

Chapter 15

Molecular Markers in the Study of Non-model Vertebrates: Their Significant Contributions to the Current Knowledge of Tetrapod Glial Cells and Fish Olfactory Neurons



Simone Bettini, Maurizio Lazzari, and Valeria Franceschini

Abstract The knowledge of the morphological and functional aspects of mammalian glial cells has greatly increased in the last few decades. Glial cells represent the most diffused cell type in the central nervous system, and they play a critical role in the development and function of the brain. Glial cell dysfunction has recently been shown to contribute to various neurological disorders, such as autism, schizophrenia, pain, and neurodegeneration. For this reason, glia constitutes an interesting area of research because of its clinical, diagnostic, and pharmacological relapses. In this chapter, we present and discuss the cytoarchitecture of glial cells in tetrapods from an evolutive perspective. GFAP and vimentin are main components of the intermediate filaments of glial cells and are used as cytoskeletal molecular markers because of their high degree of conservation in the various vertebrate groups. In the anamniotic tetrapods and their progenitors, Rhipidistia (Dipnoi are the only extant rhipidistian fish), the cytoskeletal markers show a model based exclusively on radial glial cells. In the transition from primitive vertebrates to successively evolved forms, the emergence of a new model has been observed which is believed to support the most complex functional aspects of the nervous system in the vertebrates. In reptiles, radial glial cells are prevalent, but star-shaped astrocytes begin to appear in the midbrain. In endothermic amniotes (birds and mammals), star-shaped astrocytes are predominant. In glial cells, vimentin is indicative of immature cells, while GFAP indicates mature ones.

Olfactory receptor neurons undergo continuous turnover, so they are an easy model for neurogenesis studies. Moreover, they are useful in neurotoxicity studies because of the exposed position of their apical pole to the external environment. Among vertebrates, fish represent a valid biological model in this field. In particular, zebrafish, already used in laboratories for embryological, neurobiological, genetic,

S. Bettini · M. Lazzari (✉) · V. Franceschini
Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Bologna, Italy
e-mail: maurizio.lazzari@unibo.it

© Springer Nature Switzerland AG 2019

W. Tworzydło, S. M. Bilinski (eds.), *Evo-Devo: Non-model Species in Cell and Developmental Biology*, Results and Problems in Cell Differentiation 68,
https://doi.org/10.1007/978-3-030-23459-1_15

355

and pathophysiological studies, is the reference organism in olfactory system research. Smell plays an important role in the reproductive behavior of fish, with direct influences also on the numerical consistency of their populations. Taking into account that a lot of species have considerable economic importance, it is necessary to verify if the model of zebrafish olfactory organ is also directly applicable to other fish. In this chapter, we focus on crypt cells, a morphological type of olfactory cells specific of fish. We describe hypothetical function (probably related with social behavior) and evolutive position of these cells (prior to the appearance of the vomeronasal organ in tetrapods). We also offer the first comparison of the molecular characteristics of these receptors between zebrafish and the guppy. Interestingly, the immunohistochemical expression patterns of known crypt cell markers are not overlapping in the two species.

15.1 Glial Cells

Early classical comparative studies brought to light the basic model of glial cytoarchitecture in the central nervous system (CNS) of vertebrates (Herrick 1948; Ramon y Cajal 1952). The studies of amphibian CNS highlighted the presence of glial cells whose cell bodies are located in the ependymal or periependymal layer. The long radially oriented processes of these cells pass through the neural wall to the meningeal surface where they form terminal expansions constituting the *mambra gliae limitans externa*. These elements are found both in larvae and adults (Messenger and Warner 1989) and resemble the first glial cells appearing in the embryonic brain of amniotes (Ramon y Cajal 1952).

Radial glial cells have been identified as important elements for guidance of migrating neurons during development (see Rakic 1981; Hatten 1999). Moreover, radial glial cells are the source of neurons as well as other glial elements including astrocytes, oligodendrocytes, and ependymal cells (Campbell and Götz 2002; Weissman et al. 2003; Malatesta et al. 2008; Rowitch and Kriegstein 2010). During neurogenesis, radial glial cells descend directly from neuroepithelial cells (reviewed in Götz and Huttner 2005).

Adult amniotes show a different type of glial cell, called star-shaped astrocyte due to numerous cytoplasmic processes arising from the cell body. Star-shaped astrocytes coexist with prevailing radial glial cells in reptiles (Kalman and Pritz 2001; Lazzari and Franceschini 2001). On the contrary, star-shaped astrocytes are the main type of glial cells in the endothermic amniotes, birds and mammals (Elmqvist et al. 1994; Kalman et al. 1998). The glial cytoarchitectures including star-shaped astrocytes appear functionally more complex and phylogenetically derived compared to patterns consisting of radial glial cells only.

Glial fibrillary acidic protein (GFAP), the major subunit of the gliofilaments belonging to the intermediate filament class (Eng 1985), has been recognized as a reliable marker of mature elements of the astroglial lineage (Dahl and Bignami 1985). The expression of GFAP has been reported in the glial cells of many

vertebrates (Wasowicz et al. 1994; Wicht et al. 1994; Naujoks-Manteuffel and Meyer 1996). GFAP has a considerable stability in its molecular and antigenic properties throughout the vertebrate phylogenesis (see Eng et al. 2000).

Vimentin is another cytoskeletal protein of the intermediate filament group that is used in glial cell study. It was detected in not fully differentiated cells of the astroglial lineage in reptiles (Monzon-Mayor et al. 1990; Yanes et al. 1990) and mammals (Oudega and Marani 1991; Elmquist et al. 1994; Pulido-Caballero et al. 1994). It was also found in terminally differentiated glial cells of adult teleosts and amphibians (Zamora and Mutin 1988; Cardone and Roots 1990; Rubio et al. 1992).

Immunohistochemically detected GFAP abundance and vimentin scarcity can suggest a mature condition for the glial elements. Moreover, the relative abundance of radial elements in the glial pattern is morphologically indicative of an immature condition (Kalman et al. 1998; Kalman and Pritz 2001). The relationship between GFAP/vimentin expression and amount of radial elements in the glial pattern of vertebrates is still not fully clear. Exhaustive response to developmental and phylogenetic implications of specific intermediate filament molecular markers and specific glial cell types require additional studies.

The immunohistochemical detection of intermediate filament molecular markers of glial cells brought to light the cytoarchitecture of astroglial cell elements in the vertebrate CNS (Kalman 2002). The following sections describe the characteristics of astroglial cell organization in the main groups of tetrapods (Table 15.1).

15.1.1 Lungfish

The distribution of glial intermediate filament molecular markers, GFAP and vimentin, was investigated in the CNS of the African lungfish *Protopterus annectens* using immunoperoxidase technique (Lazzari and Franceschini 2004). The Dipnoi (lungfishes) are a monophyletic group of osteichthyes (Schultze and Campbell 1986) and together with their sister group *Crossopterygii* (coelacanth) represent the unique surviving lobe-finned fish (Moy-Thomas and Miles 1971). Lungfishes are considered the closest extant relatives to terrestrial vertebrates (Forey 1986). Molecular analysis, mostly DNA sequencing, supports this assumption (Amemiya et al. 2013; Biscotti et al. 2016).

GFAP immunohistochemistry revealed a glial architecture. The cell bodies of radial cells are located at the ventricular surface (the epithelial-like monostratified layer separating the neural tissue from the cavities constituting the ventricular system of the CNS). The cell body gives rise to a long radial process, which ramify into finer fibers. These fibers form the submeningeal and perivascular glial layers. In the different cerebral regions, radial glial cells show a low degree of heterogeneity in staining intensity for GFAP. In the spinal cord, the cell bodies of the glial cells are placed in the periependymal zone. GFAP immunostaining does not reveal star-shaped astrocytes in the CNS of *Protopterus*. Vimentin immunopositivity is scarce in the nervous tissue of this lungfish.

Table 15.1 GFAP- and vimentin-immunopositive glial structure in the central nervous system of tetrapods and closest, extant relatives

	Luangfish	Urodeles		Turtles	Lizards	Crocodylians	Birds	Mammals
GFAP-immunopositivity	<i>Protopterus annectens</i>	<i>Ambystoma mexicanum</i>	<i>Triturus carnifex</i>	<i>Pelodiscus sinensis</i>	<i>Podarcis sicula</i> <i>Anolis sagrei</i> <i>Eublepharis macularius</i>	<i>Caiman crocodylus</i>	<i>Gallus domesticus</i>	Rat Dog
	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Brain	Ependymal radial glia (tanycytes): radial cells with cell bodies located at the ventricular surface and long positive processes	Ependymal radial glia (tanycytes): radial cells with cell bodies located at the ventricular surface and long positive processes	No	Predominant ependymal radial glia (tanycytes): radial cells with cell bodies located at the ventricular surface and long positive processes	Predominant ependymal radial glia (tanycytes) A few star-shaped astrocytes in the optic tectum	Predominant positive fibers from ependymal glia Non-pre-dominant star-shaped astrocytes intermingled with radial glia processes	Restricted occurrence of radial glia Predominant star-shaped astrocytes	Radial glial fibers almost disappeared Predominant star-shaped astrocytes
Spinal cord	Radial glial cells and their processes Positive ependymocytes	Radial glia with their cell bodies displaced from the ependymal layer into a periependymal position Ependymocytes with slight positivity		Radial glial cells in periependymal position A few star-shaped astrocytes	Radial astrocytes Some star-shaped astrocytes	Predominant dense network of positive radial and nonradial fibers	Numerous intensely immunostained star-shaped astrocytes	Star-shaped astrocytes

	Yes	No	Yes	No	?	Yes	Yes
Vimentin-immunopositivity Brain	Some weakly positive fibers in the gray and white matters		Ependymal radial glia (tanycytes); radial cells with cell bodies located at the ventricular surface and long positive processes			<i>P. sicula</i> and <i>E. macularius</i> : few vimentin-positive radial glial fibers	Vimentin positive glial elements at the midline of the brain (raphe and decussations)
Spinal cord	Poor vimentin-positive radial processes in the white matter		Radial glial cells and their processes Positive ependymocytes			<i>P. sicula</i> : positive ependymal cells <i>E. macularius</i> : some radial processes	Ependymal cells; (tanycytes and cuboidal cells)

Note: “?”: data not available

15.1.2 *Urodeles*

GFAP and vimentin have been used for an immunohistochemical study of glial elements in the CNS of two urodele species, *Ambystoma mexicanum* (Ambystomatidae) and *Triturus carnifex* (Salamandridae) (Lazzari et al. 1997). In *A. mexicanum* brain, GFAP-immunopositivity appears only in the ependymal cells showing a tanycytic feature (Fig. 15.1a). GFAP-positive radial processes give rise to variously extended endfeet forming the perivascular and submeningeal glial layers (Fig. 15.1b). In the spinal cord, GFAP-immunopositive radial glial cells show their cell bodies only in periependymal position. In *A. mexicanum* CNS, no vimentin immunopositive glial structures are present. In *T. carnifex* CNS, the distribution pattern of GFAP and vimentin results quite the opposite of the *A. mexicanum* condition.

15.1.3 *Turtles*

Among living animals, turtles are widely accepted as the most closely related extant vertebrates to the “progenitor” amniotes that represent the common ancestor of both reptiles and mammals (Gans and Parsons 1981; Bar et al. 2000).

The glial cytoarchitecture in the CNS of the soft-shell turtle *Trionyx sinensis* was investigated by immunoperoxidase detection of GFAP and vimentin intermediate filaments (Lazzari and Franceschini 2006). In this turtle, GFAP immunostaining reveals clear positivity in both the brain and spinal cord. The stained elements consist of long radial fibers coming from cell bodies placed in the ependymal layer displaying their tanycytic nature (Fig. 15.1c, d). In the brain, star-shaped or proper astrocytes are not detected by immunostaining; on the contrary, they appear in the spinal cord. According to the more complex turtle brain compared to amphibian condition, the ependymal radial glia of the turtle show some regional specializations concerning different size and the intensity of the immunohistochemical staining, which are comparable with the results in Emydidae (Kriegstein et al. 1986; Kalman et al. 1994, 1997).

In the spinal cord, both gray and white matters appear intensely GFAP immunopositive. The ependymal layer turns out to be immunonegative, whereas evident radial astrocytes have their cell bodies in the periependymal zone. A few GFAP-positive star-shaped astrocytes are found in the lateral column of the posterior intumescence (an enlargement of the spinal cord at the level of hind limbs). Anti-vimentin immunohistochemistry reveals no positive glial structures in the CNS of *T. sinensis*.

In the turtle, GFAP immunostaining shows a certain degree of heterogeneity significantly greater than what appears in dipnoans (Lazzari and Franceschini 2004) and urodeles (Lazzari et al. 1997). The glial pattern of turtle is simpler than the condition found in lizards (Lazzari and Franceschini 2001, 2005a, b), but clearly less

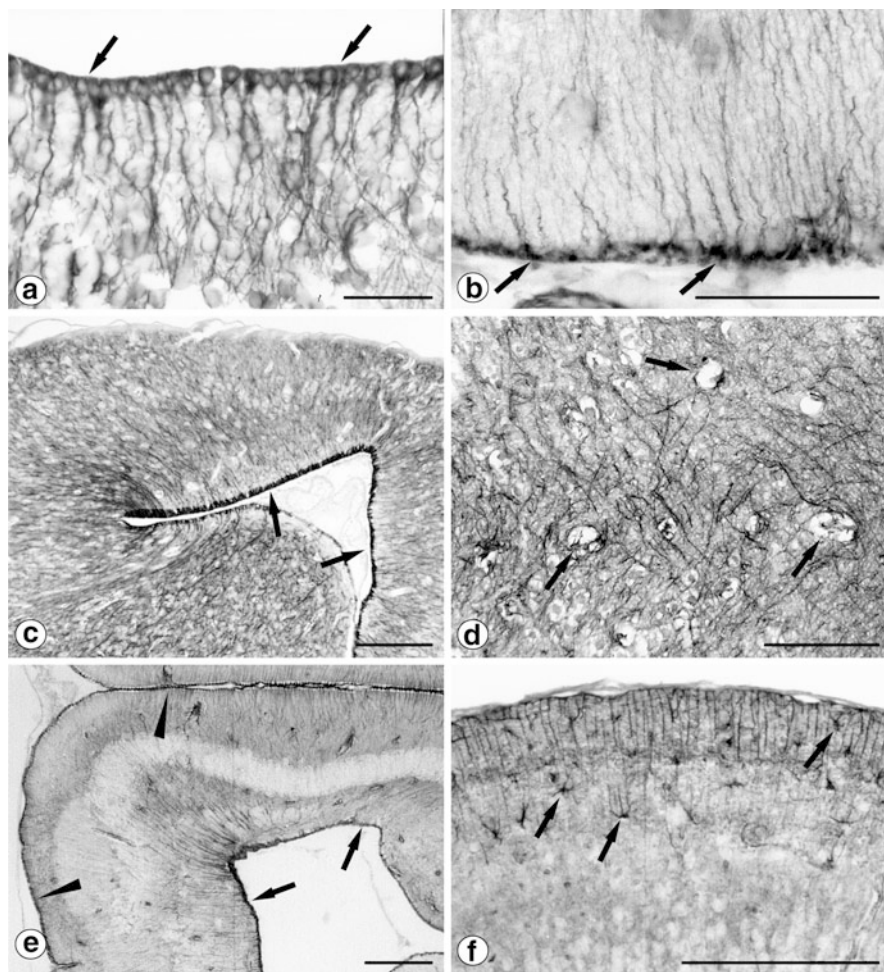


Fig. 15.1 GFAP-immunopositive structures in the brain of tetrapods. (a) In *Ambystoma mexicanum* telencephalon, radially oriented processes originate from ependymal radial glia (tanycytes) (arrows). (b) Radially oriented glial processes reach the submeningeal surface of *A. mexicanum* telencephalon where enlarged endfeet form (arrows). (c) In *Trionyx sinensis* telencephalon, tanycytes (arrows) mark the ventricular surface. Radial processes go through the neural wall. (d) In *T. sinensis* brain, glial processes give rise to perivascular glial covering (arrows). (e) In *Podarcis sicula* telencephalon, tanycytes (arrows) line the ventricular surface; glial endfeet (arrowheads) mark out the meningeal layer. (f) In *P. sicula* optic tectum, some star-shaped astrocytes (arrows) are intermingled with radial glial processes reaching the meningeal surface. Bars: 50 μm (a, b, d), 100 μm (e, f), 200 μm (c)

complex than in birds (Kalman et al. 1993a, 1998) and mammals (Elmqvist et al. 1994). According to Kalman and Pritz (2001), star-shaped astrocytes, prevailing in birds and mammalian CNS, are derived cells compared to radial glia. They give rise to a glial network which permits an increase of the neural wall thickness and

addresses the needs of the regional neuronal environment (Kalman and Pritz 2001). Star-shaped astrocytes inside a predominant radial glial model may indicate the local, more complex nervous areas (Simard et al. 2003; Mulligan and MacVicar 2004; Benediktsson et al. 2005; Volterra and Meldolesi 2005). In the adult turtle brain, the radial glial pattern and the absence of star-shaped astrocytes may represent an ancient character, shared with the stem reptile and present at early developmental stages in the mammalian brain (Clinton et al. 2014).

A more recent study on the Emydidae *Trachemys scripta elegans* (Clinton et al. 2014) has shown that radial glia are neuronal precursors in the turtle telencephalon. These results show that in the adult, radial glia retain differentiating capabilities that are similar to the activity they carry out during development.

15.1.4 Lizards

The glial pattern of lizards has been studied in different species, *Gallotia galloti* (Lacertidae, Monzon-Mayor et al. 1990; Yanes et al. 1990), *P. sicula* (Lacertidae, Lazzari and Franceschini 2001), *Agama impalearis* (Agamidae), *Eumeces algeriensis* (Scincidae), *Tarentola mauritanica* (Gekkonidae, Ahboucha et al. 2003), *Anolis sagrei* (Polychrotidae, Lazzari and Franceschini 2005a), and *Eublepharis macularius* (Eublepharidae, Lazzari and Franceschini 2005b; McDonald and Vickaryous 2018).

In adult lizards, GFAP-immunocytochemistry demonstrates the presence of three different astroglial lineage cell type: (1) ependymal radial glia or tanocytes throughout the brain; (2) proper radial glia or radial astrocytes, in the spinal cord with their cell bodies displaced from the ependymal layer to a periependymal zone; and (3) star-shaped astrocytes in the optic tectum and spinal cord (Fig. 15.1e, f).

In the spinal cord, ependymal cells are clearly vimentin-immunopositive and GFAP-negative. Only in the brain can a few vimentin-positive radial glial fibers be observed (Monzon-Mayor et al. 1990; Lazzari and Franceschini 2001, 2005b). During development, vimentin-GFAP transition has also been observed in reptiles (Monzon-Mayor et al. 1990; Yanes et al. 1990).

The presence and arrangement of star-shaped astrocytes (true astrocytes) is another point that deserves consideration in the study of saurian glia. The glial pattern containing star-shaped astrocytes is considered more complex and a phylogenetically derived state compared to the model with only radial elements. For these reasons, the glial organization of lizards may be considered a derived character compared to that of turtles.

In lizard, the intensity of the GFAP immunostaining of glial cells turned out to be not uniform throughout the neural wall. This condition recalls the GFAP-staining heterogeneity found in turtle (Kalman et al. 1994; Lazzari and Franceschini 2006).

According to Kalman and Pritz (2001), uneven distribution and different size of the astroglial structure are the main causes of the differences in regional GFAP

immunostaining of saurian brain. These authors consider differential GFAP expression less important in reptile than in birds and mammals.

Based on GFAP abundance and vimentin scarceness, the immunohistochemical reaction of adult lizard astroglial intermediate filaments is indicative of mature astroglial cell lineage. In contrast, the glial pattern of the lizard mostly based on radial elements is morphologically more immature than those found in crocodile, birds, and mammals (Kalman et al. 1998; Kalman and Pritz 2001).

However, the connections between GFAP/vimentin and radial glia/star-shaped astrocytes during CNS evolution and development are not fully clear. In lizards belonging to different families, morphology of GFAP-positive cells and their relative proportions exhibit remarkable differences (Ahboucha et al. 2003). Additional studies on the characteristic of the vertebrate astroglial elements are necessary to provide a comprehensive response.

In a recent study on the neurogenesis in the medial cortex of *E. macularius*, immunohistochemistry identified proliferating pools of neural stem/progenitor cells within the telencephalon (McDonald and Vickaryous 2018). These cells, which express GFAP and vimentin and show a clear radial morphology, are identified as the radial glia involved in neurogenesis in adult.

15.1.5 *Crocodylians*

GFAP-immunopositive structures were studied in the brain of caiman, *Caiman crocodilus*, as representative of the order Crocodylia (Kalman and Pritz 2001). The caiman astroglia were clearly GFAP-immunopositive, and similar to other reptilians, the predominant elements were radial astrocytes. The glial architecture of crocodylian brain is characterized by a regional variability that is much greater than in the other reptiles studied so far, but smaller than in birds. Kalman and Pritz (2001) suggested that this condition is indicative of a more regional specialization of the glial cytoarchitecture in caiman compared to the other reptiles. The preeminent characteristic of caiman glial system compared to the other reptiles is represented by the widespread distribution of GFAP-immunopositive star-shaped astrocytes that are absent in turtles and scarce in lizards and snakes. Kalman and Pritz (2001) pointed out that, also in caiman, star-shaped astrocytes appear mixed together with radial fiber elements and do not represent the prevailing glial elements throughout the brain. Moreover, their presence appears not related to the brain wall thickness. Although Crocodylians represent the extant reptilian group most closely related to birds, Kalman and Pritz (2001) documented that the features of the caiman glial system are only partially shared by birds and mammals. This indicates the independent, parallel evolution from the diapsids or synapsids, different ancestral groups of stem reptiles.

15.1.6 *Birds*

The immunohistochemical detection of GFAP and vimentin was studied in the domestic chicken (*Gallus domesticus*) from hatching to adulthood (Kalman et al. 1993a, b, 1998). In the telencephalon, the day-old chicken shows a vimentin-immunopositive radial fiber system. Many of these radial elements persist until adulthood and during development undergo transition from vimentin to GFAP expression.

In the posthatch telencephalon, the distribution of star-shaped astrocytes resembles the condition in the adults, except in the visual center that shows a slow maturation rate. In the diencephalon (the rear region of the forebrain) and in the mesencephalic tegmentum (the ventral part of the mesencephalon), some GFAP-positive astrocytes are present at birth, and their number increases during the first two posthatching weeks. At hatching, these districts of the chicken brain contain only a few positive fibers. Therefore, a gradual and clear substitution of radial fibers by star-shaped astrocytes does not occur. On the contrary, this substitution is evident in mammals (Kalman et al. 1998). Whereas in reptiles the GFAP-positive radial glial system persists in optic tectum throughout life, in chicken it disappears from the tectal superficial layer that appears a derived character. At hatching, numerous GFAP-positive, vimentin-negative astrocytes accompany axons in the cerebral tracts. In posthatching birds, vimentin-positive glia are observed at the midline in decussation (medial areas of the CNS where nervous fibers pass from the right side to the left one and vice versa). Probably, these vimentin-positive elements are engaged in fiber-crossing regulation. In general, disappearance of radial fibers, dominance of astrocytes, and uneven distribution of GFAP-immunopositivity characterize glial development and organization in avian brain. Since these characters are found in phylogenetically distant mammals and only rudimentary outlined in reptiles, Kalman et al. (1998) suggested that they evolved independently along parallel paths.

15.1.7 *Mammals*

In mammals, radial glial cells have almost disappeared in adults. They represent the first developmental stage of astrocytes from which fibrous and protoplasmic astrocytes originate through shortening of their long processes and displacement of their cell body away from the ependymal layer (Levitt and Rakic 1980).

In adult mammals, GFAP is regarded as the principal intermediate filament protein in astrocytes (Voigt 1989), whereas vimentin is found in ependymal cells (Dahl et al. 1981; Bignami et al. 1982).

During development, vimentin predominates in early radial glia; afterward, this vimentin-like immunoreactivity decreases as radial cells mature into astrocytes (Oudega and Marani 1991; Elmquist et al. 1994; Moore and Oglesbee 2015; Luo

et al. 2017). Therefore, in mammals, vimentin-immunoreactivity can be interpreted as marker of immature astrocytic cells and GFAP-immunoreactivity as marker of mature astrocytes. As the brain matures, radial glia become true astrocytes, and the change in intermediate filament protein expression from vimentin to GFAP may parallel this morphological transformation (Schnitzer et al. 1981; Elmquist et al. 1994). Embryonic astroglia of higher vertebrates resemble astrocytic cells of adult lower vertebrates.

In the adult mammalian CNS, radial glial cells are restricted to few locations: the retina (Müller cells), the cerebellum (Bergmann glia), and the hypothalamus where they are called tanycytes (Sild and Ruthazer 2011). New information derives from Mack et al. (2018) who investigated the astroglial cells in the cerebral cortex of the lesser hedgehog tenrec, *Echinops telfairi*. These animals belong to the group of Afrotheria representing a clade which is possibly a sister group of all other placental mammals (O'Leary et al. 2013; Averianov and Lopatin 2014). Lesser hedgehog tenrecs are considered to have the lowest encephalization index with relatively little neocortex compared to other placental mammals (Stephan et al. 1991; Krubitzer et al. 1997). The adult lesser hedgehog tenrec brain shows also continued cell proliferation (Alpar et al. 2010), reminiscent of the continued cell addition in anamniotes.

The authors concluded that the radial glia in the adult lesser hedgehog tenrec represents an immature form of astroglia that persists in these animals throughout life. It is noteworthy that radial glial structures occur at locations with continued neurogenesis (e.g., dentate gyrus and the paleocortex). Therefore, it is possible to imagine that the radial glial fibers of the adult are involved in the differentiation of new neurons. Radial glial fibers can lead differentiating neurons to appropriate locations. In adults, this activity is similar to the role of radial glia during development. The coincidence of radial glia and continued growth is found also in the brains of many anamniotes.

15.1.8 *Ensheathing Glial Cells*

GFAP and vimentin, together with other cellular markers, are useful tools also in characterizing olfactory ensheathing cells (OECs) that represent a specific type of glial cells (Pellitteri et al. 2010). Throughout life, the regeneration ability of olfactory sensory neurons (OSNs) is connected to OECs that cover olfactory axons in their route from the basal lamina of the olfactory epithelium to the glomeruli in the olfactory bulb (Pellitteri et al. 2010). In recent years, growing attention was given to OECs as they appeared able to promote and support regeneration of injured CNS (Raisman 2001; Barnett and Riddell 2004; Chung et al. 2004). In particular, studies underlined their potential role in cell-based therapy for spinal cord injury repair (Lu et al. 2002; Mackay-Sim 2005).

Recent studies are focused on OECs of anamniotes (Lazzari et al. 2013, 2014, 2016). A comparative immunohistochemical study on microsmatic (*Poecilia*

reticulata) and macrosmatic fish (*Carassius auratus*, *Danio rerio*) showed some differences between the two types of olfactory organization and some similarities between fish (anamniotes) and mammals (amniotes) OSNs (Lazzari et al. 2007, 2013, 2014; Bettini et al. 2009). *D. rerio* is a good model also in olfaction-related research. In fact, its olfactory system is histologically comparable to that of other vertebrates, and the cells of its olfactory neuroepithelium can be clearly characterized by immunological approaches (Germanà et al. 2007; Gayoso et al. 2011; Braubach et al. 2012; Lazzari et al. 2017, 2019). The immunohistochemical characterization of OECs was also studied in *A. mexicanum* (Lazzari et al. 2016). Urodeles have a relatively simple olfactory system and represent a good model for studying OECs in extant representatives of basal tetrapods. The olfactory pathways of *A. mexicanum*, representative of basal terrestrial anamniotes, share characteristics with both fish (aquatic anamniotes) and mammals (amniotes). It remains speculative whether these similarities are basal features conserved during evolution or evolved independently in different vertebrate groups. So far, the functional role of the regional and interspecific differences displayed by OEC immunolocalization remains unknown.

15.2 Olfactory Crypt Neurons

In addition to ciliated (cOSNs) and microvillous (mOSNs) neurons, the olfactory organ of fish is peculiarly composed of a third sensory cell type, called crypt cell. The crypt cells were observed for the first time in the olfactory neuroepithelium of zebrafish, *Danio rerio* (Hansen and Zeiske 1998). The cell body of the crypt cell is egg-shaped, and it is located in the upper layer of the epithelium. The most striking feature of the crypt cell is an invagination, called the “crypt,” located in the apical part of the neuron. The border of the crypt is surrounded by microvilli, while short cilia grow in its lumen. The large nucleus of the cell resides in the lower third of the soma. A thin axon emerges from the basal portion of the neuron. The last histological characteristic of crypt cells is that they are surrounded by specialized supporting cells which are particularly electron-lucent (Fig. 15.2). Although crypt cells are randomly distributed in all the lamellae of the olfactory organ, their number is lower than that of ciliated and microvillous cells.

15.2.1 *Crypt Cell Phyletic Distribution and Evolutionary Considerations*

Since their first description, crypt neurons have been observed in many actinopterygian species (Hansen et al. 2003; Hamdani and Døving 2006; Lazzari et al. 2007; Camacho et al. 2010), including freshwater and marine fish (see Hansen

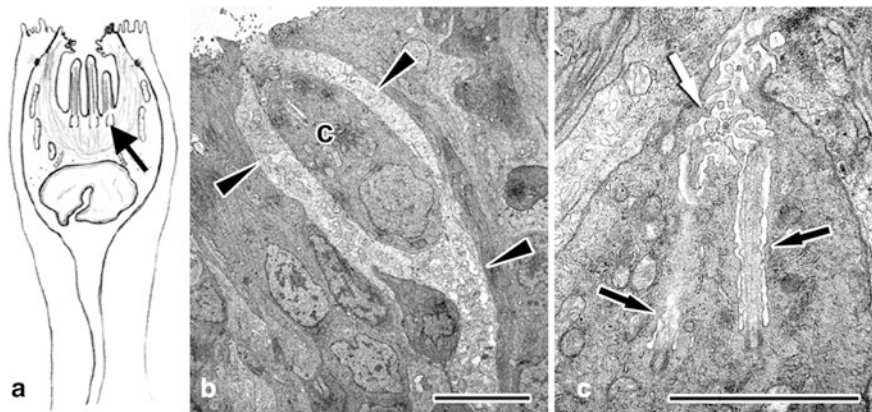


Fig. 15.2 Crypt cell morphology. (a) Schematic drawing of the crypt cell (modified from Camacho et al. 2010); cilia reside in the apical invagination, called “crypt” (arrow). (b) An ovoid crypt cell surrounded by specific electron lucent sustentacular cells (arrowheads) in the olfactory epithelium of *P. reticulata* (modified from Lazzari et al. 2007). (c) Magnification of the crypt showing cilia (black arrows) and microvilli (white arrow). Bars: 5 μm (b), 2 μm (c)

and Finger 2000 and Hansen and Zielinski 2005 for a review of species examined, and crypt cell distribution). Moreover, crypt cells were discovered in Chondrichthyes, both in sharks (*Scyliorhinus canicula*, Ferrando et al. 2006) and batoids (*Raja clavata*, Ferrando et al. 2007). Interestingly, no evidence exists about the presence of this cell type in lungfish, which suggests that the crypt neurons are ancestral traits evolved before actinopterygian radiation, but lost at the actinopterygian-sarcopterygian transition.

The evolution of air breathing in vertebrates could be a possible event that influenced the disappearance of this cells in tetrapods. Ultrastructural, histological, and ontogenetic studies on the olfactory organ of amphibians, the tetrapods most closely related to lungfish, did not reveal crypt cells in either terrestrial or aquatic adult species (see Hansen and Finger 2000 for a list of publications). The developmental analysis from early larval stage to post-metamorphic juveniles in different species of anurans (Benzekri and Reiss 2012; Jungblut et al. 2009, 2017; Quinzio and Reiss 2018) and urodeles (Franceschini et al. 2003; Saito et al. 2003; Nakada et al. 2014) with gill-bearing larvae did not report the presence of crypt cells. The olfactory system of the anuran model system *Xenopus laevis*, has two cavities, one of which is devoted to aquatic olfaction and the other to aerial olfaction, but no crypt cells have been detected in any of them (Hansen et al. 1998; Oikawa et al. 1998).

The most characteristic anatomical difference in the olfactory system between fish and amphibians is the presence in the latter of a vomeronasal system. The vomeronasal system is an accessory olfactory system possessed by most tetrapods, except birds, aquatic reptiles, aquatic mammals, and higher primates (including man) in which it is vestigial or lost (Bertmar 1981). It is composed by a vomeronasal organ (VNO), containing only mOSNs (while the main olfactory organ is exclusively

composed by cOSNs) projecting to an accessory olfactory bulb (AOB) in the rostral telencephalon (D’Aniello et al. 2017). Interestingly, a recent study (Gonzales et al. 2010) shows that the African lungfish *Protopterus dolloi* possesses, at the base of the olfactory lamellae, the epithelial crypts expressing markers of vomeronasal receptors. Cells localized in the epithelial crypts project their axons to an AOB identified in the lateral olfactory bulb, revealed also by lectin binding in a previous work (Franceschini et al. 2000). During evolution from lungfish to tetrapods, the formation of a vomeronasal organ was accompanied by a redistribution of morphological olfactory cells subtypes, with cOSNs segregated to the main olfactory organ and mOSNs to the vomeronasal organ, and crypt cells disappeared. Possible causes of crypt cell disappearance may reside in the function of the newly developed/evolving vomeronasal organ.

15.2.2 *Crypt Cells Have Similarities with the Vomeronasal Organ of Tetrapods*

Crypt cells seem to be ancestral olfactory neurons, which became replaced by microvillous cells of the vomeronasal organ during the evolutionary divergence of tetrapods (Fig. 15.3). This shift was possible probably because these two cell types show common features. In fact in *D. rerio* (Oka et al. 2012), the crypt neurons express only the G α i-coupled receptor gene *ora4* belonging to the Vomeronasal-1-Receptor (V1R)-like family.

Moreover, in sexually mature *Oncorhynchus mykiss*, crypt cells preferentially respond to gonadal extract and pheromones from the opposite sex (Bazáes and Schmachtenberg 2012), and the vomeronasal organ is presumed to detect pheromones (Halpern and Martinez-Marcos 2003). However, the vomeronasal organ can respond to a variety of other odorants with different behavioral significance in all groups of tetrapods. Recently, zebrafish crypt cells were proposed to be involved in detecting kin odor-related signal (Biechl et al. 2016). However, which chemical compounds are detected by crypt cells remain elusive because of different results obtained in various species (Vielma et al. 2008).

Important clues supporting an evolutionary relationship between crypt cells and the vomeronasal organ come from their pathways to glomerular targets in the central nervous system.

In crucian carp (Hamdani and Døving 2006) and channel catfish (Hansen et al. 2003), olfactory bulb backtracing experiments suggested a medioventral position of the crypt neuron target region where a population of secondary neurons specific for sex pheromones are located (Lastein et al. 2006). Medial glomeruli in the bulb are connected with the medial olfactory tract, mediating reproductive behavior. Interestingly, in zebrafish, axons of crypt cells project in one single glomerulus within the mediodorsal area of the olfactory bulb (Ahuja et al. 2013), a region connected with medial amygdala (Biechl et al. 2017) that in tetrapods receives input from the

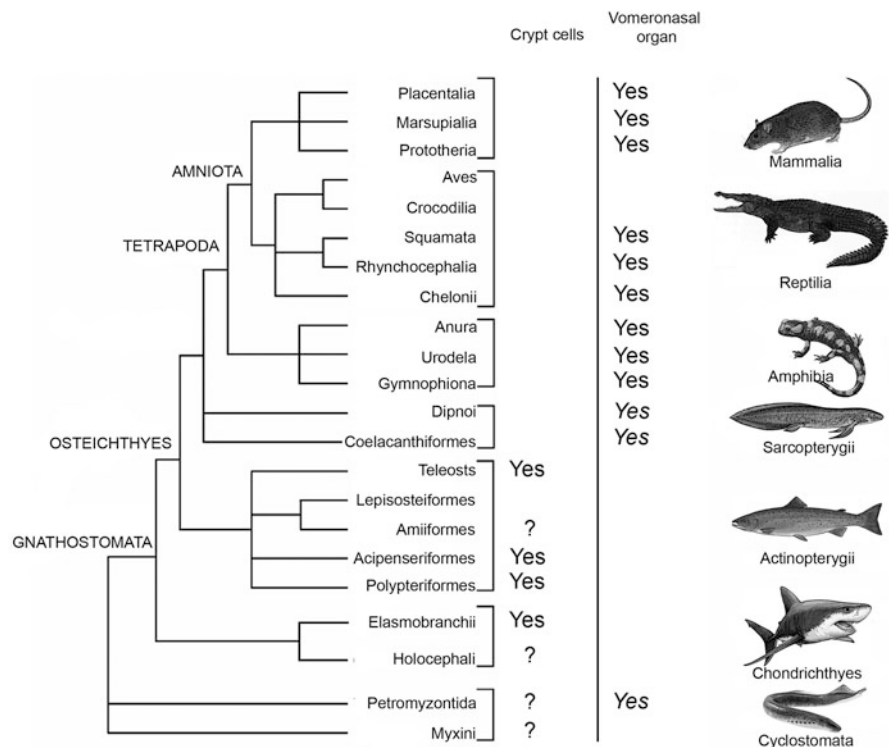


Fig. 15.3 Cladogram reporting the presence of crypt cells and vomeronasal organ in the main vertebrate groups. “Yes”: the presence has been confirmed in at least one species; “Yes”: an accessory olfactory system with segregated projections has been observed in different studies, but the organ is unique; “?”: the group has never been examined

vomeronasal organ. This finding supports the hypothesis that a primordial accessory olfactory system, which originates in crypt olfactory sensory cells, may exist in teleosts.

All together, these data reinforce the hypothesis that the crypt cells may be related to the reproductive behavior and, in general, similarly to the vomeronasal organ, to the conspecific recognition (Liu et al. 2014).

15.2.3 Developmental Studies Give Useful Information in Determining a Possible Role of Crypt Cells

Camacho et al. (2010) proposed a sequence of stages in crypt cell development, characterized by morphological profiles observed with TEM: (1) at first, immature cells appear close to the epithelial basal lamina; (2) then they differentiate into cells similar to the crypt neurons (absence of knob, envelopment by supporting cells, etc.)

but with cilia still remaining on their non-invaginated apical surface; and (3) the formation of the crypt would be the final step of their differentiation.

In vertebrates, the olfactory epithelium exhibits neurogenesis throughout the life of the animal, from the embryonic/juvenile development to the adulthood. This continuous turnover is required to restore an intact epithelium after chemical or physical injuries. However, the growth dynamics of the crypt cell population is different than cOSNs and mOSNs. In *Acipenser naccarii* (Camacho et al. 2010) and zebrafish (Sandulescu et al. 2011), the crypt cell differentiation occurs in the early days of embryonic life, but later than differentiation of cOSNs and mOSNs. Hamdani et al. (2008) demonstrated that the number of crypt cells in the olfactory epithelium of the adult crucian carp *Carassius carassius* varies dramatically throughout the year, with seasonal regression in winter and renewal during the summer spawning months. Interestingly, in *Poecilia reticulata*, a species with a year-round breeding season (Houde 1997), crypt cell density remains invariant after 90 days of life, and it is different between males and females (Bettini et al. 2012). Moreover, crypt cells reach their adult density, through sex-specific dynamics, at the end of gonad development. These findings may suggest that crypt neurons are involved in mediating behavior related to reproduction and confirm their possible sensitivity to sex pheromones. However, the onset of crypt cell differentiation occurs very early (Bettini et al. 2012; Camacho et al. 2010; Ferrando et al. 2007; Sandulescu et al. 2011) so they might be involved also in the nonreproductive functions, perhaps in association with cOSNs and mOSNs.

15.2.4 *Crypt Cell Markers*

In all fish examined in the literature, crypt cell morphology has been fully described by TEM studies, and in various species (e.g., channel catfish, crucian carp, sturgeon, zebrafish), they have been visualized also by retrograde transport of fluorescent dye (Hansen et al. 2003; Hamdani and Døving 2006; Camacho et al. 2010; Ahuja et al. 2013). The use of specific immunohistochemical staining in the olfactory system is important for numerous fields of research, for example, environmental studies on the differential effects of pollutants on olfactory neuronal populations (Bettini et al. 2016; Lazzari et al. 2017, 2019). However, only in the zebrafish model antigenic properties of crypt neurons have been studied.

15.2.5 *The Zebrafish Model*

Germanà et al. (2004) reported that crypt neurons in zebrafish olfactory lamellae are specifically immunostained by antiserum against S100, a neural calcium-binding protein that stimulates cell proliferation and migration and inhibits apoptosis and differentiation (Donato et al. 2013). Thus, the anti-S100 serum was used in several

subsequent studies (Gayoso et al. 2011, 2012; Sato et al. 2005). However, in the last decade, some studies (Ahuja et al. 2013; Kress et al. 2015; Oka et al. 2012) showed that the specificity of anti-S100 varies according to tissue processing: in fresh-frozen sections, the cells with crypt-neuron morphology appeared specifically stained, while, in fixed conditions, other cell types, possibly microvillous neurons, were also stained. Moreover, while anti-S100-positive microvillous cells express the *s100z* gene, anti-S100-positive crypt cells do not express *S100* genes, raising some doubts on which crypt cell antigen is recognized by the antiserum. In addition, the dorsomedial glomerular field of zebrafish olfactory bulb receives afferents from the crypt cells, as revealed by DiI retrolabeling (Gayoso et al. 2012), but their axons are S100-negative (Gayoso et al. 2011). This uncertainty precludes the use of S100-like immunoreactivity in various studies and procedures, including visualization of axonal terminal regions.

The first molecular characterization of crypt cells was made by Catania et al. (2003), who discovered that anti-TrkA, specific for a neurotrophin receptor, labeled only zebrafish crypt cells in the olfactory epithelium, but this antibody was not investigated further at the time. Ten years later, Ahuja et al. (2013) demonstrated that anti-TrkA-immunohistochemistry constitutes a robust and sensitive marker for zebrafish crypt cells, and nowadays, it is the only marker with such specificity.

15.2.6 What About Other Fish?

In scientific research, the use of only one animal model (zebrafish, in this case) allows direct comparisons of data, and this facilitates the determination of cause and effect relationship and the formulation of hypothesis. On the other hand, the characteristics described in the model could not be extended to the majority of other species. For this reason, recently, for the first time, the antigenic properties of crypt cells in another fish, *P. reticulata* (guppy) was examined (Bettini et al. 2017). *P. reticulata* possesses some anatomical and histological characteristics of the olfactory organ different from zebrafish (Lazzari et al. 2007). Crypt cell markers in the guppy appeared to overlap only partially with zebrafish markers (Fig. 15.4). While no olfactory neuron was labeled by anti-TrkA, the anti-S100 immunohistochemistry gave a pattern comparable to what has been reported in zebrafish. In addition, in the guppy, the calretinin, a known marker of zebrafish cOSNs and mOSNs (Germanà et al. 2007), was expressed also by some S100-positive crypt neurons. However, other anti-S100-labeled crypt cells remained immunonegative. This revealed two distinct subpopulations of the same morphotype recognizable for their protein content (calretinin-positive and calretinin-negative crypt neurons). It is possible that this heterogeneity in protein expression also reflects some functional differences. All that considered, this recent study revealed that the molecular features of olfactory crypt neurons described in the fish model *D. rerio* cannot be extended to all teleosts, and the investigation of other phyletic taxa is necessary to provide more conclusive answers on the evolution of olfactory crypt cells in fish.

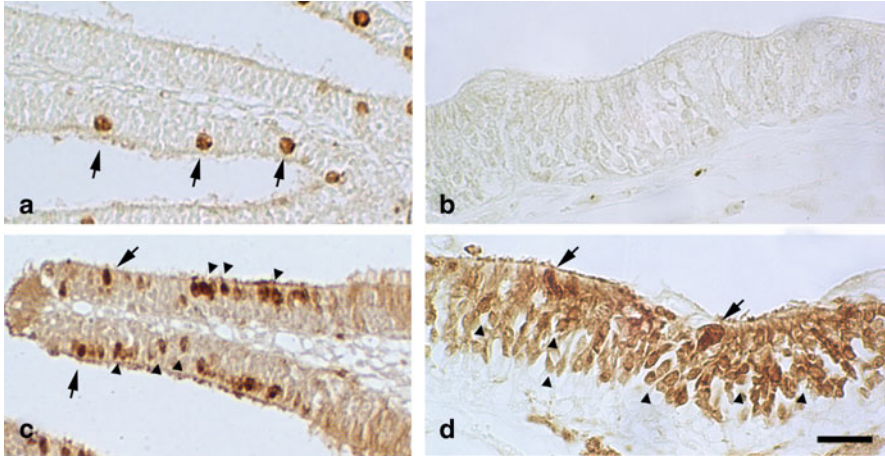


Fig. 15.4 Comparison between TrkA (a–b) and S100 (c–d) immunohistochemistry in *D. rerio* (a, c) and *P. reticulata* (b, d). TrkA is a specific marker for crypt cell (arrows) in zebrafish but not in *P. reticulata*; S100-immunohistochemistry stains crypt cells (arrows) and microvillous cells with short dendrites in the medial layer of the epithelium (arrowheads) in both species. Bar: 20 μ m

References

- Ahboucha S, Laalaoui A, Didier-Bazes M, Montange M, Cooper HM, Gamrani H (2003) Differential patterns of glial fibrillary acidic protein-immunolabeling in the brain of adult lizards. *J Comp Neurol* 464:159–171
- Ahuja G, Ivandic I, Saltürk M, Oka Y, Nadler W, Korsching SI (2013) Zebrafish crypt neurons project to a single, identified mediodorsal glomerulus. *Sci Rep* 3:2063–2071
- Alpar A, Künzle H, Gärtner U, Popkova Y, Bauer U, Grosche J, Reichenbach A, Härtig W (2010) Slow age-dependent decline of doublecortin expression and BrdU labeling in the forebrain from lesser hedgehog tenrecs. *Brain Res* 1330:9–19
- Amemiya CT, Alfoldi J, Lee AP, Fan S, Philippe H et al (2013) The African coelacanth genome provides insights into tetrapod evolution. *Nature* 496:311–316
- Averianov AO, Lopatin AV (2014) High-level systematics of placental mammals: current status of the problem. *Biol Bull* 41:801–816
- Bar I, Lambert de Rouvroit C, Goffinet AM (2000) The evolution of cortical development. An hypothesis based on the role of the Reelin signaling pathway. *Trends Neurosci* 23:633–638
- Barnett SC, Riddell JS (2004) Olfactory ensheathing cells (OECs) and the treatment of CNS injury: advantages and possible caveats. *J Anat* 204:57–67
- Bazáas A, Schmachtenberg O (2012) Odorant tuning of olfactory crypt cells from juvenile and adult rainbow trout. *J Exp Biol* 215:1740–1748
- Benediktsson AM, Schachtele SJ, Green SH, Dailey ME (2005) Ballistic labeling and dynamic imaging of astrocytes in organotypic hippocampal slice cultures. *J Neurosci Methods* 141:41–53
- Benzekri NA, Reiss JO (2012) Olfactory metamorphosis in the coastal tailed frog *Ascaphus truei* (Amphibia, Anura, Leiopelmatidae). *J Morphol* 273:68–87
- Bertmar G (1981) Evolution of vomeronasal organs in vertebrates. *Evolution* 35:359–366
- Bettini S, Lazzari M, Ciani F, Franceschini V (2009) Immunohistochemical and histochemical characteristics of the olfactory system of the guppy, *Poecilia reticulata* (Teleostei, Poeciliidae). *Anat Rec* 292:1569–1576

- Bettini S, Lazzari M, Franceschini V (2012) Quantitative analysis of crypt cell population during postnatal development of the olfactory organ of the guppy, *Poecilia reticulata* (Teleostei, Poeciliidae), from birth to sexual maturity. *J Exp Biol* 215:2711–2715
- Bettini S, Lazzari M, Ferrando S, Gallus L, Franceschini V (2016) Histopathological analysis of the olfactory epithelium of zebrafish (*Danio rerio*) exposed to sublethal doses of urea. *J Anat* 228:59–69
- Bettini S, Milani L, Lazzari M, Maurizii MG, Franceschini V (2017) Crypt cell markers in the olfactory organ of *Poecilia reticulata*: analysis and comparison with the fish model *Danio rerio*. *Brain Struct Funct* 222:3063–3074
- Biechl D, Tietje K, Gabriele Gerlach G, Wullmann MF (2016) Crypt cells are involved in kin recognition in larval zebrafish. *Sci Rep* 6:24590
- Biechl D, Tietje K, Ryu S, Grothe B, Gerlach G, Wullmann MF (2017) Identification of accessory olfactory system and medial amygdala in the zebrafish. *Sci Rep* 7:44295
- Bignami A, Raju T, Dahl D (1982) Localization of vimentin, the non-specific intermediate filament protein, in embryonal glia and in early differentiating neurons. *Dev Biol* 91:286–295
- Biscotti MA, Gerdol M, Canapa A, Forconi M, Olmo E, Pallavicini A, Barucca M, Scharl M (2016) The lungfish transcriptome: a glimpse into molecular evolution events at the transition from water to land. *Sci Rep* 6:21571
- Braubach OR, Fine A, Croll RP (2012) Distribution and functional organization of glomeruli in the olfactory bulbs of zebrafish (*Danio rerio*). *J Comp Neurol* 520:2317–2339
- Camacho S, Ostos-Garrido MV, Domezain A, Carmona R (2010) Study of the olfactory epithelium in the developing sturgeon. Characterization of the crypt cells. *Chem Senses* 35:147–156
- Campbell K, Götz M (2002) Radial glia: multi-purpose cells for vertebrate brain development. *Trends Neurosci* 25:235–238
- Cardone B, Roots BJ (1990) Comparative immunohistochemical study of glial filament proteins (glial fibrillary acidic protein and vimentin) in goldfish, octopus and snail. *Glia* 3:180–192
- Catania S, Germanà A, Laurà R, Gonzalez-Martinez T, Ciriaco E, Vega JA (2003) The crypt neurons in the olfactory epithelium of the adult zebrafish express TrkA-like immunoreactivity. *Neurosci Lett* 350:5–8
- Chung RS, Woodhouse A, Fung S, Dickson TC, West AK, Vickers JC, Chuah MI (2004) Olfactory ensheathing cells promote neurite sprouting of injured axons in vitro by direct cellular contact and secretion of soluble factors. *Cell Mol Life Sci* 61:1238–1245
- Clinton BK, Cunningham CL, Kriegstein AR, Noctor SC, Martínez-Cerdeño V (2014) Radial glia in the proliferative ventricular zone of the embryonic and adult turtle, *Trachemys scripta elegans*. *Neurogenesis (Austin)* 1(1):e970905
- D’Aniello B, Semin GR, Scandurra A, Pinelli C (2017) The vomeronasal organ: a neglected organ. *Front Neuroanat* 11:70
- Dahl D, Bignami A (1985) Intermediate filaments in nervous tissue. In: Shay JW (ed) *Cell and muscle motility*, vol 6. Plenum Press, New York, pp 75–96
- Dahl D, Rueger DC, Bignami A, Weber K, Osborn M (1981) Vimentin, the 57,000 molecular weight protein in fibroblast filaments in the major cytoskeletal protein in immature glia. *Eur J Cell Biol* 24:191–196
- Donato R, Cannon BR, Sorci G, Riuizzi F, Hsu K et al (2013) Functions of S100 proteins. *Curr Mol Med* 13:24–57
- Elmqvist JK, Swanson JJ, Sakaguchi DS, Ross LR, Jacobson CD (1994) Developmental distribution of GFAP and vimentin in the Brazilian opossum brain. *J Comp Neurol* 344:283–296
- Eng LF (1985) Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filament in differentiated astrocytes. *J Neuroimmunol* 8:203–214
- Eng LF, Ghirnikar RS, Lee YL (2000) Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). *Neurochem Res* 25:1439–1451
- Ferrando S, Bottaro M, Gallus L, Girosi L, Vacchi M, Tagliaferro G (2006) Observations of crypt neuron-like cells in the olfactory epithelium of a cartilaginous fish. *Neurosci Lett* 403:280–282
- Ferrando S, Bottaro M, Pedemonte F, De Lorenzo S, Gallus L, Tagliaferro G (2007) Appearance of crypt neurons in the olfactory epithelium of the skate *Raja clavata* during development. *Anat Rec* 290:1268–1272

- Forey PL (1986) Relationship of lungfishes. *J Morphol Suppl* 1:75–91
- Franceschini V, Lazzari M, Ciani F (2000) Cell surface glycoconjugates in the olfactory system of lungfish *Protopterus annectens*. *Acta Zool* 81:131–137
- Franceschini V, Lazzari M, Ciani F (2003) Surface glycoconjugates in the olfactory system of *Ambystoma mexicanum*. *J Morphol* 256:301–305
- Gans C, Parsons T (1981) *Biology of the reptilia: morphology*. Academic Press, New York
- Gayoso JÁ, Castro A, Anadón R, Manso MJ (2011) Differential bulbar and extrabulbar projections of diverse olfactory receptor neuron populations in the adult zebrafish (*Danio rerio*). *J Comp Neurol* 519:247–276
- Gayoso JÁ, Castro A, Anadón R, Manso MJ (2012) Crypt cells of the zebrafish *Danio rerio* mainly project to the dorsomedial glomerular field of the olfactory bulb. *Chem Senses* 37:357–369
- Germanà A, Montalbano G, Laurà R, Ciriaco E, del Valle ME, Vega JA (2004) S100 protein-like immunoreactivity in the crypt olfactory neurons of the adult zebrafish. *Neurosci Lett* 371:196–198
- Germanà A, Paruta S, Germanà GP, Ochoa-Erena FJ, Montalbano G, Vega JA (2007) Differential distribution of S100 protein and calretinin in mechanosensory and chemosensory cells of adult zebrafish (*Danio rerio*). *Brain Res* 1162:48–55
- González A, Morona R, López JM, Moreno N, Northcutt RG (2010) Lungfishes, like tetrapods, possess a vomeronasal system. *Front Neuroanat* 4:1–11
- Götz M, Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* 6:777–788
- Halpern M, Martínez-Marcos A (2003) Structure and function of the vomeronasal system: an update. *Prog Neurobiol* 70:245–318
- Hamdani H, Døving KB (2006) Specific projection of the sensory crypt cells in the olfactory system in crucian carp, *Carassius carassius*. *Chem Senses* 31:63–67
- Hamdani H, Lastein S, Gregersen F, Døving KB (2008) Seasonal variations in olfactory sensory neurons—fish sensitivity to sex pheromones explained? *Chem Senses* 33:119–123
- Hansen A, Finger TE (2000) Phyletic distribution of crypt-type olfactory receptor neurons in fishes. *Brain Behav Evol* 55:100–110
- Hansen A, Zeiske E (1998) The peripheral olfactory organ of the zebrafish, *Danio rerio*: an ultrastructural study. *Chem Senses* 23:39–48
- Hansen A, Zielinski BS (2005) Diversity in the olfactory epithelium of bony fishes: development, lamellar arrangement, sensory neuron cell types and transduction components. *J Neurocytol* 34:183–208
- Hansen A, Reiss JO, Gentry CL, Burd GD (1998) Ultrastructure of the olfactory organ in the clawed frog, *Xenopus laevis*, during larval development and metamorphosis. *J Comp Neurol* 398:273–288
- Hansen A, Rolen SH, Anderson K, Morita Y, Caprio J, Finger TE (2003) Correlation between olfactory receptor cell type and function in the channel catfish. *J Neurosci* 23:9328–9339
- Hatten ME (1999) Central nervous system neuronal migration. *Annu Rev Neurosci* 22:511–539
- Herrick JC (1948) *The brain of the tiger salamander, Ambystoma tigrinum*. Chicago University Press, Chicago
- Houde AE (1997) Reproductive biology and sexual behavior. In: Krebs JR, Clutton-Brock T (eds) *Sex, color and mate choice in guppies* (Monographs in behavior and ecology). Princeton University Press, Princeton
- Jungblut LD, Paz DA, López-Costa JJ, Pozzi AG (2009) Heterogeneous distribution of G protein alpha subunits in the main olfactory and vomeronasal systems of *Rhinella (Bufo) arenarum* tadpoles. *Zool Sci* 26:722–728
- Jungblut LD, Reiss JO, Paz DA, Pozzi AG (2017) Quantitative comparative analysis of the nasal chemosensory organs of anurans during larval development and metamorphosis highlights the relative importance of chemosensory subsystems in the group. *J Morphol* 278:1208–1219
- Kalman M (2002) GFAP expression withdraws—a trend of glial evolution. *Brain Res Bull* 57 (3/4):509–511

- Kalman M, Pritz MB (2001) Glial fibrillary acidic protein-immunopositive structures in the brain of a crocodylian, *Caiman crocodilus*, and its bearing on the evolution of astroglia. *J Comp Neurol* 431:460–480
- Kalman M, Szekely A, Csillag A (1993a) Distribution of glial fibrillary acidic protein-immunoreactive structures in the brain of the domestic chicken (*Gallus domesticus*). *J Comp Neurol* 330:221–237
- Kalman M, Szekely A, Csillag A (1993b) GFAP and vimentin in the developing chicken brain. *Anat Anz (Suppl)* 175:130–131
- Kalman M, Kiss A, Majorossy K (1994) Distribution of glial fibrillary acidic protein-immunopositive structures in the brain of the red-eared freshwater turtle (*Pseudemys scripta elegans*). *Anat Embryol* 189:421–434
- Kalman M, Martin-Partido G, Hidalgo-Sanchez M, Majorossy K (1997) Distribution of glial fibrillary acidic protein-immunopositive structures in the developing brain of the turtle *Mauremys leprosa*. *Anat Embryol* 196:47–65
- Kalman M, Szekely AD, Csillag A (1998) Distribution of glial fibrillary acidic protein and vimentin-immunopositive elements in the developing chicken brain from hatch to adulthood. *Anat Embryol* 198:213–235
- Kress S, Biechl D, Wullmann MF (2015) Combinatorial analysis of calcium-binding proteins in larval and adult zebrafish primary olfactory system identifies differential olfactory bulb glomerular projection fields. *Brain Struct Funct* 220:1951–1970
- Kriegstein AR, Shen JM, Eshbar N (1986) Monoclonal antibodies to the turtle cortex reveal neuronal subsets, antigenic cross-reactivity with the mammalian neocortex, and forebrain structures sharing a pallial derivation. *J Comp Neurol* 254:330–340
- Krubitzer L, Künzle H, Kaas J (1997) Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J Comp Neurol* 379:399–414
- Lastein S, Hamdani H, Døving KB (2006) Gender distinction in neural discrimination of sex pheromones in the olfactory bulb of crucian carp, *Carassius carassius*. *Chem Senses* 31:69–77
- Lazzari M, Franceschini V (2001) Glial fibrillary acidic protein and vimentin immunoreactivity of astroglial cells in the central nervous system of adult *Podarcis sicula* (Squamata, Lacertidae). *J Anat* 198:67–75
- Lazzari M, Franceschini V (2004) Glial fibrillary acidic protein and vimentin immunoreactivity of astroglial cells in the central nervous system of the African lungfish, *Protopterus annectens* (Dipnoi: Lepidosirenidae). *J Morphol* 262:741–749
- Lazzari M, Franceschini V (2005a) Astroglial cells in the central nervous system of the brown anole lizard, *Anolis sagrei*, revealed by intermediate filament immunohistochemistry. *J Morphol* 265:325–334
- Lazzari M, Franceschini V (2005b) Intermediate filament immunohistochemistry of astroglial cells in the leopard gecko, *Eublepharis macularius*. *Anat Embryol* 210:275–286
- Lazzari M, Franceschini V (2006) Glial cytoarchitecture in the central nervous system of the soft-shell turtle, *Trionyx sinensis*, revealed by intermediate filament immunohistochemistry. *Anat Embryol* 211:497–506
- Lazzari M, Franceschini V, Ciani F (1997) Glial fibrillary acidic protein and vimentin in radial glia of *Ambystoma mexicanum* and *Triturus carnifex*: an immunocytochemical study. *J Brain Res* 38:187–194
- Lazzari M, Bettini S, Ciani F, Franceschini V (2007) Light and transmission electron microscopy study of the peripheral olfactory organ of the guppy, *Poecilia reticulata* (Teleostei, Poeciliidae). *Microsc Res Tech* 70:782–789
- Lazzari M, Bettini S, Franceschini V (2013) Immunocytochemical characterization of olfactory ensheathing cells in fish. *Brain Struct Funct* 218:539–549
- Lazzari M, Bettini S, Franceschini V (2014) Immunocytochemical characterisation of olfactory ensheathing cells of zebrafish. *J Anat* 224:192–206
- Lazzari M, Bettini S, Franceschini V (2016) Immunocytochemical characterisation of ensheathing glia in the olfactory and vomeronasal systems of *Ambystoma mexicanum* (Caudata: Ambystomatidae). *Brain Struct Funct* 221:955–967

- Lazzari M, Bettini S, Milani L, Maurizii MG, Franceschini V (2017) Differential response of olfactory sensory neuron populations to copper ion exposure in zebrafish. *Aquat Toxicol* 183:54–62
- Lazzari M, Bettini S, Milani L, Maurizii MG, Franceschini V (2019) Differential nickel-induced responses of olfactory sensory neuron populations in zebrafish. *Aquat Toxicol* 206:14–23
- Levitt P, Rakic P (1980) Immunoperoxidase localization of glial fibrillary acidic protein in radial glial cells and astrocytes of the developing rhesus monkey brain. *J Comp Neurol* 193:815–840
- Liu Y, Zhang J, Liu D, Zhang J (2014) Vomeronasal organ lesion disrupts social odor recognition, behaviors and fitness in golden hamsters. *Integr Zool* 9:255–264
- Lu J, Féron F, Mackay-Sim A, Waite PM (2002) Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. *Brain* 125:14–21
- Luo H, Wu XQ, Zhao M, Wang Q, Jiang GP et al (2017) Expression of vimentin and glial fibrillary acidic protein in central nervous system development of rats. *Asian Pac J Trop Med* 10:1185–1189
- Mack AF, Künzle H, Lange M, Mages B, Reichenbach A, Härtig W (2018) Radial glial elements in the cerebral cortex of the lesser hedgehog Tenrec. *Brain Struct Funct* 223:3909–3917
- Mackay-Sim A (2005) Olfactory ensheathing cells and spinal cord repair. *Keio J Med* 54:8–14
- Malatesta P, Appolloni I, Calzolari F (2008) Radial glia and neural stem cells. *Cell Tissue Res* 331:165–178
- McDonald RP, Vickaryous MK (2018) Evidence for neurogenesis in the medial cortex of the leopard gecko, *Eublepharis macularius*. *Sci Rep* 8:9648
- Messenger NJ, Warner AE (1989) The appearance of neural and glial cell markers during early development of the nervous system in the amphibian embryo. *Development* 107:43–54
- Monzon-Mayor M, Yanes C, Ghandour MS, De Barry J, Gombos G (1990) GFAP and vimentin immunohistochemistry in the adult and developing midbrain of the lizard *Gallotia galloti*. *J Comp Neurol* 295:569–579
- Moore SA, Oglesbee MJ (2015) Spinal cord ependymal responses to naturally occurring traumatic spinal cord injury in dogs. *Vet Pathol* 52:1108–1117
- Moy-Thomas JA, Miles RS (1971) Paleozoic fishes. WB Saunders, Philadelphia
- Mulligan SJ, MacVicar BA (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* 431:195–199
- Nakada T, Hagino-Yamagishi K, Nakanishi K, Yokosuka M, Saito TR, Toyoda F, Hasunuma I, Nakakura T, Kikuyama S (2014) Expression of G proteins in the olfactory receptor neurons of the newt *Cynops pyrrhogaster*: their unique projection into the olfactory bulbs. *J Comp Neurol* 522(15):3501–3519
- Naujoks-Manteuffel C, Meyer DL (1996) Glial fibrillary acidic protein in the brain of the caecilian *Typhlonectes natans* (Amphibia, Gymnophiona): an immunocytochemical study. *Cell Tissue Res* 283:51–58
- O’Leary MA, Bloch JJ, Flynn JJ, Gaudin TJ, Giallombardo A, Giannini NP, Goldberg SL, Kraatz BP, Luo ZX, Meng J, Ni X, Novacek MJ, Perini FA, Randall Z, Rougier GW, Sargis EJ, Silcox MT, Simmons NB, Spaulding M, Velazco PM, Weksler M, Wible JR, Cirranello AL (2013) The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* 339:662–667
- Oikawa T, Suzuki K, Saito TR, Takahashi KW, Taniguchi K (1998) Fine structure of three types of olfactory organs in *Xenopus laevis*. *Anat Rec* 252:301–310
- Oka Y, Saraiva LR, Korsching SI (2012) Crypt neurons express a single V1R-related ora gene. *Chem Senses* 37(3):219–227
- Oudega M, Marani E (1991) Expression of vimentin and glial fibrillary acidic protein in the developing rat spinal cord: an immunocytochemical study of the spinal cord glial system. *J Anat* 179:97–114
- Pellitteri R, Spatuzza M, Stanzani S, Zaccheo D (2010) Biomarkers expression in rat olfactory ensheathing cells. *Front Biosci* 2:289–298
- Pulido-Caballero J, Jiménez-Sampedro F, Echevarría-Aza D, Martínez-Millán L (1994) Postnatal development of vimentin-positive cells in the rabbit superior colliculus. *J Comp Neurol* 343:102–112

- Quinzio SI, Reiss JO (2018) The ontogeny of the olfactory system in ceratophryid frogs (Anura, Ceratophryidae). *J Morphol* 279:37–49
- Raisman G (2001) Olfactory ensheathing cells: another miracle cure for spinal cord injury. *Nat Rev Neurosci* 2:369–375
- Rakic P (1981) Neuronal-glia interactions during development. *TINS* 4:184–187
- Ramon y Cajal S (1952) *Histologie du système nerveux de l'homme et des vertébrés*, vol. 1. Consejo superior de Investigaciones Científicas, Instituto Ramon Y Cajal, Madrid
- Rowitch DH, Kriegstein AR (2010) Developmental genetics of vertebrate glial-cell specification. *Nature* 468:214–222
- Rubio M, Suarez I, Bodega G, Fernandez B (1992) Glial fibrillary acidic protein and vimentin immunohistochemistry in the posterior rhombencephalon of the Iberian barb (*Barbus comiza*). *Neurosci Lett* 134:203–206
- Saito S, Matsui T, Kobayashi N, Wakisaka H, Mominoki K, Matsuda S, Taniguchi K (2003) Lectin histochemical study on the olfactory organ of the newt, *Cynops pyrrhogaster*, revealed heterogeneous mucous environments in a single nasal cavity. *Anat Embryol (Berl)* 206:349–356
- Sandulescu CM, Teow RY, Hale ME, Zhang C (2011) Onset and dynamic expression of S100 proteins in the olfactory organ and the lateral line system in zebrafish development. *Brain Res* 1383:120–127
- Sato Y, Miyasaka N, Yoshihara Y (2005) Mutually exclusive glomerular innervation by two distinct types of olfactory sensory neurons revealed in transgenic zebrafish. *J Neurosci* 25 (20):4889–4897
- Schnitzer J, Franke WW, Schachner M (1981) Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. *J Cell Biol* 90:435–447
- Schultze HP, Campbell KSW (1986) Characterization of the Dipnoi, a monophyletic group. *J Morphol Suppl* 1:25–37
- Sild M, Ruthazer ES (2011) Radial glia: progenitor, pathway partner. *Neuroscience* 17:288–302
- Simard M, Arcuino G, Takano T, Liu OS, Nedergaard M (2003) Signaling at the glio-vascular interface. *J Neurosci* 23:9254–9262
- Stephan H, Baron G, Frahm H (1991) Comparative brain characteristics. In: *Insectivora. Comparative brain research in mammals*, vol 1. Springer, New York
- Vielma A, Ardiles A, Delgado L, Schmachtenberg O (2008) The elusive crypt olfactory receptor neuron: evidence for its stimulation by amino acids and cAMP pathway agonists. *J Exp Biol* 211:2417–2422
- Voigt T (1989) Development of glial cells in the cerebral walls of ferrets: direct tracing of their transformation from radial glia into astrocytes. *J Comp Neurol* 289:74–88
- Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 6:626–640
- Wasowicz M, Pierre J, Reperant J, Ward R, Vesselkin NP, Versaux-Botteri C (1994) Immunoreactivity to glial fibrillary acidic protein (GFAP) in the brain and spinal cord of the lamprey (*Lampetra fluviatilis*). *J Brain Res* 35:71–78
- Weissman T, Noctor SC, Clinton BK, Honig LS, Kriegstein AR (2003) Neurogenic radial glial cells in reptile, rodent and human: from mitosis to migration. *Cereb Cortex* 13:550–559
- Wicht H, Derouiche A, Korf H-W (1994) An immunocytochemical investigation of glial morphology in the Pacific hagfish: radial and astrocyte-like glia have the same phylogenetic age. *J Neurocytol* 23:565–576
- Yanes C, Monzon-Mayor M, Ghandour MS, De Barry J, Gombos G (1990) Radial glia and astrocytes in developing and adult telencephalon of the lizard *Gallotia galloti* as revealed by immunohistochemistry with anti-GFAP and anti-vimentin antibodies. *J Comp Neurol* 295:559–568
- Zamora AJ, Mutin M (1988) Vimentin and glial fibrillary acidic protein filaments in radial glia of the adult urodele spinal cord. *Neuroscience* 27:279–288