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# Comparative Study of the Anatomy and Laminar Organization in the Olfactory Bulb of Three Orders of Freshwater Teleosts

By

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With 21 Figures

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**Abstract**

The anatomy and lamination of the olfactory bulb in *Cyprinus carpio, Tinca tinca, Barbus bocagei* (Fam. Cyprinidae, Or. Cypriniformes); *Salmo gairdneri* (Fam. Salmonidae, Or. Salmoniformes); and *Gambusia affinis* (Fam. Poeciliidae, Or. Cyprinodontiformes), all of them freshwater teleosts, are studied. These species show significative differences on the location, size, morphology, and lamination of their olfactory bulbs. The presence of a new stratum in the olfactory bulb of *Salmo gairdneri* and a completely different laminar organization in the olfactory bulb of *Gambusia affinis* are described for the first time. The anatomical and histological peculiarities of this structure in the orders studied could be the basis for different experimental approaches.

## 1. Introduction

The first relay station in the olfactory pathway, the olfactory bulb, has received a good deal of attention since many years (VAN GEHUCHTEN and MARTIN 1891, BLANES 1898, CATOIS 1901, RAMÓN Y CAJAL 1911, CROSBY and HUMPHREY 1938, 1939, PRICE and POWELL 1970, LOPEZ-MASCARAQUE et al. 1986). In teleosts, there are early works on the histology of the olfactory bulb (ARIËNS KAPPERS 1906, SHELDON 1912, HOLMGREN 1920) and comparative studies of the whole philogenetic scale (ALLISON 1953, NIEUWENHUYS 1967, ANDRES 1970, GARRIDO 1978). In recent years, considerable interest has been aroused concerning this centre; thus, studies have been conducted on electrophysiological aspects (HARA and GORBMAN 1967, HUVE et al. 1983, STACEY and KYLE 1983), on its connections (BARTHELD et al. 1984, LEVINE and DETHIER 1985, BARTHELD and MEYER 1986) and on its cytoarchitecture, modulation, and transmission circuitry (ICHIKAWA 1976, ICHIKAWA and UEDA 1979, KOSAKA 1980, KOSAKA and HAMA 1979a–c, 1980, 1982, 1982–1983, VELASCO 1980, OKA 1980, 1983, ALONSO et al. 1986, 1987, 1988b, LARA et al. 1987). These studies have provided considerable information on the general structure of the olfactory bulb and its connections. In this sense, important differences have been reported between bony fish and higher vertebrates, mainly macrosmatic mammals. Thus, works have been reported on details of the teleostean olfactory

bulb, such as neuron types and different pathways of projections, clearly different from those of mammals. In this way, it has been suggested that the teleostean olfactory bulb would have evolved, at least partly, in a different way from that of higher vertebrates (Kosaka and Hama 1979b, Alonso et al. 1986, 1987, 1988b).

However, the majority of the above mentioned works have been centered exclusively on the olfactory bulb of Cyprinid fish (in particular, *Carassius auratus* and *Cyprinus carpio*), being very few references on the organization of this nerve centre in other groups. Despite, this, some data have been published on the forebrain diversity in different species of Teleosts (Bannister 1972), although they have focused mainly on the telencephalic hemispheres.

In order to know the interspecific variability of the teleostean olfactory bulb, we carry out a study on the anatomy and laminar organization of this nerve centre in species belonging to 3 orders of freshwater teleosts. The results would be useful for choosing the best species to be used in different experimental approaches.

## 2. Materials and Methods

The study was based on 20 specimens of *Cyprinus carpio* L. (length range 16 to 35 cm; weigth range 120 to 2,500 g), 20 specimens of *Tinca tinca* (L.) (15 to 19 cm; 65 to 115 g), 25 specimens of *Barbus bocagei* Steindachner (19 to 35 cm; 110 to 600 g); 25 specimens of *Gambusia affinis* (B et G) (1.5 to 4 cm; 0.5 to 3.5 g), and 25 specimens of *Salmo gairdneri* Richardson (21 to 26 cm; 160 to 220 g), collected from the River Tormes (Salamanca, Spain) and from fisheries "La Flecha" (Salamanca).

The animals were anaesthetized with 0.03% MS-222 *(Sandoz)*, (0.01% in the case of *Gambusia affinis*). After removal, the olfactory bulbs of half of the specimens of each species were submerged in Bouin's fluid, embedded in paraffin, and sectioned at 10 µm along the longitudinal, transversal and sagittal planes. The sections were stained, respectively, with hematoxylin-eosin, Nissl stain and by the Heidenhain modification of the Kultschitzky technique. In the case of *Gambusia affinis*, owing to the difficulty involved in extracting the brain, the whole head was fixed and embedded thereafter following the protocol described for the other species. The brains of the other half of the specimens were fixed either by immersion of by intracardiac perfusion with 2% paraformaldehyde-2% glutaraldehyde in 0.12 mol/l phosphate buffer ($pH = 7.4$). The pieces were then embedded in Spurr's resin, sectioned at 1 µm along the above-mentioned planes and stained with Ziehl's fuchsin (Alonso et al. 1988a) or toluidine blue.

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Fig. 1. Dorsal view of the brain of a cyprinoid fish. × 2. *ob* olfactory bulb, *arrows* olfactory tracts

Fig. 2. Transversal section of the head of *Gambusia affinis*. × 17. *ob* olfactory bulb, *th* telencephalic hemisphere

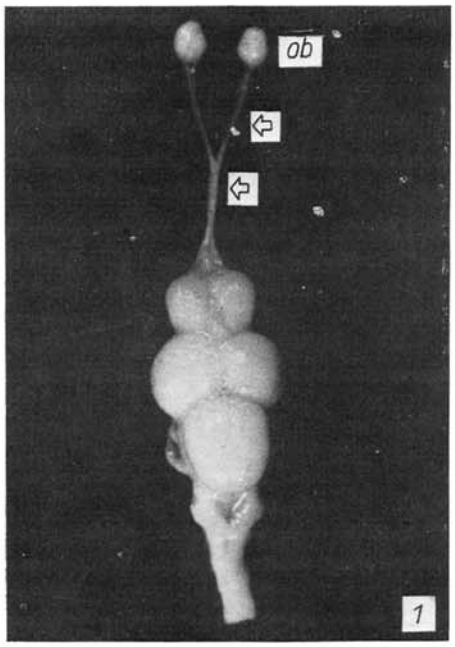
Fig. 3. Olfactory tract of *Cyprinus carpio*. Hematoxylin-Eosin, × 150. *L* Lateral olfactory tract, *M* Medial olfactory tract, *m* meninx, *t* tela, *v* ventricle

Fig. 4. Caudal region of the olfactory bulb of *Tinca tinca*. Ziehl's fuchsin, × 250.

Fig. 5. Olfactory tract *(arrows)* in the caudal region of the olfactory bulb of *Gambusia affinis*. Heidenhain, × 470

Fig. 6. Telencephalon of *Gambusia affinis*. Hematoxylin-Eosin, × 75. *ob* olfactory bulb, *on* olfactory nerve, *th* telencephalic hemisphere

Fig. 7. Fascicles of the olfactory nerve *(arrows)* entering into the olfactory bulb of *Barbus bocagei*. Hematoxylin-Eosin, × 80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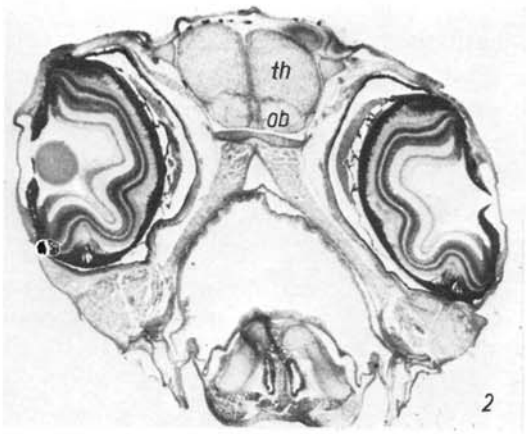
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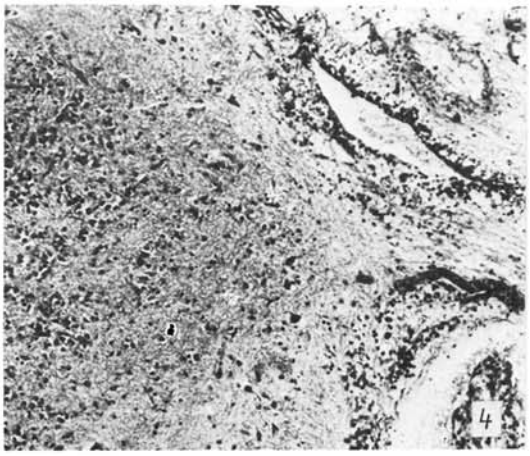
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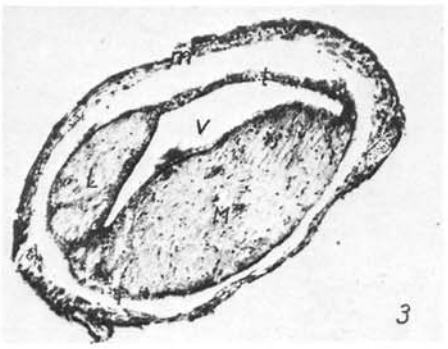
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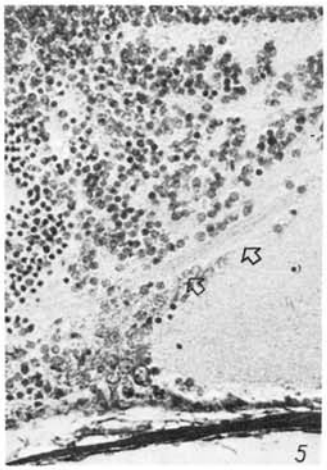
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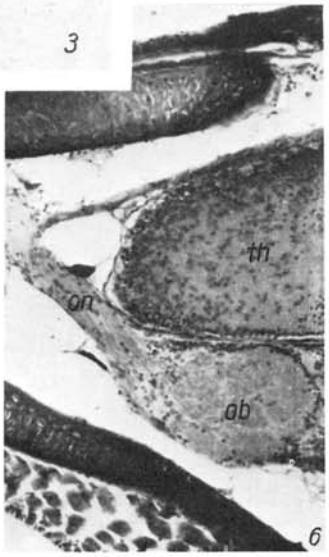
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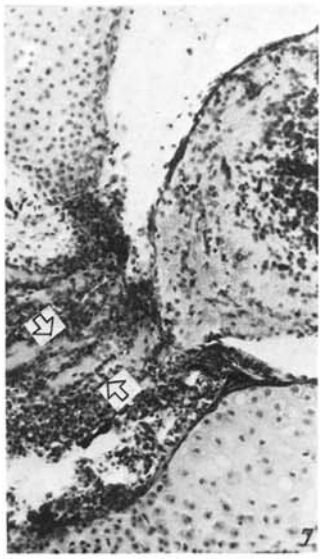
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### 3. Results

The 3 groups studied, Family Cyprinidae (Or. Cypriniformes), Family Poeciliidae (Or. Cyprinodontiformes), and Family Salmonidae (Or. Salmoniformes) had highly significant differences in the structure of their olfactory bulbs. Thus, in the Cyprinoids *Barbus bocagei, Cyprinus carpio,* and *Tinca tinca,* the olfactory bulb is situated in an extremely rostral position, very close to the olfactory epithelium (Fig. 1). In these species, the olfactory nerves are very short ranging from 0.5 to 2 mm in length. The fibrous fascicles, which constitute the olfactory nerves, are clearly identifiable (Fig. 7), they are relatively independent and intermingle profusely. The olfactory nerve enters the bulb through its anterior portion, being the fibres distributed over the whole of its surface except in the caudal region, showing a greater degree of development in the ventral and lateral regions. Ending the posterior region of the cyprinoid olfactory bulb arises an olfactory tract (Fig. 4), principally composed of myelinated axons stemming from the mitral cells and, probably, from the ruffed cells; centrifugal fibres from other brain areas can also be seen among them. The olfactory tracts are several centimeters in length, according to the size of the specimen. 2 portions can be distinguished on them: the lateral olfactory tract and the medial olfactory tract. The olfactory tract exhibits on its dorsal portion, over both subdivisions, a ventricle communicating with both the bulbar and telencephalic ventricles (Fig. 3).

In the other 2 families studied, Salmonidae and Poeciliidae, the olfactory bulb is appended to the telencephalic hemispheres (Fig. 2), the olfactory nerves are extremely long (Fig. 6) and, on the contrary, the olfactory tracts are short and internal (Fig. 5). In *Salmo gairdneri,* the olfactory nerves are compact and follow a fairly straight course. Their implantation cone is very marked (Fig. 14) and the olfactory fibres extend over this, particularly on its rostral and dorsal portions. The olfactory tracts course horizontally, penetrating into the hemispheres. In *Gambusia affinis,* the olfactory nerves follow a pronounced curved path due to the location of the olfactory bulb (Fig. 6). Into this latter organ, they are distributed through the anterior and ventral portions, especially in this latter. The olfactory tracts course obliquely in an upwards direction (Fig. 5).

The form and location of the olfactory bulb also vary. In the case of the cypriniform fish, the bulb is located distally from the brain showing a subspheroid or ovoid shape (Fig. 8). In the

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Fig. 8. Longitudinal section of the olfactory bulb of a cyprinoid fish. Hematoxylin-Eosin, × 20

Fig. 9. Longitudinal section of the olfactory bulb of a salmonid fish. Hematoxylin-Eosin, × 20

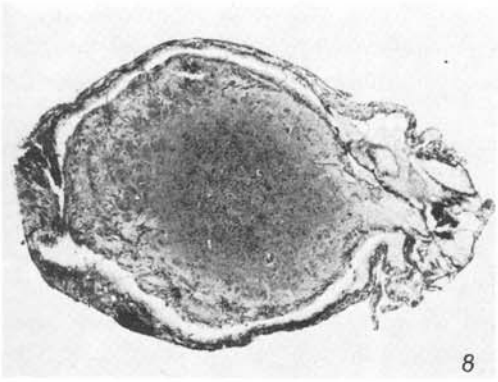
Fig. 10. Bulbar ventricle. *Tinca tinca.* Toluidine blue, × 200. *f* fibres of the olfactory tract

Fig. 11. Mitral cells and ruffed cells *(arrows)* in the plexiform layer. *Cyprinus carpio.* Nissl, × 200

Fig. 12. Ependymary *(el)* and subependymary *(sel)* layers in the olfactory bulb of *Salmo gairdneri.* Nissl, × 750

Fig. 13. Outer strata of the carp olfactory bulb. Ziehl's fuchsin, × 230. *onfl* olfactory nerve fibre layer, *gl* glomerular layer

Fig. 14. Implantation cone of the olfactory nerve *(on)* in the olfactory bulb of the rainbow trout *Salmo gairdneri.* Hematoxylin-Eosin, × 350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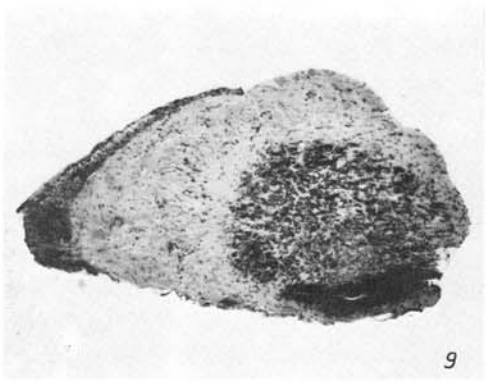
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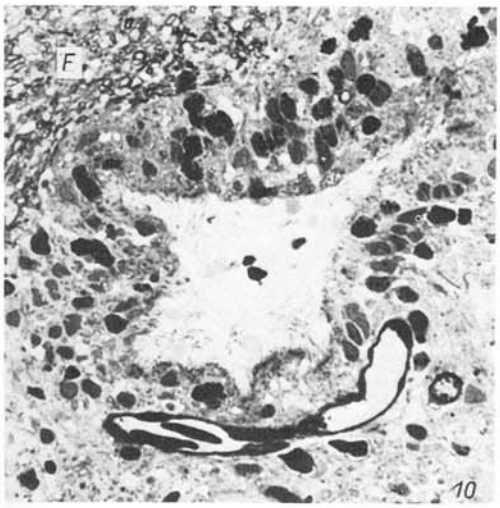
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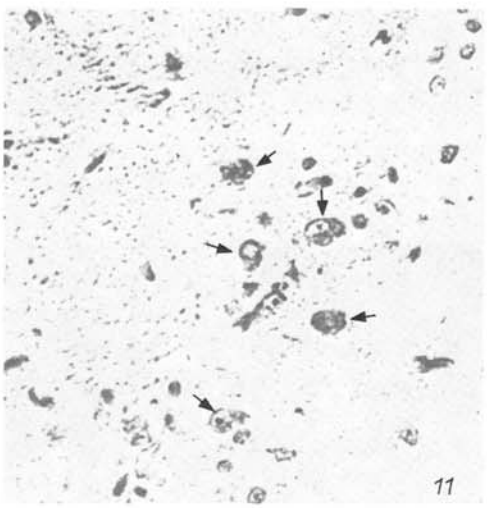
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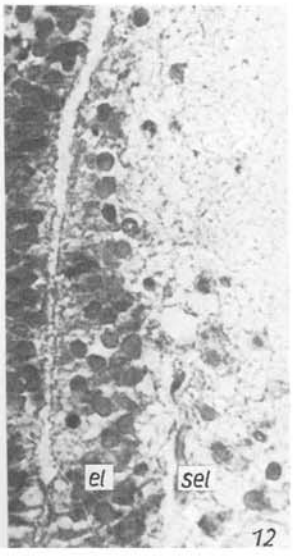
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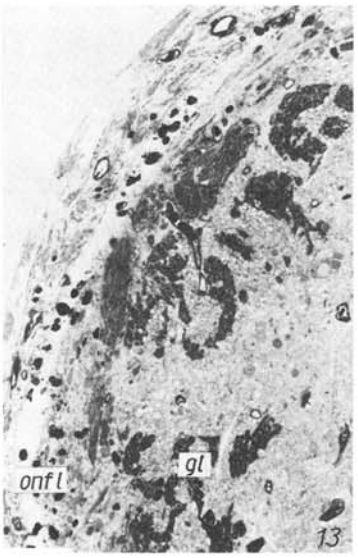
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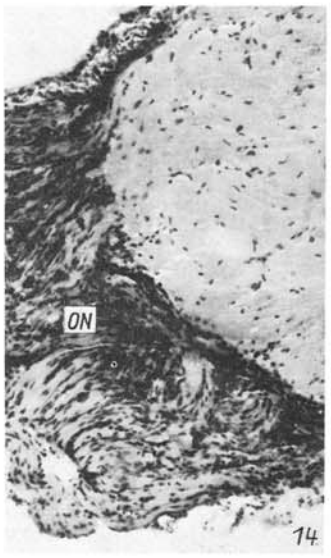
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salmoniformes, it is located anteriorly to the telencephalic hemispheres being pyramid-shaped with its apex protruding forwards (Fig. 9). Finally in *Gambusia affinis,* the rounded or sligthly oval bulbs are placed ventrally to the telencephalic hemispheres, which are very elongate and cover them dorsally (Figs. 2, 6).

In all the species studied, the histological sections reveal the presence of a ventricle that is the continuation of the ventricle of the telencephalic hemispheres (Fig. 10). In the cyprinoids the ventricle is situated in caudo-ventral position. It has the shape of a cleft and its anterior surface exhibits a cone-like swelling whose base extends towards the rostral end of the olfactory bulb. In *Salmo gairdneri,* the ventricle is also posterior and its development is much more marked; it shows expansions that penetrate deeply into the bulb forming the lateral or ventricular recesses. In Poeciliidae, the development of the ventricle is minimum and this cavity is almost absent, being the ependymary layer surrounding it, fused with each other. The ventricle is dorsally and caudally situated.

Regarding the lamination in the different species, in the cyprinoids, which are the best known, the following strata were observed from the outermost part to the innermost one:

### 3.1. Olfactory Nerve Fibre Layer

It is constituted by the entrance into the bulb and distribution over its surface of the olfactory axons from the olfactory mucosa (Fig. 7).

### 3.2. Glomerular Layer

It is the point of the sensory information passage from the 1 to the 2 neuron on the olfactory pathway, through synapses among the olfactory axons and the mitral cells. The glomeruli, at least in the species studied, are not clearly separated and form a set that we denominate the glomerular area (Fig. 13).

### 3.3. Plexiform Layer

This is formed by a dense neuropil in which large somata of mitral and ruffed cells can be seen. However, these cells do not constitute a well-defined stratum and can also be observed in other layers (Fig. 11).

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Fig. 15. Transversal section of the olfactory bulb of *Salmo gairdneri.* Hematoxylin-Eosin, × 85. *gcl* granule cell layer

Fig. 16. Plexiform layer in the olfactory bulb of *Tinca tinca.* Toluidine Blue, × 600

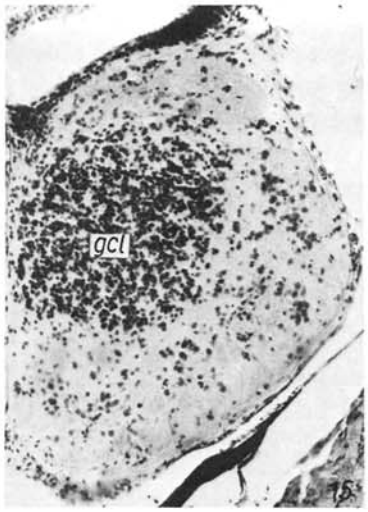
Fig. 17. Ependymary *(el)* and subependymary layers *(sel). Salmo gairdneri.* Toluidine blue, × 950

Fig. 18. Laminar organization in the olfactory bulb of *Gambusia affinis.* Hematoxylin-Eosin, × 125. *gc* granule cells, *of* olfactory fibres, *pl* plexiform layer

Fig. 19. Plexiform layer in the olfactory bulb of *Gambusia affinis.* Hematoxylin-Eosin, × 450

Fig. 20. Ganglionar cells of the terminal nerve in the olfactory bulb of *Barbus bocagei.* Nissl, × 160

Fig. 21. Ganglionar cells of the terminal nerve in the olfactory nerve of *Tinca tinca.* Nissl, × 60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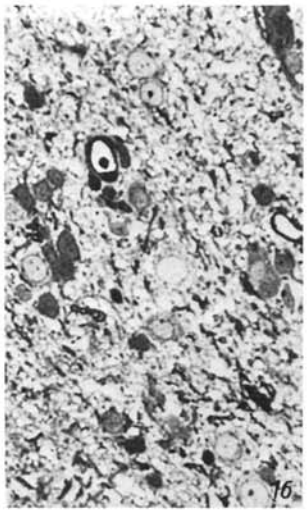
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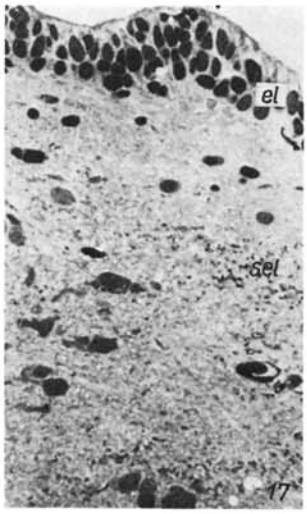
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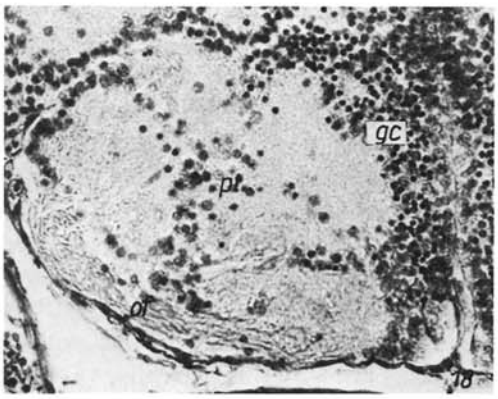
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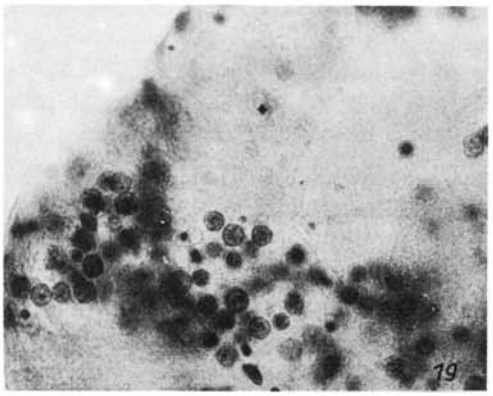
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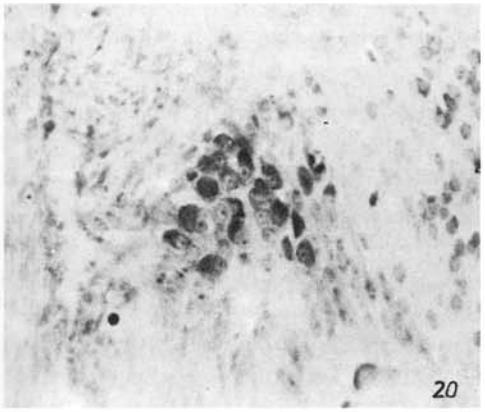
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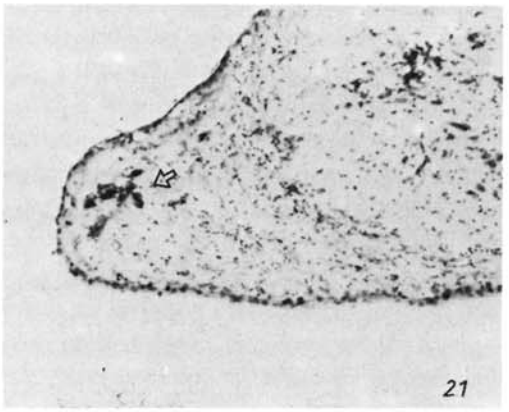
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### 3.4. Granule Cell Layer

This is the central and best-developed layer. It is characterized by the presence of large numbers of small neurons, the granule cells, that are densely grouped (Fig. 15). The caudal portion of this stratum is in contact with the ependymary layer surrounding the ventricle (Fig. 10).

All these layers are arranged more or less concentrically, except that in the posterior zone, at the exit of the olfactory tract, neither olfactory nerve fibres nor glomeruli are found.

In *Salmo gairdneri* the layering is fairly similar. The strata are also concentric, the following can be distinguished:

*a*. Olfactory Nerve Fibre Layer,
*b*. Glomerular Layer (extremely well developed in the dorsal portion),
*c*. Plexiform Layer (with the mitral cells arranged frequently in small groups),
*d*. Granule Cell Layer,
*e*. Subependymary Layer (Figs. 12, 17),
*f*. Ependymary Layer.

### 3.5. Subependymary Layer

The Subependymary Layer (Fig. 17) has not been described previously and exhibits 2 clearly distinguishable areas: a posterior or caudal zone of great cellular density, identified as a zone of cell proliferation, and an anterior or rostral zone formed of a dense neuropil in contact with the granule cell layer; we consider this to be a zone of cell migration.

In *Gambusia affinis,* the layering exhibits peculiar characteristics: the distribution is not concentric and there is an olfactory nerve fibre layer with a preferentially ventral and slightly rostral orientation (Fig. 18). These fibres penetrate as bundles, forming a glomerular layer with the same kind of distribution although both strata are difficult to separate from one another. In this species, the glomerular layer has contours that are not as well defined as in the other 2 orders studied. The plexiform layer is in *Gambusia affinis* the best developed one. It is situated in the centre of the bulb being composed of a dense neuropil in which abundant isolated neurons can be found (Fig. 19).

The granule cell layer forms a pole in the dorsal-caudal position. Between this and the ependymary layer that surrounds the ventricle, which in this species is almost completely obliterated, there is an area of cell proliferation although it is not possible to distinguish clearly a subependymary layer.

In adult specimens of all the species studied, the presence was observed of a zone of cellular proliferation located immediately under the ventricle. This zone did not show an uniform distribution, being better developed its anterior portion. We have observed the presence of cells in all the phases of mitoses; these were more numerous in *Salmo gairdneri,* less in *Gambusia affinis,* and much scarcer in the cyprinoids. Likewise, in a very few specimens, we observed the occurrence of cells in divisional process at the subpial glial border located above the Olfactory Nerve Fibre Layer.

The only type of neurons clearly identifiable with the employed techniques by its enormous size was the so-called giant cells of the olfactory bulb or ganglion cells of the Terminal Nerve

(Fig. 20). These show a varying distribution and are frequent in the olfactory nerve of the cyprinoids (Fig. 21); in the olfactory bulb, they are as abundant in Cyprinidae as in Salmonidae, whereas in Poeciliidae they are found close to the olfactory tract, forming dense, compact groups with no clear limits between the caudal part of the bulb and the rostral portion of the telencephalic hemispheres.

A detailed study of the neuronal typology in the different taxonomic groups using silver impregnation methods and the electron microscopy will be dealt with in a forthcoming publication.

#### 4. Discussion

The olfactory bulb is a structure that is common to the whole of the subphylum of vertebrates with the exception of the *Amphioxus* (Allison 1953, Garrido 1978). Allison (1953) reported that in general, the microscopic structure of the olfactory bulb is similar in all the animals and that even though variations exist in the laminar organization and in the distribution of the main neuronal types, it is possible to distinguish the same structural organization in all of them. However, recent researchs (Kosaka and Hama 1979a–c, 1982–1983, Alonso et al. 1986, 1987, 1988b) has pointed to the existence, in the olfactory bulb of bony fish, of neuronal types and circuits which are not present in higher vertebrates. Accordingly, it has been suggested that the evolution of this nerve centre in teleosts and in mammals seems, at least in part, to have diverged. Among Teleosts, although we think that their anatomy is consistent with a general scheme, our own results point to the existence of significant variations among the different orders and species.

As it has been reported in our results, the olfactory bulb may be located in 2 different positions:

1. *Apposed to the telencephalic hemispheres:* This is the case of Cyclostomes, most Teleosts (among them the Salmoniformes and Cyprinodontiformes studied by us), turtles, birds, and many mammals (van Gehuchten and Martin 1891, Blanes 1898, Jensen 1930, Crosby and Humphrey 1939, Heier 1948, Allison 1953, Nieuwenhuys 1962, 1967, Bauchot et al. 1973, Lopez-Mascaraque et al. 1986). One disposition, considered by Nieuwenhuys (1967) to be a case apart, is that of the amphibians in which the olfactory bulbs are not independent structures but rather occupy a part of the telencephalic hemispheres (Herrick 1910, 1924, Hoffman 1963).

2. *Separated from the rest of the brain:* This is the case of most cartilaginous fish, lung fish, some families of teleosts (among them the Cyprinidae studied by us), Crossopterygii, some amphibians, most reptiles, and primates (Ariëns Kappers 1906, Crosby 1917, Holmgren and Horst 1925, Nieuwenhuys 1967, Garcia-Verdugo et al. 1986).

Regarding the presence of olfactory bulbs with long olfactory tracts, it has been observed in 3 families of teleosts: Cyprinidae, Mormyridae, and Siluridae. The specimens belonging to these families have a much faster transmission to the telencephalon than other groups, since the longer distance is covered by the olfactory tract of myelinic nature. By contrast, in the other groups, the longest part of the pathway is covered by the amyelinic fibres comprising the olfactory nerve. Regardless of other factors that might also be important, our opinion is that this suggests the olfactory system being much more efficient in the 1 case than in the 2nd one.

With respect to the location of the olfactory bulbs in most vertebrates, they are situated in the rostral or rostro-ventral portion of the telencephalic hemispheres. However, in Selachii (Nieuwenhuys 1967), they are disposed laterally to the hemispheres and in *Gambusia affinis,* we have observed a ventral location.

The shape of the olfactory bulb also varies considerably: round or oval morphologies are typical of bulbs with short olfactory nerves and long olfactory tracts; whereas when they are apposed to the telencephalon dome-shaped or pyramidal olfactory bulbs are more common. In *Gambusia affinis,* there is an exception since its olfactory bulb is located most caudally showing a fairly regular oval shape.

Accessory olfactory bulbs, which receive afferences from the axons of the bipolar neurons located in the vomeronasal organ, are found in gymnophiones, anurans, most reptiles, and many mammals (Allison 1953, Nieuwenhuys 1967, Garrido 1978). In the olfactory bulb of the studied teleosts, we have not observed any similar kind of formation, nor a possible precursor of this organ. Regarding the architectural organization and layering in all animals, the olfactory bulb shows a lamination that varies in its extension and cell density. The accepted typology for the olfactory bulb of macrosmatic mammals (Ramón y Cajal 1911) divided it into 7 different layers:

1. Superficial Nerve Layer,
2. Olfactory Glomeruli Layer,
3. External Plexiform Layer,
4. Mitral Cell Layer,
5. Internal of Deep Plexiform Layer,
6. Granule Cells and White Matter Layer.
7. Epithelial or Ependymary Layer.

This kind of olfactory bulb organization is considered to be the most complete and complex of those reached on the phylogenetic scale. In bony fish, the olfactory bulb exhibits a less complex layering with fewer strata, mainly due to the fact that the mitral cells never form a thin, sheet-like layer and thus, it is impossible to distinguish between an internal and an external plexiform layer.

The 1st stratum (Olfactory Nerve Fibre Layer) is accepted for the whole of the vertebrates (Allison 1953, Andres 1970, Garrido 1978) although with certain differences, as we have seen in our own observations, in its development and arrangement.

The 2nd stratum is called the Glomerular Layer (Andres 1970, Ichikawa 1976, Kosaka and Hama 1979a, b; and our results) in disagreement with the Olfactory Glomeruli Layer described for higher vertebrates (Ramón y Cajal 1911) since the absence of periglomerular cells does it impossible to identify individual glomeruli. To consider as separate an external cell layer and a glomerular layer may well lead to confusion since many external cells (mainly mitral, ruffed, perinest, and displaced granule cells) can also be found in the glomerular layer. Nevertheless, the principal function of this area is comparable, being the zone of convergence and relay of the olfactory information. However, differences in the modulation circuits of this region can be found (Kosaka and Hama 1982–1983, Alonso et al. 1987).

The 3rd stratum is known as the plexiform layer (Kosaka and Hama 1979a; and our

observations), whereas Andres (1970) and Ichikawa (1976) named it the Mitral Cell Layer. We consider this latter denomination to be less appropriate because these neurons are also found in more external and internal strata; and also because there are other neuronal types such as ruffed and perinest cells, which are also typically present in this stratum. Kosaka and Hama (1979c) group the plexiform and glomerular layers together into a single stratum that they call the intermediate or 2nd layer. We think that these 2 layers are clearly distinguishable between each other, both with the light and electron microscope, and hence describe them as being separate in this study.

Andres (1970) preferred to use the term Plexiform Layer for both the Plexiform and the Granule Cell layers because he considers the neuropil as being primarily responsible for the characterization of the strata. Thus, the granule cells would be situated within the periventricular layer and in the plexiform layer. Oka (1983) calls this stratum the Internal Cell Layer. The same denomination is used by Huve et al. (1983) who proposed the term External Plexiform Layer to refer to a new stratum which would include the plexiform and glomerular layers. However, these authors also differentiate a glomerular layer. Our findings do not seem to support such terminology; thus, the term internal cell layer referred to the granule cell layer does it difficult to establish a limit with the adjacent zone of the telencephalic hemispheres in the no-cyprinoid species. This adjacent zone does in fact exhibit numerous cells, but not granule cells. We think that although the Granule Cell Layer shows other cellular types such as short axon cells and stellate cells, it is not a problem regarding their actual denomination since the granule cells are, in this stratum, the main and most numerous neurons.

Furthermore, apart from these 4 main strata, we consider the existence of another 2 layers: the subependymary layer and the ependymary layer.

In a preliminary hypothesis, according to our studies, the subependymary layer would be included within the layering of the olfactory bulb. We believe that hitherto it has not been described since most studies have been focused on cyprinids and, moreover, generally on *Carassius auratus,* in which the subependymary layer does not exist. According to our results, this stratum exists and would play an important role in the processes of cell proliferation and migration, instead in the adult life. In this sense, this layer exhibits a maximum degree of development in species which exhibit a highest number of cells undergoing division. If such cells, during a gradual process of migration, begin to be placed in the caudal portion of the granule cell layer, either to increase the number of cells or for a process of turnover, the subependymary layer will decrease in volume until it finally disappears. This could be the case of the cyprinoids where the number of cells undergoing division is much lower.

The last stratum, the ependymary layer, has been described by several different authors and corresponds to layer 7 of Ramón y Cajal (1911) and to layer 5 of Andres (1970). Oka (1983) has pointed out that there is no ependymary layer in *Carassius auratus*. However, our results seem to show that although it is very reduced, this stratum is indeed present in all the cyprinoids studied.

Regarding cell types, both glial and neuronal, the techniques employed in the present study did not permit inequivocal identification, although within each stratum, there seemed to be characteristic neurons that were similar with respect to their shape and size.

We hope that the terminology proposed for the lamination of the olfactory bulb could unify

the different terms proposed in the literature, making also easily comparable with descriptions of the mammalian olfactory bulb.

Overall, the considerable variability of the olfactory bulb in teleosts is related to the phylogeny and the importance of this sense in the behaviour of the species. There are characteristics, such as the extension of the ventricle, that are related with the evolutionary development that species have reached, whereas other, such as long olfactory tracts, appear in groups that are phylogenetically quite distant from another. The great importance of the olfactory system for bony fish in behaviour such as feeding (Pipping 1927), sexual communication (Stacey and Kyle 1983), avoidance from predators (Frisch 1941), and orientation including the location of parent streams during migration (Hasler and Wisby 1951, Solomon 1973) means that further detailed studies should be conduced both qualitatively and quantitatively on the structure, physiology, and behaviour related with this important nerve center.

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