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Ruffed cells in the olfactory bulb of freshwater teleosts. I. Golgi impregnation*

J. R. ALONSO, J. LARA, J. J. MIGUEL AND J. AIJÓN
Department of Cytology-Histology, Faculty of Biology, 37008 *Salamanca, Spain*

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INTRODUCTION

Ruffed cells have only been described in the olfactory bulb of the goldfish *Carassius auratus* (Kosaka, 1980; Kosaka & Hama, 1979*a*, *b*, 1982–3), the catfish *Parasilurus asotus* and the sea eel *Conger myriaster* (Kosaka & Hama, 1980). Kosaka & Hama (1979*b*), consider this cell to be a new type of neuron whose distinctive characteristic is the presence on the initial portion of the axon of a series of protrusions with a ruff-like appearance around the shaft.

We have observed such cells in the course of a systematic study of the organisation of the olfactory bulb of five species belonging to family Cyprinidae: the Mediterranean barbel *Barbus meridionalis*, the crucian carp *Carassius carassius*, the Iberian nose *Chondrostoma polylepis*, the carp *Cyprinus carpio* and the tench *Tinca tinca*, and of one species belonging to family Salmonidae: the rainbow trout *Salmo gairdneri*, all of them freshwater teleosts. These observations support the idea put forward by Kosaka & Hama (1980) that one could be dealing with a neuronal type present in all teleosts.

In the present work we describe the morphological characteristics of this neuron in the above-mentioned species.

MATERIALS AND METHODS

The material employed in this study consisted of the olfactory bulbs of 20 specimens of Mediterranean barbel, 10 specimens of crucian carp, 15 specimens of Iberian nose, 10 specimens of carp, 20 specimens of tench and 10 specimens of rainbow trout, all of them adult.

After anaesthesia with MS-222 (Sandoz) at 0·03 % the olfactory bulbs were removed *in vivo* and immediately submerged in fixative solution. Six bulbs from each species, fixed in Bouin's solution, were embedded in paraffin, cut in 10 μm sections along the transverse, longitudinal and sagittal planes and stained alternatively with haematoxylin–eosin and according to the Nissl technique. These were used as controls for the structure of the olfactory bulbs.

The remaining bulbs were processed according to the Golgi–Colonnier (Colonnier, 1964) or Golgi–Meyer (Meyer, 1982) procedures. After impregnation, the pieces were rapidly passed through a graded alcohol series up to absolute alcohol (10–15 sec.). Following this they were submerged in a bath of 8 % celloidin dissolved in alcohol–ether (1 : 1) and then dried in a stream of warm air so as to form a thin layer of celloidin around each piece. These were then placed in embedding moulds filled with liquid agar, obtained by dissolving agar in powdered form (7 %) in hot water (60–70 °C);

* Reprint requests to Dr J. Aijón.

after orientating the pieces they were left to cool at room temperature. The blocks thus obtained were cut on a Vibratome at 75–125 μm along the transverse, longitudinal and sagittal planes. The sections were collected in 30% ethanol. Following this the pieces were dehydrated (this caused the agar to separate), cleared in xylene and mounted in Entellan (Merck).

Fifty cells showing optimum impregnation were chosen from all the ruffed cells observed in the different species. These were drawn with the aid of a camera lucida and measured with a MOP-Videoplan semiautomatic planimeter (Kontron). In all cases the diameter of the soma, the diameter of the dendritic field, the diameter of the ruff, the overall length of the initial portion of the axon and the length of each of the parts of this initial portion were determined.

RESULTS

The olfactory bulb of the species studied in this work is a paired structure, spherical or slightly ovoid in shape in the Cypriniformes and with a more piriform shape in Salmoniformes, and about 1·3–2·1 mm in diameter. There is a different location in Cypriniformes and Salmoniformes. In the first group, it is found in the anterior part of the head of the fish, closely apposed to the olfactory mucosa and joined to the rest of the brain by two fibrous bundles – the olfactory tracts. In *Salmo gairdneri*, the olfactory bulbs are apposed to the telencephalic hemispheres and there are very long olfactory nerves.

Microscopically, in the sections stained with haematoxylin–eosin or Nissl, the bulb consisted of four concentric layers which from the superficial to the deep parts are:

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

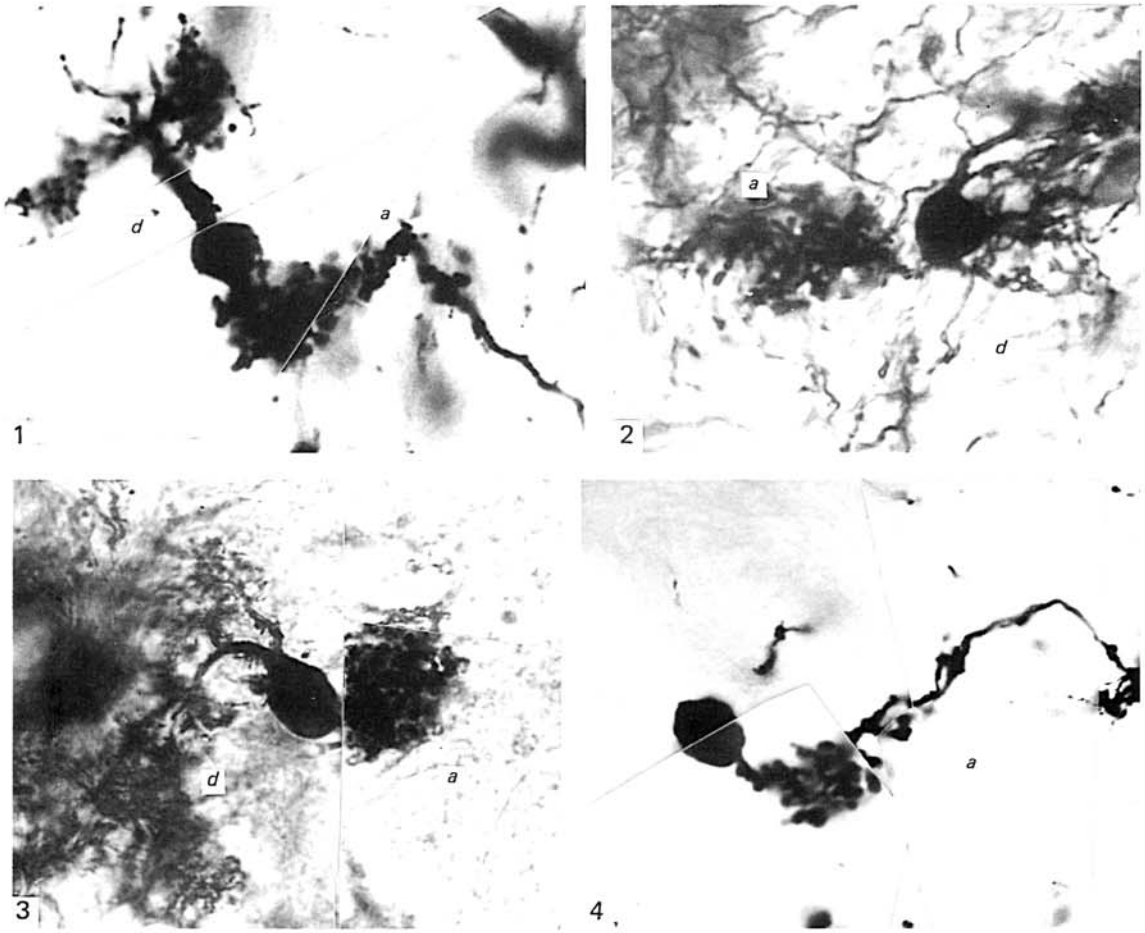
We could not identify the somata of the ruffed cells in the species studied in this work in routine histological sections, because it was not possible to distinguish them from the somata of the mitral cells.

After applying the Golgi techniques in the six species studied, ruffed cells were seen throughout the plexiform and mitral cell and glomerular layers, especially in their inner portions. Their disposition was similar to that of the mitral cells, although the ruffed cells were usually found at greater depths. Only on rare occasions was it possible to see ruffed cells near the fibres of the olfactory nerve. Close to the ruffed cells, mitral and perinest cells and abundant glia could be seen. The prolongations observed in the vicinity of the ruffed cells arose from perinest cells or, mainly, from internally situated granule cells and seemed to establish contacts.

The ruffed cells had an ovoid or spherical soma whose diameter varied between 9–16 μm in *Barbus meridionalis* (Fig. 1) and *Tinca tinca* (Fig. 2); 11–17 μm in *Chondrostoma polylepis* (Fig. 3) and 12–20 μm in *Carassius carassius* (Fig. 4), *Cyprinus carpio* (Fig. 5) and *Salmo gairdneri*. Two kinds of prolongation arose from the cell body, one of which was dendritic in nature while the other was an axon with certain peculiar details (Figs. 1–10).

The dendrites were of two kinds: thick and thin. The former, with a diameter of 2–4 μm left the cell body and formed a spherical or cone-shaped dendritic field with a diameter ranging between 8 and 54 μm, located in the neighbourhood of the soma.

The thick dendrites branched and intermingled with each other. They had varicose

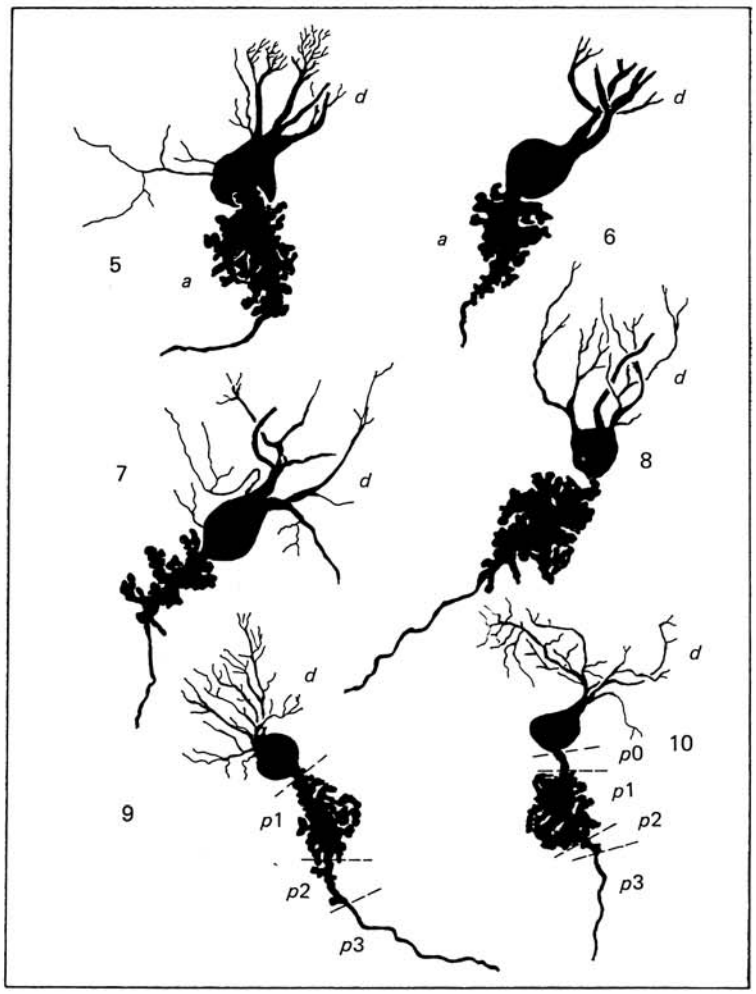


Figs. 1–4. Ruffed cells of different species (×850). *a*, Axon; *d*, dendrite. 1, *Barbus meridionalis* (Golgi–Colonnier); 2, *Tinca tinca* (Golgi–Meyer); 3, *Chondrostoma polylepis* (Golgi–Meyer); 4, *Carassius carassius* (Golgi–Colonnier).

ramifications from which arose small appendages (Figs. 1, 3) above all in the thicker portions of the dendrites. Considering their morphology, these appendages recalled the spines observed in other neurons of the olfactory bulb of these teleosts since they were seen to be both sessile and pedunculated. In the latter case, the stalk appeared as a very fine, almost filiform expansion at the end of which there was a rounded, triangular or polyhedral head. The sessile appendages were lateral expansions of the dendrites and also varied in their morphology, presenting more or less thickened and flattened heads.

The thinner dendrites were not present in all the species studied by us. They had a filamentous appearance with few branches and in most cases they were seen to arise directly from the soma of the cells. Their arrangement was often independent of the dendritic field formed by the thick dendrites (Figs. 5, 7). According to our observations, the dendrites of the ruffed cells were distributed in four different patterns.

(1) There was a single thick dendrite trunk which branched at a certain distance from the soma (Fig. 1) giving rise to abundant fine prolongations over a short distance which intermingled with each other constituting a dendritic field with a diameter of



Figs. 5–10. Camera lucida drawings of ruffed cells (× 1250). *a*, Axon; *d*, dendrite. *p*0, Part 0 of the initial portion of the axon; *p*1, Part 1 of the IP; *p*2, Part 2 of the IP; *p*3, Part 3 of the IP. 5, *Cyprinus carpio* (Golgi–Meyer); 6, *Salmo gairdneri* (Golgi–Colonnier); 7, *Carassius carassius* (Golgi–Colonnier); 8, *Tinca tinca* (Golgi–Meyer); 9, *T. tinca* (Golgi–Meyer); 10, *T. tinca* (Golgi–Meyer).

8–35 μm. This was the pattern observed in the Mediterranean barbel and in most of the ruffed cells of the Iberian nose.

(2) There was a single thick dendritic trunk branching at different parts of its course and constituting a dendritic field some 15–47 μm in diameter. No thin dendrites could be seen (Fig. 6). This was the arrangement observed in most of the ruffed cells from the tench and the rainbow trout and in some of those found in the Iberian nose.

(3) This pattern was characterised by one thick dendrite and several thinner ones, all of them arising from different parts of the soma. The dendritic field was more diffuse than in the previous cases and had a diameter of 19–54 μm (Fig. 7). This was characteristic of the carp, the crucian carp and of some ruffed cells of the tench.

(4) Characterised by various dendritic prolongations of similar nature leaving the soma. The ramifications were less abundant and constituted a dendritic field with a diameter varying between 25 and 50 μm (Fig. 8). This was the least common type and only appeared in some ruffed cells of the tench and the crucian carp.

The most remarkable structural characteristic of the ruffed cell was its axon. With

Table 1. *Length of the initial portion of the axon* (μm)

	Part '0'	Part 1	Part 2	Part 3	Total
B. meridionalis	—	23–37	13–26	27–48	70–140
C. carassius	—	22–28	8–11	21–60	60–130
C. carpio	—	12–30	5–14	21–46	50–125
Ch. polylepis	—	16–23	9–17	13–50	50–125
S. gairdneri	2–5	18–26	8–13	15–50	55–115
T. tinca	5–9	18–32	7–18	14–52	70–150

the silver chromate impregnation techniques employed it was possible clearly to distinguish the ruffed cells from the rest of the bulbar neurons. The axon of these cells, which was impregnated for variable lengths, showed in its initial portion a group of closely intermingled protrusions which, as a whole, formed a field with a round or ovoid contour – the ruff – whose diameter ranged between 15 and 40 μm in the six species studied. The axon always stemmed from the soma and in many cases arose from the pole opposite that from which the dendrites emerged. The initial portion of the axon could be divided into three parts according to its structural features (Figs. 9, 10).

Part 1, the closest to the soma, showed many elaborate protrusions arising from the axonal shaft. These intermingled with one another to constitute the most important part of the ruff, forming a spherical field around the shaft. The protrusions were difficult to identify individually and were irregular and bead-shaped in appearance with widened parts separated by constrictions or filiform portions. They ended in rounded heads. Their course was tortuous and they exhibited a dense packaging of the protrusions inside the ruff, which was compact and had clearly defined borders.

Part 2 was the middle part of the initial portion of the axon. There were several collateral protrusions scattered along the shaft which extended laterally for variable distances. These protrusions were smaller than those observed in Part 1 and frequently appeared as bulges on the stalk or were present with a thin peduncle connected to a rounded head. The bead-like protrusions were much rarer than in Part 1. They normally appeared perpendicular to the axonal axis but, when long, usually had irregular trajectories.

Part 3, the last and longest part of the initial portion was cylindrical in appearance, with smooth contours and no protrusions. It followed an undulating course and its staining ceased suddenly, probably on account of the appearance of a myelin sheath, which hid the final target of the axon.

In *Tinca tinca* and *Salmo gairdneri*, it was sometimes possible to observe a part '0' situated between the soma and Part 1 of the initial portion of the axon. This portion had a smooth and somewhat undulating appearance. In this same species, Part 2 was sometimes very reduced or was even indistinguishable.

The dimensions of the different parts of the initial portion in each of the species studied are shown in Table 1.

DISCUSSION

Up to the present this kind of neuron has only been described in certain species of teleosts by one research group (Kosaka, 1980; Kosaka & Hama, 1979*a*, *b*, 1980, 1982–3). It is not thought to exist in other classes of vertebrates.

The ruffed cell appears as a cell type characteristic of the olfactory bulb of teleosts,

clearly distinguishable by silver impregnation techniques from the rest of the bulbar neurons. Its location is characteristically in the glomerular and plexiform and in the mitral cell layers common to all the species studied by us and to those described by other authors.

The ruffed cells are relatively large neurons, comparable in their dimensions in the organ studied only to the mitral cells which are usually slightly larger. The somata vary in size although they are similar to those reported for *Conger myriaster* (Kosaka & Hama, 1980) and *Carassius auratus* (Kosaka & Hama, 1979*b*) and slightly smaller than those of *Parasilurus asotus* (Kosaka & Hama, 1980). A similar situation is observed with respect to the dimensions of the ruff. The dendritic arborisation exhibits certain features common to all the specimens studied, but has other features which are different. In all cases the dendrites of the ruffed cells branch in the neighbourhood of the soma, unlike the mitral cells whose dendrites are involved with areas fairly distant from their cell body.

Regarding the types of distribution of the dendrites examined in this work, the existence of thick and thin varieties, principally our Type 3, which are to be found in the carp, the crucian carp and some cells of the tench, has been reported earlier in the ruffed cells of *Parasilurus asotus* (Kosaka & Hama, 1980), while in *Conger myriaster* (Kosaka & Hama, 1980) there is a dendritic trunk branching after a certain distance; this would include our Types 1 and 2 belonging to the barbel, the nose, the trout and the tench. Finally, the description in *Carassius auratus* (Kosaka & Hama, 1979*a*) of several similar dendrites arising from the cell body would fit in with our Type 4 which appears in certain cells of *Carassius carassius* and *Tinca tinca*. We believe that the proposed classification yields new data which help to complete the classification of the ruffed cells in all the species studied and which show variations within species of the same family and analogies with other families which are fairly separated phylogenetically. The existence of appendages on the dendrites has been described in a very similar way for *Carassius auratus* (Kosaka & Hama, 1979*b*) and *Parasilurus asotus* (Kosaka & Hama, 1980).

The morphology of the axon of the ruffed cells offers certain characteristics common to all the species studied by us. The disposition of the protrusions of the initial portion coincides with that reported for the most closely related species – *Carassius auratus* – in which three parts may be differentiated (Kosaka, 1980; Kosaka & Hama, 1979*b*). In *Parasilurus asotus* (Kosaka & Hama, 1980) only two parts have been described and, though in some cells of the *Tinca tinca* it is difficult to delimit Part 2 in the vast majority of ruffed cells in this species, this part does exist even though it is short. In the only other species studied up to the present, *Conger myriaster*, the arrangement is different, the first part being completely smooth (Kosaka & Hama, 1980). In *Tinca tinca*, the species in which we have observed the greatest number of variations, there is a smooth part '0' between the soma and the ruff. It is also present in some ruffed cells of *Salmo gairdneri*. However, in the cases in which it does exist, this portion never runs for any great distance. The techniques employed in this work have not allowed us to discover the final target of the axon; nevertheless, in the part which can be distinguished the axon seems to follow a similar trajectory to that of the mitral cells, which together with the presence of a myelin sheath suggests that the ruffed cells may direct their axons towards higher centres.

Furthermore, the differences between the diameter of the dendritic fields and between the lengths of the different parts of the initial portion in the species studied by us, compared with the corresponding species of Kosaka & Hama (1979*b*, 1980), could be due to the different techniques used for the study of these cells.

The existence is thus confirmed, probably generally in Teleosts, of the ruffed cell with a characteristic morphology and location. Nevertheless, there are variations in the number, kind and branching pattern of the dendrites and in the disposition of the protrusions in the initial portion of the axon. Despite this it is possible to assert the existence of a characteristic typology in the prolongations which is unrelated to the fact that the specimens examined belonged to a particular order of Teleosts.

SUMMARY

The olfactory bulbs of *Barbus meridionalis*, *Carassius carassius*, *Chondrostoma polylepis*, *Cyprinus carpio*, *Tinca tinca* and *Salmo gairdneri* were examined by two variants of the Golgi technique. We report, for the first time, the existence of ruffed cells in these species. This neuronal type shows a characteristic morphology and location. Overall, these cells exhibit a generalised pattern, although there are qualitative and quantitative variations between the various species studied.

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