl: glomerular layer
pm: plexiform and mitral cell layer
gr: granule cell layer

long peduncles, is very rare and normally found in dense groups near the neuronal body. The third kind could feasibly be a quite different kind of spines, or perhaps some modification of the normal pedunculated kind, or maybe an artefact.

In the terminal zones of the dendrites, it is occasionally possible to observe the presence of varicosities; that is, of thin zones followed by thicker ones, producing a "string-of-beads" effect. Likewise, the terminal end of certain dendrites may display a spherical vesicle which is continued by a short thin branch. Its size is bigger than that of the thicker portions of the varicosities and much smaller than the bulge near the soma described in subtype IIa.

Discussion

The morphology and arrangement of the granule cells in the olfactory bulb of *Cyprinus carpio* and *Barbus meridionalis* are different to those of higher vertebrates and at the same time display greater variety.

Overall, the granule cells make up an irregular meshwork in which the outermost ones, fundamentally those of type I and subtype IIa, project their dendrites, often in parallel to the surface of the bulb, while the innermost ones, subtype IIb and type III, do so perpendicularly. This is in contrast to the situation of macrosmatic mammals in which the olfactory bulb displays a more precise laminar ordering and where the granule cells may be seen to adopt a perpendicular orientation with respect to the principal axis of the bulb (Garrido 1978).

The distinction between the main and accessory prolongations described for granule cells in mammals (Van Gehuchten et Martin 1891, Price and Powell 1970, Schneider and Macrides 1978, Mori and Kishi 1982) is not valid as a general model for teleosts, as may be deduced from the typification proposed here. Accordingly, our types I, II and subtype IIIa are not compatible with this model; only subtype IIIb could respond to such characteristics, though in contrast to this is the fact that on no occasion in our preparations was it possible to observe spines in the internal prolongations which might be compared with the accessory ones according to their position and smaller diameter than the main prolongations.

The existence of granule cells which stain at different intensities by the Nissl technique, light and dark granule cells, coincides with similar observations made regarding the Nucleus Rotundus of the carp (Cuadrado et al. 1984) and has likewise been described in the olfactory bulb of the rat (Struble and Walters 1982).

With respect to the differences described in the surface or deep distribution of the differen kinds of granule cells mentioned, Orona, et al. (1983) have reported that different subpopulations of these neurons, according to their situation, innervate different regions of the external plexiform layer. In contrast, other investigators (Price and Powell 1970, Schneider and Macrides 1978) did not report any differences within the granule cells as a whole. Struble and Walters (1982) describe that in the rat, 70% of the light granule cells of the main olfactory bulb appear in the inner half, which which would be evidence of rudimentary stratification. On

the other hand, Davis et al. (1982) were able to observe differences in the immunoreactivity between neurons of deep and superficial strata of the granule cell layer. They indicated that the somatostatin-positive neurons are probably short-axon cells, while the met-enkephalin positive neurons would be granule cells from superficial areas. However, Bogan et al. (1982) describe neurons which, though they react with met-enkephalin, are situated at deep regions of the granule cell layer. The study of these characteristics would confirm the different distributions of the different kinds of granule cells described in this article.

According to our observations, we believe that the olfactory bulb of *Cyprinus carpio* and *Barbus meridionalis* is not a poorly developed structure in the fish, as has been proposed by several authors for teleosts in general (Allison 1953, Garrido 1978), since though its laminar disposition is diffuse, it does show a considerable degree of organization. In this sense, our results agree with those of Kosaka and Hama (1982) who suggested that in teleosts the olfactory bulb has developed in a different direction to that of mammals, leading to an "apparently simple" though functionally complex organization.

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