

# An update on anatomy and function of the teleost olfactory system

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About half of all extant vertebrates are teleost fishes. Although our knowledge about anatomy and function of their olfactory systems still lags behind that of mammals, recent advances in cellular and molecular biology have provided us with a wealth of novel information about the sense of smell in this important animal group. Its paired olfactory organs contain up to five types of olfactory receptor neurons expressing OR, TAAR, VR1- and VR2-class odorant receptors associated with individual transduction machineries. The different types of receptor neurons are preferentially tuned towards particular classes of odorants, that are associated with specific behaviors, such as feeding, mating or migration. We discuss the connections of the receptor neurons in the olfactory bulb, the differences in bulbar circuitry compared to mammals, and the characteristics of second order projections to telencephalic olfactory areas, considering the everted ontogeny of the teleost telencephalon. The review concludes with a brief overview of current theories about odor coding and the prominent neural oscillations observed in the teleost olfactory system.

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## Abstract

About half of all extant vertebrates are teleost fishes. Although our knowledge about anatomy and function of their olfactory systems still lags behind that of mammals, recent advances in cellular and molecular biology have provided us with a wealth of novel information about the sense of smell in this important animal group. Its paired olfactory organs contain up to five types of olfactory receptor neurons expressing OR, TAAR, VR1- and VR2-class odorant receptors associated with individual transduction machineries. The different types of receptor neurons are preferentially tuned towards particular classes of odorants, that are associated with specific behaviors, such as feeding, mating or migration. We discuss the connections of the receptor neurons in the olfactory bulb, the differences in bulbar circuitry compared to mammals, and the characteristics of second order projections to telencephalic olfactory areas, considering the everted ontogeny of the teleost telencephalon. The review concludes with a brief overview of current theories about odor coding and the prominent neural oscillations observed in the teleost olfactory system.

## Introduction

The sense of smell plays an important role in fishes, mediating behaviors and physiological responses related to food search and feeding, social interaction, mating, detection of predators and contamination, or migration and search for spawning sites (Sorensen & Caprio, 1998; Bone & Moore, 2008). As opposed to higher vertebrates (amphibians, reptiles and mammals), which usually have access to a limited degree of light even in moonless nights, fish encounter situations of total darkness easily in turbid or deeper waters and underwater caves, where their olfactory sense may provide a degree of orientation. Olfactory information has long been known to be important for the homing of migrating salmonids and other fishes (Dittman & Quinn, 1996; Bett & Hinch, 2016). There is evidence for salmonids that some amino acids dissolved in the water of native rivers could mediate migratory conducts (Yamamoto, Hino & Ueda, 2010; Yamamoto, Shibata & Ueda, 2013). However, in general, the identity of the odors mediating orientation behavior in teleosts remains a topic of active investigation. On the other hand, amino acids are considered the most relevant odorants indicating the presence of a food source to teleosts, given

the behavioral responses they generate (Valentinčič, Lamb & Caprio, 1999; Whitlock, 2006; Calvo-Ochoa & Byrd-Jacobs, 2019), and the fact that teleosts are generally sensitive to most amino acids, with electrophysiological screening thresholds ranging from micromolar to nanomolar concentrations ( $10^{-6}$  –  $10^{-8}$  M) (Ivanova & Caprio, 1993; Nikonov & Caprio, 2007; Bazáes, Olivares & Schmachtenberg, 2013; Nikonov et al., 2017; Sato & Sorensen, 2018). Nucleic acids and polyamines are also related to food odor, with evidence that several nucleotides, including ATP and adenosine, are able to evoke olfactory responses in channel catfish (*Ictalurus punctatus*), zebrafish (*Danio rerio*) and goldfish (*Carassius auratus*) (Hansen et al., 2003; Rolen et al., 2003; Wakisaka et al., 2017).

Sensitivity thresholds for different types of odorants depend evidently on the species, but also on the type of assay, behavioral versus electrophysiological, and for the latter, whether individual neurons or bulk responses are recorded from the olfactory epithelium, bulb or telencephalon. Studies using electroolfactogram (EOG) recordings have indicated that fish are sensitive to very low concentrations of certain odorants, presenting responses to some amino acids at thresholds in the order of  $10^{-9}$  M, and below  $10^{-11}$  M for certain bile salts, steroid hormones and prostaglandins (Reutter & Kapoor, 2005; Bone & Moore, 2008). Bile salts are good candidates for social odors, as they are constantly released by fishes (Buchinger, Li & Johnson, 2014). However, controversy persists as to what degree mixtures of bile salts are species-specific and may therefore be considered pheromonal odors (Sorensen & Stacey, 2004; Giaquinto & Hara, 2008). Recently, using EOG recordings in the rainbow trout, responses to lactic acid, pyruvic acid and four B vitamins with thresholds in the nanomolar range been reported for the first time in rainbow trout, adding these compounds to the growing list of food odors. Lactate and pyruvate are important components of cellular metabolism, while B vitamins, which are synthesized by phytoplankton and algae, are essential for teleost development, which might explain their sensitive detection (Valdés et al., 2015).

Some odorants trigger an instinctive avoidance response. For example, an imminent risk of predation can be detected by fish through the presence of passively released molecules when a conspecific is attacked and injured, which prevail in the environment for much longer than visual or mechanical signals. The skin of teleosts presents a type of cells called *club cells* that release a combination of chemical warning signals in the context of predation or parasitism, generating changes in the behavior of rainbow trout and other teleost species. These substances include

glycosaminoglycans of chondroitin, an important component of the skin's mucosa (Poulin, Marcogliese & McLaughlin, 1999; Speedie & Gerlai, 2008; Wisenden et al., 2009; Mathuru et al., 2012).

Here, we provide an update on anatomy and function of the teleost olfactory system, including the important advances achieved during the last years. The review is intended to complement prior major revisions of the relevant literature on fish olfaction, covering peripheral coding and olfactory bulb circuitry (Satou, 1990; Laberge & Hara, 2001), the olfactory gene repertoire (Korsching, 2009), the variety of olfactory receptor neurons and their respective connections (Hansen et al., 2005; Hamdani & Døving, 2007; Bazáes, Olivares & Schmachtenberg, 2013; Yoshihara, 2014) and the generation of olfactory behaviors (Kermen et al., 2013; Yoshihara, 2014).

## Survey methodology

Initially, a PubMed search was conducted for the terms “teleost” and “olfactory”, yielding 304 results. Twenty-two notable and highly cited articles on vertebrate olfaction were also included in the general data base. Four books were additionally considered: “Fish Chemosenses” (Reutter & Kapoor, 2005); “Biology of Fishes” (Bone & Moore, 2008); “Comparative Vertebrate Neuroanatomy” (Butler & Hodos, 2005) and “Sensory Systems Neuroscience” (Hara & Zielinski, 2007). A total of 116 references was finally selected and included in the review. It should be noted that while the field of teleost olfaction is small compared to other biomedical areas, it is still too large to be comprehensively covered in one review, and no attempt was made in that direction. Particularly, an inter-species comparison was beyond the focus of this work, since the large number of teleosts and their huge variety would render any such attempt hopeless. Instead, we focused on the most popular model species: Channel catfish, rainbow trout, goldfish and zebrafish.

## Olfactory epithelium and nerve

The nasal cavities of teleost fishes, including salmonids such as rainbow trout (*Oncorhynchus mykiss*), are located on the dorsal anterior face of the head (Fig. 1A, inset). Each cavity has two

91 openings, allowing entry and outflow of water, propelled by the movement of the fish, muscular  
 92 pumping actions, ciliary beating of supporting cells, and water currents (Doving et al., 1977).  
 93 Unlike higher vertebrates, there is no connection between the olfactory and the respiratory  
 94 systems, and respiratory sniffing and its implications for odor detection are absent, although  
 95 some fishes may use muscular contractions for active olfactory sampling (Doving et al., 1977;  
 96 Nevitt, 1991). Teleost fishes lack a separate vomeronasal organ, and the sole olfactory  
 97 epithelium is located on the floor of the nasal cavity, in most species arranged in the form of  
 98 sheets or lamellae, which radiate from a central ridge or *raphe* and give rise to an olfactory  
 99 *rosette* (Hansen & Zielinski, 2005) (Fig. 1A). The number and size of the lamellae increase  
 100 throughout development of the teleost, but remain relatively constant after the specimen reaches  
 101 maturity. The lamellae are composed of two pseudostratified epithelial layers that wrap a thin  
 102 sheet of stroma. Sensory and non-sensory cells are irregularly interspersed within the epithelia,  
 103 as are mucus-producing goblet cells (Bazáes, Olivares & Schmachtenberg, 2013) (Fig. 1B).  
 104 The olfactory epithelium consists of three main types of cells: Olfactory receptor neurons  
 105 (ORNs), supporting or sustentacular cells, and basal cells, which are capable to divide and  
 106 regenerate the epithelium after injury (Iqbal & Byrd-Jacobs, 2010) (Fig. 1D). In teleosts, there  
 107 are four established types of ORNs intermingled in the olfactory epithelium: Ciliated and  
 108 microvillous receptor neurons, olfactory crypt cells and *Kappe* cells, the latter discovered in  
 109 zebrafish (Hansen et al., 2003; Bazáes, Olivares & Schmachtenberg, 2013; Ahuja et al., 2015). A  
 110 putative fifth type of ORN, termed “pear-shaped”, was also recently described in zebrafish, and  
 111 shown to function as an adenosine sensor (Wakisaka et al., 2017). Olfactory cilia and microvilli  
 112 contain the olfactory receptor proteins that are exposed to chemical stimuli in the water. Ciliated  
 113 and microvillous ORNs are bipolar cells, with an apical cylindrical dendrite that extends to the  
 114 surface of the lamellae (Fig. 1B, inset) and an axon that projects towards the submucosa forming  
 115 the fascicles of the olfactory nerve. These cell types are morphologically and molecularly similar  
 116 to the ciliated and microvillous ORNs of the main olfactory and vomeronasal organs of higher  
 117 vertebrates (Farbman, 2000; Hansen et al., 2003). Olfactory crypt cells, which are located in  
 118 more apical regions of the epithelium, have an oval shape with cilia and microvilli enclosed in an  
 119 apical invagination, and are completely enveloped by one or two supporting cells of glial  
 120 characteristics (Hansen & Finger, 2000; Schmachtenberg, 2006; Bazaes & Schmachtenberg,  
 121 2012). The *Kappe* cells, found in the most apical epithelial positions, have a similar shape as

crypt cells, but are slightly more slender and do not present cilia (Ahuja et al., 2015). Finally, little is known about the recently described pear-shaped zebrafish ORN, which is morphologically similar to crypt and *Kappe* cells, but displays different molecular signatures, such as expression of olfactory marker protein (OMP) (Wakisaka et al., 2017). These latter two cell types have hitherto not been described in teleosts other than zebrafish. If they do indeed qualify to be recognized as separate ORN types remains to be confirmed.

## Olfactory bulb and tract

The axonal projections of vertebrate ORNs traverse the skull in olfactory nerve fascicles that enter the olfactory bulbs and converge in spherical neuropil complexes termed glomeruli (Mombaerts et al., 1996). The teleost olfactory bulb has several similarities and some differences with respect to the olfactory bulb of other vertebrates (Satou, 1990; Kermen et al., 2013; Nagayama, Homma & Imamura, 2014; Calvo-Ochoa & Byrd-Jacobs, 2019) (Fig. 1C). Recently, an entire olfactory bulb of zebrafish larvae has been reconstructed from serial section electron microscopy, including the skeletons of over 1000 neurons, 75% of which were identified as mitral cells (Wanner et al., 2016; Wanner, Genoud & Friedrich, 2016). In the teleost olfactory bulb, four layers can be identified from the periphery towards the center: Olfactory nerve layer, glomerular layer, mitral cell layer and granular layer (Satou, 1990). The outermost layer of this structure corresponds to the layer formed by the axonal endings of the ORNs, which are distributed profusely over the surface of the bulb before traversing deeper into the glomeruli of the glomerular layer, where they form glutamatergic synapses with the dendrites of the mitral cells, whose cell bodies are located in the subjacent layer (Fujita, Satou & Ueda, 1988). The glomerular layer of teleosts also contains nerve endings from higher telencephalic centers and projections of granule cells, GABAergic cells whose cell bodies are located in the layer that bears the same name and with which the mitral cells share dendrodendritic synapses (Satou, 1990). As opposed to mammals, mitral cells can innervate multiple glomeruli in teleost fish, but not all of them do (Laberge & Hara, 2001; Fuller, Yettaw & Byrd, 2006). Bulbar glomeruli are surrounded by short axon cells and periglomerular cells, which participate in this first stage of olfactory processing, together with neuromodulatory efferent projections from the telencephalon making synapses with granular cells in the granular layer of the bulb (Nieuwenhuys, Donkelaar

& Nicholson, 1998) (Fig. 1F).

The tufted cells of mammals are either absent or indistinguishable from mitral cells in the teleost olfactory bulb (Edwards & Michel, 2002). However, a second type of projection neuron termed ruffed cells has been observed in fish that is not present in mammals (Kosaka & Hama, 1979, 1981). The ruffed cells, which project to the telencephalon together with the mitral cells, also present dendrites in the glomerular layer but only appear to make synapses with granule cells (Kosaka & Hama, 1979; Satou, 1990). These cells are thought to play an important role in olfactory processing and in the generation of prominent olfactory oscillations in the teleost bulb, since their response is of opposite polarity compared to that of mitral cells (Zippel, 1998) (Fig. 1E). The axons of the mitral cells give rise to the olfactory tracts that carry the information to other relay centers in the telencephalon (Nieuwenhuys, Donkelaar & Nicholson, 1998) (Fig. 1F). Studies using tracers, electrophysiology, genetics and behavior in rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*) and other teleosts have allowed to determine the presence of two subdivisions of the olfactory tract: the lateral olfactory tract and the medial olfactory tract (Folgueira, Anadón & Yáñez, 2004a; Hamdani & Døving, 2007; Miyasaka et al., 2009, 2014). These can be further subdivided into fiber bundles which conserve the chemotopic organization of the olfactory bulb to some degree, thus maintaining the odotopically organized processing of different types of odors (Finger, 1975; Satou, 1990). In crucian carp, ablation studies of the different olfactory tracts that project to various telencephalic regions indicated that the medial bundle of the median olfactory tract mediates escape behavior in response to odors considered as alarm signals (Hamdani et al., 2000), while the lateral bundle of the median olfactory tract relays odor information related to reproductive behavior (Weltzien et al., 2003). Finally, the lateral olfactory tract has been reported to convey information about odorants related to feeding behaviors in this species (Hamdani, Alexander & Døving, 2001).

## The odor transduction process

In the ciliary and microvillous membranes of vertebrate ORNs, odorant receptor proteins belonging to the OR superfamily of G protein-coupled receptors are expressed (Buck & Axel, 1991). In addition, a subset of receptor neurons express another class of odorant receptor



proteins, termed trace-amine associated receptors (TAARs) (Liberles & Buck, 2006). A third class of receptor proteins, the vomeronasal receptors (VRs), is expressed within the vomeronasal organ of many higher vertebrates, and in the olfactory epithelium of fishes, where it may serve to detect conspecific odors and pheromones (Silva & Antunes, 2016). It was demonstrated in several teleost species that the different types of olfactory receptor proteins are expressed differentially in the specific ORN types, which in turn present different transduction processes associated with diverse types of G-proteins (Hansen et al., 2003; Hansen, Anderson & Finger, 2004). While ciliated ORNs generally express receptor proteins of the OR and TAAR types, which are associated with  $G_{\alpha_{olf}}$  proteins, microvillous receptor neurons present V1R-type receptors associated with  $G_{\alpha_i}$ , and V2R receptors coupled to the G-proteins  $G_{\alpha_o}$  (Fig. 1D),  $G_{\alpha_q}$ ,  $G_{\alpha_{q/11}}$  and  $G_{\alpha_{i-3}}$ . Crypt cells express V1R type receptors coupled to  $G_{\alpha_o}$ ,  $G_{\alpha_{q/11}}$  and  $G_{\alpha_{i1b}}$ , while *Kappe* cells, although their class of odorant receptor proteins remains unknown, are thought to be associated with  $G_{\alpha_o}$  proteins (Hansen et al., 2003; Hansen, Anderson & Finger, 2004; Hansen & Zielinski, 2005; Bazáes, Olivares & Schmachtenberg, 2013; Ahuja et al., 2015).

The olfactory receptor gene repertoire has been analyzed in several teleost species, revealing a significant degree of variation and diversity, with 189 functional genes and 31 pseudogenes detected in zebrafish, the highest number in teleosts counted to date (Korsching, 2009; Yoshihara, 2014). In the case of salmonid fishes, a total of 60 functional genes and 51 pseudogenes have been found in *Salmo salar*, which are distributed among the different families of olfactory receptors in such a way that 24 functional OR-type receptors have been found together with 24 pseudogenes, 7 genes of functional V1R-type receptors and 1 pseudogene, and 29 genes of V2R-type receptors with 26 pseudogenes (Johnstone et al., 2012). More recently, the discovery of 27 putative functional genes and 25 putative pseudogenes of the TAAR family has been added to this list (Tessarolo et al., 2014).

The conformational change that the receptor undergoes due to the binding of an odorant induces the activation of the respective G proteins, which dissociate their alpha subunit. In ciliated ORNs of teleosts, as in those of higher vertebrates (Buck & Axel, 1991), the alpha subunit of the  $G_{\alpha_{olf}}$  protein triggers the activation of the enzyme adenylate cyclase, generating an increase in cyclic AMP (cAMP) that functions as a second messenger (Hansen et al., 2003; Schmachtenberg & Bacigalupo, 2004). cAMP causes the activation of cationic cyclic nucleotide-gated channels (CNGCs), allowing the influx of sodium and to a lesser extent of calcium, which depolarizes the

cell membrane directly and by an amplifying effect, through the activation of nearby calcium-dependent chloride channels (Kurahashi & Yau, 1994; Reisert & Reingruber, 2019). In addition, calcium contributes to the termination of the response through an adaptive reduction of the sensitivity of CNGCs to cyclic nucleotides by means of calcium-calmodulin activation, until intracellular calcium levels return to pre-stimulation levels mainly by action of the sodium-calcium exchanger (Matthews & Reisert, 2003). In *O. mykiss*, the function of calcium-activated chloride channels (termed either Anoctamin 2 or TMEM16B) has been demonstrated directly with patch clamp recordings of ORNs and by electroolfactogram (EOG) recordings of the olfactory organs, indicating that the olfactory transduction process operates in the same way in freshwater fish as in higher vertebrates (Sato & Suzuki, 2000). In seawater, the large extracellular sodium chloride concentration would make the amplificatory outward chloride currents both unnecessary and impossible (Osorio & Schmachtenberg, 2013), however, the absence of Anoctamin 2 or TMEM16B channels from ORNs of marine teleosts remains to be shown. The transduction pathways of the other teleost ORN classes are less well understood, and have been reviewed elsewhere (Bazáes, Olivares & Schmachtenberg, 2013).

## The teleost telencephalon

In the brain, sensory input is analyzed and compared with available memories, integrated between different sensory modalities; new memories are formed and behavioral responses may be initiated. Between the main groups of vertebrates, there is a marked difference in the development of the brain, which makes comparative neuroanatomical studies between species difficult. While in vertebrates including lampreys, cartilaginous fish, amphibians and amniotes, the telencephalon develops from the neural tube through a process called *evagination*, in teleost fishes a process called *eversion* is generated, in which structures of the brain are distributed differently (Butler, 2000; Butler & Hodos, 2005; Folgueira et al., 2012). Evagination is produced by the expansion of the lumen of the neural tube, which gives rise to the lateral ventricles inside the telencephalon. The eversion process, however, generates a telencephalon without cavities, developing a ventricular surface located dorsally (Nieuwenhuys, 2011). Although the sections of the neural tube that give rise to the different functional cortical regions of species generating the evagination process are homologous to those found in teleost fishes, once the eversion process is

completed during embryonic development, the functional regions, including those that process olfactory information, present a disposition that differs greatly from the other groups of vertebrates (Butler & Hodos, 2005; Ito & Yamamoto, 2009; Folgueira et al., 2012). A distinctive feature of the mammalian telencephalon is the presence of a neocortex or six-layer pallium, which is considered responsible for superior cognitive functions. The pallium of the teleost fishes lacks a six-layer cortex, and even laminar structures. Instead, the teleost telencephalon contains zones and nuclei that are neuroanatomically and functionally analogous to the pallial and subpallial structures of evaginating vertebrates (Ito & Yamamoto, 2009).

In the teleost telencephalon, two regions defined by topographic criteria can be recognized: The dorsal area (*Area Dorsalis Telencephali*), and the ventral area (*Area Ventralis Telencephali*) considered homologous to the pallium and the subpallium, respectively, of the remainder of vertebrates. Both telencephalic regions were shown to receive bulbar projections in zebrafish (Miyasaka et al., 2014). These telencephalic areas have been subdivided into zones and nuclei by various authors, among which the ventral area of the telencephalon can be distinguished into a central (Vc), ventral (Vv), dorsal (Vd), lateral (Vl); supracomissural (Vs) and paracomissural (Vp) nucleus, while the dorsal area can be subdivided into a central (Dc), dorsal (Dd), lateral (Dl), medial (Dm) and posterior (Dp) zone (Nieuwenhuys, Donkelaar & Nicholson, 1998; Wullimann & Rink, 2002; Folgueira, Anadón & Yáñez, 2004b; Biechl et al., 2017) (Fig. 1F). It should be noted however, that in rainbow trout, the dorsal and the dorsolateral region of the dorsal area of the telencephalon (Dd + Dl-d), have been proposed to form a homogeneous region according to cytoarchitectonic and neurochemical criteria (Folgueira, Anadón & Yáñez, 2004b). The differences in the development of the teleost telencephalon make it difficult to identify areas that are functionally homologous to the telencephalon of other vertebrates. Studies analyzing neurochemistry, connectivity, gene expression and development in several species of teleosts suggest that the nuclei located dorsally within the *Area Ventralis*, Vc and Vd, represent the striatum of other vertebrates. Also, the Vs represents subpallial tonsillar regions, and Vv and Vl, located ventrally in the telencephalon, could represent the septum, with Vv being a homologue of the lateral septum, the *nucleus accumbens* and the *substantia innominata*, which are related to the reward system. In the dorsal area of the telencephalon, Dc represents the pallium, the most medial part of Dm is thought to be analogous to portions of the amygdala, and Dl is likely homologous to the hippocampus.

Studies in different species of teleosts have established the pallial regions and the subpallial nuclei that receive projections from the olfactory bulb: Dp, Dl, Vv, Vs, Vp, Vc, Vl, among which the Vv region stands out related to the reward systems, and Dp related to the identity of odorants. Dp is the main target of the secondary olfactory fibers (Fig. 1F), comparable to the primary olfactory cortex or piriform cortex of higher vertebrates (Wullimann, Rupp & Reichert, 1996; Nieuwenhuys, Donkelaar & Nicholson, 1998; Cerdá-Reverter, Zanuy & Muñoz-Cueto, 2001; Wullimann & Rink, 2002; Folgueira, Anadón & Yáñez, 2004a,b; Butler & Hodos, 2005; Mueller et al., 2011; Kermen et al., 2013; Biechl et al., 2017). Olfactory stimulation causes patterns of activity in specific sectors of the olfactory bulb and in the Vv and Dp sectors of the telencephalon in channel catfish and zebrafish (Nikonov, Finger & Caprio, 2005; Yakshi et al., 2009). Blumhagen showed that extracellular recordings of the Dp region of zebrafish are synchronized with the activity of mitral cells after olfactory stimulation (Blumhagen et al., 2011). This is in line with evidence supporting the notion that the Vv and Dp regions of the teleost telencephalon are analogous to structures related to olfactory processing and learning in higher vertebrates (Butler & Hodos, 2005; Ito & Yamamoto, 2009; Mueller et al., 2011).

## Olfactory coding in teleosts

The coding of olfactory information regarding stimulus identity, intensity, time course and spatial origin occurs at different levels of the olfactory system, allowing parallel and differentiated processing of different aspects of the olfactory stimuli. Initial coding occurs at the level of the olfactory epithelium and is determined by the types and numbers of ORNs that are activated (Kang & Caprio, 1995; Duchamp-Viret, Duchamp & Chaput, 2000). The information is odotopically organized in the olfactory bulb and modified by efferent input and complex circuits formed by local interneurons, as in higher vertebrates (Li et al., 2019). Finally, a percept is thought to be generated at the level of several telencephalic regions (Wilson & Mainen, 2006). In the olfactory epithelium, ORNs choose and express one allele of the olfactory receptor gene repertoire (Khan, Vaes & Mombaerts, 2011), and receptor neuron groups of variable size that express a common olfactory receptor gene send their axons to the same pair of glomeruli in the bulb. Since odorant receptor proteins are generally of a broad spectrum and activated by different, albeit mostly structurally similar molecules, each odor activates a practically unique

combination of ORNs in the olfactory epithelium, and of glomeruli in the olfactory bulb (Malnic et al., 1999; Wilson & Mainen, 2006). The combination of the afferents of many ORNs in the olfactory bulb also causes an increase in signal strength and helps reduce the noise associated with local fluctuations in odorant concentration (Kay & Stopfer, 2006).

In fish, the projections of the diverse types of ORNs reach different sectors of the olfactory bulb, although there is also evidence that a small subpopulation of ORNs projects directly to the ventral region of the telencephalon (Hara & Zielinski, 2007; Tierney, 2015).

Electrophysiological studies in trout and channel catfish have confirmed that different types of odorants are processed in different parts of the olfactory bulb, generating a gross general odotopy (Friedrich & Korsching, 1997; Hara & Zhang, 1998; Nikonov & Caprio, 2004, 2005; Nikonov, Finger & Caprio, 2005; Rolen & Caprio, 2007). Using retrograde tracers in channel catfish, it was observed that the ciliated ORNs project their axons to medial regions of the olfactory bulb, which are activated by bile salts, and ventral regions, which respond to amino acids, while the microvillous ORNs project mainly to the dorsal surface of the bulb, where mainly responses to amino acids (anterior region) and nucleotides (posterior region) are observed. These separate innervation patterns of the two main types of teleost ORNs were beautifully confirmed in transgenic zebrafish (Sato, Miyasaka & Yoshihara, 2005).

Furthermore, immunohistochemical studies of zebrafish crypt cells have shown that these project to the single posterior mediodorsal glomerulus 2 (mdG2) in the olfactory bulb (Ahuja et al., 2013), which may be involved in pheromone and/or kin recognition (Bazaes & Schmachtenberg, 2012; Biechl et al., 2016). *Kappe* cells also project to a single glomerulus, posterior mediodorsal glomerulus 5 (mdG5) in zebrafish (Hansen et al., 2003, 2005; Ahuja et al., 2013, 2015; Kress, Biechl & Wullimann, 2014), but their function(s) remain enigmatic to date. Thus, the general odotopic map of vertebrate olfactory bulbs is complemented by a coarser ORN type and odorant class-dependent map in teleost fishes.

Odors usually represent complex mixtures that involve many odorants, and the perception of odors does not generally allow the identification of individual components, unless they are limited in number, as elegantly demonstrated in the catfish *Ameiurus melas* (Valentincic et al., 2011). Instead, the components are united generating a recognition based on patterns (Laurent, 2002). The olfactory system is able to decompose odors with different degrees of resolution, from a qualitative point of view, thus recognizing the "family" of odorants with which the

perceived odor is related. Changes in odor concentration can also be determined, which may affect the perceived smell quality (Friedrich & Laurent, 2001; Laurent, 2002). This process of decoding the depends on the complete olfactory system, involving an analysis at the telencephalic level, which acts as a secondary relay of odor information (Yaksi et al., 2009). The first relay, the bulb, performs the task of de-correlating the information, thus increasing the coding space, implying a decrease in the overlap, over time, between odorant mixtures (Friedrich, Laurent & Rainer, 2004). In this process, patterns of oscillatory activity are generated in response to odor stimulation, which are the result of the summation of synaptic activity of multiple afferent neurons (Laberge & Hara, 2001; Friedrich, Habermann & Laurent, 2004; Buzsáki & Draguhn, 2004; Schoppa, 2006). In teleost fishes, olfactory stimulation causes patterns of transient oscillatory activity in specific sectors of the olfactory bulb and in the Vv and Dp sectors of the telencephalon in channel catfish, zebrafish and rainbow trout (Nikonov, Finger & Caprio, 2005; Yaksi et al., 2009; Olivares, 2019) (Fig. 1E). The most prominent oscillations are observed in the 7-12 Hz range, which may functionally correspond to the beta-range in mammals (Satou & Ueda, 1978; Satou, 1990; Kay et al., 2009). As early as in 1938, Adrian had been able to observe and record oscillations in the central olfactory system of decapitated fish, using different methods of mechanical and chemical stimulation (Adrian & Ludwig, 1938). Blumhagen et al. (2011), showed that local field potential recordings of the Dp region of zebrafish are synchronized with the activity of mitral cells recorded during olfactory stimulation (Blumhagen et al., 2011). There is mounting evidence indicating that the origin of oscillations in the olfactory system is the olfactory bulb (Satou & Ueda, 1978; Kay, 2014). Although the role of oscillations evoked by odors in the olfactory system of vertebrates is not yet understood, one attractive possibility is that the synchronization of neural networks at specific frequencies allows to isolate transient communication pathways between distant brain regions (Buzsáki & Draguhn, 2004). The different types of oscillations observed in the olfactory system differ in frequency and power depending on the behavioral state of the animal, however, the frequency ranges used for the classification of these signals are species-specific, and generally significantly lower in fishes than in mammals (Kay et al., 2009).

## Conclusions

The teleost olfactory system displays important cellular, anatomical and functional differences compared to other classes of vertebrates. These are in part due to the aqueous nature of their habitat, the presence of a sole type of olfactory organ compared to up to five in mammals, and the separation of the olfactory from the respiratory system in fishes. Ciliated and microvillous ORNs have conserved features across vertebrates, whereas olfactory crypt, pear-shaped and *Kappe* cells appear unique to fishes. The teleost olfactory bulb lacks tufted cells but contains ruffed cells instead, and mitral cells may innervate more than one glomerulus. Olfactory tracts to the telencephalon preserve the bulbar odotopy among their bundles and innervate telencephalic areas that are functionally but not anatomically homologous to higher vertebrates. Prominent field potential oscillations, of lower frequency than in mammals and lacking respiratory rhythms, are observed in response to odorant stimulation, originating in the olfactory bulb and expanding through the olfactory tracts to olfactory telencephalic areas. Future studies will, among others, define the species-specific odors guiding migratory behavior in certain teleosts, notably in salmonids, clarify how olfactory memories are created and stored, define the roles of pear-shaped and *Kappe* cells, and hopefully elucidate the function of the olfactory oscillations, which has remained enigmatic for decades.

# References

- Adrian ED, Ludwig C. 1938. Nervous discharges from the olfactory organs of fish. *The Journal of physiology* 94:441–60.
- Ahuja G, Ivandic I, Saltürk M, Oka Y, Nadler W, Korsching SI. 2013. Zebrafish crypt neurons project to a single, identified mediodorsal glomerulus. *Scientific reports* 3:2063. DOI: 10.1038/srep02063.
- Ahuja G, Nia SB, Zapilko V, Shiriagin V, Kowatschew D, Oka Y, Korsching SI. 2015. Kappe neurons, a novel population of olfactory sensory neurons. *Scientific Reports* 4:1–8. DOI: 10.1038/srep04037.
- Bazáes A, Olivares J, Schmachtenberg O. 2013. Properties, Projections, and Tuning of Teleost Olfactory Receptor Neurons. *Journal of Chemical Ecology* 39:451–464. DOI: 10.1007/s10886-013-0268-1.
- Bazaes A, Schmachtenberg O. 2012. Odorant tuning of olfactory crypt cells from juvenile and adult rainbow trout. *Journal of Experimental Biology* 215:1740–1748. DOI: 10.1242/jeb.067264.
- Bett NN, Hinch SG. 2016. Olfactory navigation during spawning migrations: a review and introduction of the Hierarchical Navigation Hypothesis. *Biological reviews of the Cambridge Philosophical Society* 91:728–759. DOI: 10.1111/brv.12191.
- Biechl D, Tietje K, Gerlach G, Wullimann MF. 2016. Crypt cells are involved in kin recognition in larval zebrafish. *Scientific Reports* 6:24590. DOI: 10.1038/srep24590.
- Biechl D, Tietje K, Ryu S, Grothe B, Gerlach G, Wullimann MF. 2017. Identification of accessory olfactory system and medial amygdala in the zebrafish. *Scientific Reports* 7:1–17. DOI: 10.1038/srep44295.
- Blumhagen F, Zhu P, Shum J, Schärer Y-PZ, Yaksi E, Deisseroth K, Friedrich RW. 2011. Neuronal filtering of multiplexed odour representations. *Nature* 479:493–8. DOI: 10.1038/nature10633.
- Bone Q, Moore R. 2008. *Biology of Fishes*. Saunders College Publishing. DOI: 10.1080/17451000902889821.
- Buchinger TJ, Li W, Johnson NS. 2014. Bile salts as semiochemicals in fish. *Chemical Senses* 39:647–654. DOI: 10.1093/chemse/bju039.
- Buck L, Axel R. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* 65:175–187. DOI: 10.1016/0092-8674(91)90418-X.
- Butler AB. 2000. Topography and topology of the teleost telencephalon: A paradox resolved. *Neuroscience Letters* 293:95–98. DOI: 10.1016/S0304-3940(00)01497-X.
- Butler AB, Hodos W. 2005. *Comparative vertebrate neuroanatomy: Evolution and Adaptation*. John Wiley & Sons, Inc., Hoboken, New Jersey.
- Buzsáki G, Draguhn A. 2004. Neuronal oscillations in cortical networks. *Science (New York, N.Y.)* 304:1926–9. DOI: 10.1126/science.1099745.
- Calvo-Ochoa E, Byrd-Jacobs CA. 2019. The olfactory system of zebrafish as a model for the study of neurotoxicity and injury: Implications for neuroplasticity and disease. *International Journal of Molecular Sciences* 20. DOI: 10.3390/ijms20071639.
- Cerdá-Reverter JM, Zanuy S, Muñoz-Cueto JA. 2001. Cytoarchitectonic study of the brain of a perciform species, the sea bass (*Dicentrarchus labrax*). I. The telencephalon. *Journal of Morphology* 247:217–228.
- Dittman A, Quinn T. 1996. Homing in Pacific salmon: mechanisms and ecological basis. *The*



- 427 *Journal of Experimental Biology* 199:83–91.
- 428 Doving KB, Dubois-Dauphin M, Holley A, Jourdan F. 1977. Functional Anatomy of the
- 429 Olfactory Organ of Fish and the Ciliary Mechanism of Water Transport. *Acta Zoologica*
- 430 58:245–255.
- 431 Duchamp-Viret P, Duchamp A, Chaput MA. 2000. Peripheral odor coding in the rat and frog:
- 432 quality and intensity specification. *The Journal of Neuroscience* 20:2383–2390. DOI:
- 433 citeulike-article-id:9521592.
- 434 Edwards JG, Michel WC. 2002. Odor-stimulated glutamatergic neurotransmission in the
- 435 zebrafish olfactory bulb. *Journal of Comparative Neurology* 454:294–309. DOI:
- 436 10.1002/cne.10445.
- 437 Farbman AI. 2000. Cell biology of olfactory epithelium. In: Finger TE, Silver WL, Restrepo D
- 438 eds. *The Neurobiology of taste and smell*. Wiley, New York, 131–158.
- 439 Finger TE. 1975. The distribution of the olfactory tracts in the bullhead catfish, *Ictalurus*
- 440 *nebulosus*. *Journal of Comparative Neurology* 161:125–141. DOI: 10.1002/cne.901610110.
- 441 Folgueira M, Anadón R, Yáñez J. 2004a. An experimental study of the connections of the
- 442 telencephalon in the rainbow trout (*Oncorhynchus mykiss*). I: Olfactory bulb and ventral
- 443 area. *Journal of Comparative Neurology* 480:180–203. DOI: 10.1002/cne.20340.
- 444 Folgueira M, Anadón R, Yáñez J. 2004b. Experimental study of the connections of the
- 445 telencephalon in the rainbow trout (*Oncorhynchus mykiss*). II: Dorsal area and preoptic
- 446 region. *Journal of Comparative Neurology* 480:204–233. DOI: 10.1002/cne.20341.
- 447 Folgueira M, Bayley P, Navratilova P, Becker TS, Wilson SW, Clarke JD. 2012. Morphogenesis
- 448 underlying the development of the everted teleost telencephalon. *Neural Development* 7:1–
- 449 13.
- 450 Friedrich RW, Habermann CJ, Laurent G. 2004. Multiplexing using synchrony in the zebrafish
- 451 olfactory bulb. *Nature Neuroscience* 7:862–871. DOI: 10.1038/nn1292.
- 452 Friedrich RW, Korsching SI. 1997. Combinatorial and chemotopic odorant coding in the
- 453 zebrafish olfactory bulb visualized by optical imaging. *Neuron* 18:737–752. DOI:
- 454 10.1016/S0896-6273(00)80314-1.
- 455 Friedrich RW, Laurent G. 2001. Dynamic optimization of odor representations by slow temporal
- 456 patterning of mitral cell activity. *Science (New York, N.Y.)* 291:889–94. DOI:
- 457 10.1126/science.291.5505.889.
- 458 Friedrich RW, Laurent G, Rainer W. 2004. Dynamics of olfactory bulb input and output activity
- 459 during odor stimulation in zebrafish. *Journal of neurophysiology* 91:2658–69. DOI:
- 460 10.1152/jn.01143.2003.
- 461 Fujita I, Satou M, Ueda K. 1988. Morphology of physiologically identified mitral cells in the
- 462 carp olfactory bulb: A light microscopic study after intracellular staining with horseradish
- 463 peroxidase. *Journal of Comparative Neurology* 267:253–268. DOI:
- 464 10.1002/cne.902670208.
- 465 Fuller CL, Yettaw HK, Byrd CA. 2006. Mitral cells in the olfactory bulb of adult zebrafish
- 466 (*Danio rerio*): morphology and distribution. *Journal of Comparative Neurology* 499:218–
- 467 230.
- 468 Giaquinto P, Hara TJ. 2008. Discrimination of bile acids by the rainbow trout olfactory system:
- 469 Evidence as potential pheromone. *Biological Research* 41:33–42. DOI: 10.4067/S0716-
- 470 97602008000100005.
- 471 Hamdani EH, Alexander G, Døving KB. 2001. Projection of sensory neurons with microvilli to
- 472 the lateral olfactory tract indicates their participation in feeding behaviour in crucian carp.

- 473 *Chemical senses* 26:1139–1144.
- 474 Hamdani EH, Døving KB. 2007. The functional organization of the fish olfactory system.
- 475 *Progress in Neurobiology* 82:80–86. DOI: 10.1016/j.pneurobio.2007.02.007.
- 476 Hamdani EH, Stabell OB, Alexander G, Døving KB. 2000. Alarm reaction in the crucian carp is
- 477 mediated by the medial bundle of the medial olfactory tract. *Chemical senses* 25:103–9.
- 478 Hansen A, Anderson KT, Finger TE. 2004. Differential distribution of olfactory receptor neurons
- 479 in goldfish: Structural and molecular correlates. *Journal of Comparative Neurology*
- 480 477:347–359. DOI: 10.1002/cne.20202.
- 481 Hansen A, Finger TE. 2000. Phyletic distribution of crypt-type olfactory receptor neurons in
- 482 fishes. *Brain, Behavior and Evolution* 55:100–110. DOI: 10.1159/000006645.
- 483 Hansen A, Rolen SH, Anderson K, Morita Y, Caprio J, Finger TE. 2003. Correlation between
- 484 olfactory receptor cell type and function in the channel catfish. *The Journal of Neuroscience*
- 485 23:9328–9339. DOI: 23/28/9328 [pii].
- 486 Hansen A, Rolen SH, Anderson K, Morita Y, Caprio J, Finger TE. 2005. Olfactory receptor
- 487 neurons in fish: Structural, molecular and functional correlates. *Chemical Senses* 30
- 488 SUPPL.:2005. DOI: 10.1093/chemse/bjh239.
- 489 Hansen A, Zielinski BS. 2005. Diversity in the olfactory epithelium of bony fishes:
- 490 Development, lamellar arrangement, sensory neuron cell types and transduction
- 491 components. *Journal of Neurocytology* 34:183–208. DOI: 10.1007/s11068-005-8353-1.
- 492 Hara TJ, Zhang C. 1998. Topographic Bulbar Projections and Dual Neural Pathways of the
- 493 Primary Olfactory Neurons in Salmonid Fishes. *Neuroscience* 82:301–313.
- 494 Hara TJ, Zielinski B. 2007. *Sensory Systems Neuroscience*. Academic Press, Elsevier. DOI:
- 495 10.1016/S1546-5098(06)25006-4.
- 496 Iqbal T, Byrd-Jacobs C. 2010. Rapid degeneration and regeneration of the zebrafish olfactory
- 497 epithelium after triton x-100 application. *Chemical Senses* 35:351–361. DOI:
- 498 10.1093/chemse/bjq019.
- 499 Ito H, Yamamoto N. 2009. Non-laminar cerebral cortex in teleost fishes? *Biology letters* 5:117–
- 500 21. DOI: 10.1098/rsbl.2008.0397.
- 501 Ivanova TT, Caprio J. 1993. Odorant receptors activated by amino acids in sensory neurons of
- 502 the channel catfish *Ictalurus punctatus*. *J. Gen. Physiol.* 102:1085–1105.
- 503 Johnstone KA, Lubieniecki KP, Koop BF, Davidson WS. 2012. Identification of olfactory
- 504 receptor genes in Atlantic salmon *Salmo salar*. *Journal of Fish Biology* 81:559–575. DOI:
- 505 10.1111/j.1095-8649.2012.03368.x.
- 506 Kang J, Caprio J. 1995. In Vivo Responses of Single Olfactory Receptor Neurons in the Channel
- 507 Catfish, *Ictalurus punctatus*. *Journal of Neurophysiology* 73:172–177.
- 508 Kay LM. 2014. Circuit oscillations in odor perception and memory. *Progress in Brain Research*
- 509 208:223–251. DOI: 10.1016/B978-0-444-63350-7.00009-7.
- 510 Kay LM, Beshel J, Brea J, Martin C, Rojas-Libano D, Kopell N. 2009. Olfactory oscillations: the
- 511 what, how and what for. *Trends in Neurosciences* 32:207–214. DOI:
- 512 10.1016/j.tins.2008.11.008.
- 513 Kay LM, Stopfer M. 2006. Information processing in the olfactory systems of insects and
- 514 vertebrates. *Seminars in Cell and Developmental Biology* 17:433–442. DOI:
- 515 10.1016/j.semcdb.2006.04.012.
- 516 Kermen F, Franco LM, Wyatt C, Yaksi E. 2013. Neural circuits mediating olfactory-driven
- 517 behavior in fish. *Frontiers in neural circuits* 7:62. DOI: 10.3389/fncir.2013.00062.
- 518 Khan M, Vaes E, Mombaerts P. 2011. Regulation of the probability of mouse odorant receptor

gene choice. *Cell* 147:907–921. DOI: 10.1016/j.cell.2011.09.049.

Korsching S. 2009. The Molecular Evolution of Teleost Olfactory Receptor Gene Families. In: *Chemosensory Systems in Mammals, Fishes, and Insects*. 221–238. DOI: 10.1007/400.

Kosaka T, Hama K. 1979. Ruffed cell: A new type of neuron with a distinctive initial unmyelinated portion of the axon in the olfactory bulb of the goldfish (*Carassius auratus*). *The Journal of Comparative Neurology* 186:301–320. DOI: 10.1002/cne.901930109.

Kosaka T, Hama K. 1981. Ruffed cell: A new type of neuron with a distinctive initial unmyelinated portion of the axon in the olfactory bulb of the goldfish (*Carassius auratus*). *The Journal of Comparative Neurology* 201:571–587. DOI: 10.1002/cne.901930109.

Kress S, Biechl D, Wullimann MF. 2014. Combinatorial analysis of calcium-binding proteins in larval and adult zebrafish primary olfactory system identifies differential olfactory bulb glomerular projection fields. *Brain Structure and Function* 220:1951–1970. DOI: 10.1007/s00429-014-0765-1.

Kurahashi T, Yau KW. 1994. Olfactory Transduction: Tale of an unusual chloride current. *Current Biology* 4:256–258. DOI: 10.1016/S0960-9822(00)00058-0.

Laberge F, Hara TJ. 2001. Neurobiology of fish olfaction: A review. *Brain Research Reviews* 36:46–59. DOI: 10.1016/S0165-0173(01)00064-9.

Laurent G. 2002. Olfactory network dynamics and the coding of multidimensional signals. *Nature reviews. Neuroscience* 3:884–895. DOI: 10.1038/nrn964.

Li A, Rao X, Zhou Y, Restrepo D. 2019. Complex neural representation of odor information in the olfactory bulb. *Acta Physiologica*:e13333. DOI: 10.1111/apha.13333.

Liberles SD, Buck LB. 2006. A second class of chemosensory receptors in the olfactory epithelium. *Nature* 442:645–650. DOI: 10.1038/nature05066.

Malnic B, Hirono J, Sato T, Buck LB. 1999. Combinatorial receptor codes for odors. *Cell* 96:713–23. DOI: 10.1016/S0092-8674(00)80581-4.

Mathuru AS, Kibat C, Cheong WF, Shui G, Wenk MR, Friedrich RW, Jesuthasan S. 2012. Chondroitin fragments are odorants that trigger fear behavior in fish. *Current Biology* 22:538–544. DOI: 10.1016/j.cub.2012.01.061.

Matthews HR, Reisert J. 2003. Calcium, the two-faced messenger of olfactory transduction and adaptation. *Current Opinion in Neurobiology* 13:469–475. DOI: 10.1016/S0959-4388(03)00097-7.

Miyasaka N, Arganda-Carreras I, Wakisaka N, Masuda M, Sömbül U, Seung HS, Yoshihara Y. 2014. Olfactory projectome in the zebrafish forebrain revealed by genetic single-neuron labelling. *Nature Communications* 5. DOI: 10.1038/ncomms4639.

Miyasaka N, Morimoto K, Tsubokawa T, Higashijima S -i., Okamoto H, Yoshihara Y. 2009. From the Olfactory Bulb to Higher Brain Centers: Genetic Visualization of Secondary Olfactory Pathways in Zebrafish. *Journal of Neuroscience* 29:4756–4767. DOI: 10.1523/JNEUROSCI.0118-09.2009.

Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R. 1996. Visualizing an olfactory sensory map. *Cell* 87:675–686. DOI: 10.1016/S0092-8674(00)81387-2.

Mueller T, Dong Z, Berberoglu MA, Guo S. 2011. The dorsal pallium in zebrafish, *Danio rerio* (Cyprinidae, Teleostei). *Brain research* 1381:95–105. DOI: 10.1016/j.brainres.2010.12.089.

Nagayama S, Homma R, Imamura F. 2014. Neuronal organization of olfactory bulb circuits. *Frontiers in Neural Circuits* 8:1–19. DOI: 10.3389/fncir.2014.00098.

Nevitt GA. 1991. Do fish sniff? A new mechanism of olfactory sampling in pleuronectid

- flounders. *The Journal of Experimental Biology* 157:1–18.
- Nieuwenhuys R. 2011. The development and general morphology of the telencephalon of actinopterygian fishes: Synopsis, documentation and commentary. *Brain Structure and Function* 215:141–157. DOI: 10.1007/s00429-010-0285-6.
- Nieuwenhuys R, Donkelaar HJ Ten, Nicholson C. 1998. Chapter 15 Holosteans and Teleosts. In: *The Central Nervous System of Vertebrates Volume 2*. Springer, 900–913.
- Nikonov AA, Butler JM, Field KE, Caprio J, Maruska KP. 2017. Reproductive and metabolic state differences in olfactory responses to amino acids in a mouth brooding African cichlid fish. *The Journal of Experimental Biology* 220:2980–2992. DOI: 10.1242/jeb.157925.
- Nikonov AA, Caprio J. 2004. Odorant Specificity of Single Olfactory Bulb Neurons to Amino Acids in the Channel Catfish. *J Neurophysiol* 92:386–397.
- Nikonov AA, Caprio J. 2005. Processing of odor information in the olfactory bulb and cerebral lobes. *Chemical Senses* 30 SUPPL.:317–318. DOI: 10.1093/chemse/bjh242.
- Nikonov AA, Caprio J. 2007. Responses of Olfactory Forebrain Units to Amino Acids in the Channel Catfish Res. *Journal of Neurophysiology* 97:2490–2498. DOI: 10.1152/jn.01198.2006.
- Nikonov A a, Finger TE, Caprio J. 2005. Beyond the olfactory bulb: an odotopic map in the forebrain. *Proceedings of the National Academy of Sciences of the United States of America* 102:18688–18693. DOI: 10.1073/pnas.0505241102.
- Olivares J. 2019. Analysis of Olfactory Responses in the Telencephalon of the rainbow trout *Oncorhynchus mykiss*. PhD thesis. Universidad de Valparaiso.
- Osorio R, Schmachtenberg O. 2013. Calcium-activated chloride channels do not contribute to the odorant transduction current in the marine teleost *Isacia conceptionis*. *Journal of Fish Biology* 83:1468–1473.
- Poulin R, Marcogliese DJ, McLaughlin JD. 1999. Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *Journal of Fish Biology* 55:47–53. DOI: 10.1006/jfbi.1999.0970.
- Reisert J, Reingruber J. 2019. Ca<sup>2+</sup>-activated Cl<sup>-</sup> current ensures robust and reliable signal amplification in vertebrate olfactory receptor neurons. *Proc Natl Acad Sci USA* 116:1053–1058.
- Reutter K, Kapoor BG. 2005. *Fish Chemosenses*. Science Publishers, Enfield, USA.
- Rolen SH, Caprio J. 2007. Processing of bile salt odor information by single olfactory bulb neurons in the channel catfish. *Journal of neurophysiology* 97:4058–4068. DOI: 10.1152/jn.00247.2007.
- Rolen SH, Sorensen PW, Mattson D, Caprio J. 2003. Polyamines as olfactory stimuli in the goldfish *Carassius auratus*. *The Journal of experimental biology* 206:1683–1696. DOI: 10.1242/jeb.00338.
- Sato Y, Miyasaka N, Yoshihara Y. 2005. Mutually Exclusive Glomerular Innervation by Two Distinct Transgenic Zebrafish. *Science And Technology* 25:4889–4897. DOI: 10.1523/JNEUROSCI.0679-05.2005.
- Sato K, Sorensen PW. 2018. The chemical sensitivity and electrical activity of individual olfactory sensory neurons to a range of sex pheromones and food odors in the goldfish. *Chemical Senses* 43:249–260. DOI: 10.1093/chemse/bjy016.
- Sato K, Suzuki N. 2000. The contribution of a Ca<sup>2+</sup>-activated Cl<sup>-</sup> conductance to amino-acid-induced inward current responses of ciliated olfactory neurons of the rainbow trout. *J Exp Biol* 203:253–262.

- Satou M. 1990. Synaptic organization, local neuronal circuitry, and functional segregation of the teleost olfactory bulb. *Progress in Neurobiology* 34:115–142. DOI: 10.1016/0301-0082(90)90004-Z.
- Satou M, Ueda K. 1978. Synchronized rhythmic discharges of the secondary olfactory neurons in carp. *Brain Research* 158:313–329.
- Schmachtenberg O. 2006. Histological and electrophysiological properties of crypt cells from the olfactory epithelium of the marine teleost *Trachurus symmetricus*. *Journal of Comparative Neurology* 495:113–121. DOI: 10.1002/cne.20847.
- Schmachtenberg O, Bacigalupo J. 2004. Olfactory transduction in ciliated receptor neurons of the Cabinza grunt, *Isacia conceptionis* (teleostei: haemulidae). *European Journal of Neuroscience* 20:3378–3386.
- Schoppa NE. 2006. Synchronization of olfactory bulb mitral cells by precisely timed inhibitory inputs. *Neuron* 49:271–83. DOI: 10.1016/j.neuron.2005.11.038.
- Silva L, Antunes A. 2016. Vomeronasal Receptors in Vertebrates and the Evolution of Pheromone Detection. *Annual Review of Animal Biosciences* 5:353–370. DOI: 10.1146/annurev-animal-022516-022801.
- Sorensen PW, Caprio J. 1998. Chemoreception. In: *The Physiology of Fishes*, edited by Evands D.H. 375–405.
- Sorensen PW, Stacey NE. 2004. Brief review of fish pheromones and discussion of their possible uses in the control of non-indigenous teleost fishes. *New Zealand Journal of Marine and Freshwater Research* 38:399–417. DOI: 10.1080/00288330.2004.9517248.
- Speedie N, Gerlai R. 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). *Behavioural Brain Research* 188:168–177. DOI: 10.1016/j.bbr.2007.10.031.
- Tessarolo JA, Tabesh MJ, Nesbitt M, Davidson WS. 2014. Genomic Organization and Evolution of the Trace Amine-Associated Receptor (TAAR) Repertoire in Atlantic Salmon (*Salmo salar*). *G3-Genes|Genomes|Genetics* 4:1135–1141. DOI: 10.1534/g3.114.010660.
- Tierney KB. 2015. Olfaction in Aquatic Vertebrates. *Handbook of Olfaction and Gustation: Third Edition*:547–564. DOI: 10.1002/9781118971758.ch23.
- Valdés J, Olivares J, Ponce D, Schmachtenberg O. 2015. Analysis of olfactory sensitivity in rainbow trout (*Oncorhynchus mykiss*) reveals their ability to detect lactic acid, pyruvic acid and four B vitamins. *Fish Physiology and Biochemistry* 41:879–885. DOI: 10.1007/s10695-015-0054-9.
- Valentinčič T, Lamb C., Caprio J. 1999. Expression of a Reflex Biting/Snapping Response to Amino Acids Prior to First Exogenous Feeding in Salmonid Alevins. *Physiology & Behavior* 67:567–572. DOI: 10.1016/S0031-9384(99)00112-2.
- Valentincic T, Miklavc P, Kralj S, Zgonik V. 2011. Olfactory discrimination of complex mixtures of amino acids by the black bullhead *Ameiurus melas*. *Journal of Fish Biology* 79:33–52. DOI: 10.1111/j.1095-8649.2011.02976.x.
- Wakisaka N, Miyasaka N, Koide T, Masuda M, Hiraki-Kajiyama T, Yoshihara Y. 2017. An Adenosine Receptor for Olfaction in Fish. *Current Biology* 27:1437-1447.e4. DOI: 10.1016/j.cub.2017.04.014.
- Wanner AA, Genoud C, Friedrich RW. 2016. 3-Dimensional Electron Microscopic Imaging of the Zebrafish Olfactory Bulb and Dense Reconstruction of Neurons. *Scientific Data* 3:1–15. DOI: 10.1038/sdata.2016.100.
- Wanner AA, Genoud C, Masudi T, Siksou L, Friedrich RW. 2016. Dense EM-based reconstruction of the interglomerular projectome in the zebrafish olfactory bulb. *Nature*

- 657 *Neuroscience* 19:816–825. DOI: 10.1038/nn.4290.
- 658 Weltzien FA, Höglund E, Hamdani EH, Døving KB. 2003. Does the lateral bundle of the medial
- 659 olfactory tract mediate reproductive behavior in male crucian carp? *Chemical Senses*
- 660 28:293–300. DOI: 10.1093/chemse/28.4.293.
- 661 Whitlock KE. 2006. The sense of scents: olfactory behaviors in the zebrafish. *Zebrafish* 3:203–
- 662 213. DOI: 10.1089/zeb.2006.3.203.
- 663 Wilson RI, Mainen ZF. 2006. Early Events in Olfactory Processing. *Annual Review of*
- 664 *Neuroscience* 29:163–201. DOI: 10.1146/annurev.neuro.29.051605.112950.
- 665 Wisenden BD, Rugg ML, Korpi NL, Fuselier LC. 2009. Lab and field estimates of active time of
- 666 chemical alarm cues of a cyprinid fish and an amphipod crustacean. *Behaviour* 146:1423–
- 667 1442. DOI: 10.1163/156853909X440998.
- 668 Wullimann MF, Rink E. 2002. The teleostean forebrain: a comparative and developmental view
- 669 based on early proliferation, Pax6 activity and catecholaminergic organization. *Brain*
- 670 *research bulletin* 57:363–70.
- 671 Wullimann MF, Rupp B, Reichert H. 1996. *Neuroanatomy of the Zebrafish Brain*. Basel -
- 672 Boston - Berlin: Birkhäuser Verlag. DOI: 10.1007/978-3-0348-8979-7.
- 673 Yaksi E, von Saint Paul F, Niessing J, Bundschuh ST, Friedrich RW. 2009. Transformation of
- 674 odor representations in target areas of the olfactory bulb. *Nature neuroscience* 12:474–482.
- 675 DOI: 10.1038/nn.2288.
- 676 Yamamoto Y, Hino H, Ueda H. 2010. Olfactory imprinting of amino acids in lacustrine sockeye
- 677 salmon. *PLoS ONE* 5:1–9. DOI: 10.1371/journal.pone.0008633.
- 678 Yamamoto Y, Shibata H, Ueda H. 2013. Olfactory Homing of Chum Salmon to Stable
- 679 Compositions of Amino Acids in Natal Stream Water. *Zoological Science* 30:607–612.
- 680 DOI: 10.2108/zsj.30.607.
- 681 Yoshihara Y. 2014. Zebrafish Olfactory System. In: Mori K ed. *The Olfactory System: From*
- 682 *Odor Molecules to Motivational Behaviors*. Springer Japan, 71–96. DOI: 10.1007/978-4-
- 683 431-54376-3\_5.
- 684 Zippel HP. 1998. Mitral cells and ruffed cells. *Annals of the New York Academy of*
- 685 *Sciences*:533–534.
- 686

# Figure 1

Rainbow trout (*Oncorhynchus mykiss*) as model system for teleost olfactory research.

Fig. 1. Rainbow trout (*Oncorhynchus mykiss*) as model system for teleost olfactory research.

(A) Isolated olfactory rosette of a juvenile specimen. Inset: Exposed olfactory organs of *O. mykiss*. (B) Toluidine blue-stained cryosection of the olfactory lamellae from *O. mykiss*. Inset: Ultrastructural detail of the dendritic endings of a microvillous (m) and a ciliated (c) ORN. (C) Horizontal cryosection through the olfactory bulbs, stained with toluidine blue. (D) Transverse section through the sensory neuroepithelium, immunohistochemically labeled for Ga<sub>o</sub> (red) to mark microvillous ORNs, and for PCNA (green) to label basal cells. Modified from Bazáes et al., 2013. (E) Synchronous responses to a mixture of amino acids, recorded from the olfactory epithelium (EOG), olfactory bulb (OB), and telencephalic areas Vv and Dp in a live anesthetized specimen of *O. mykiss*. Note prominent field potential oscillations during the odor response. From Olivares 2019, PhD thesis, Universidad de Valparaiso. (F) Top: Schematic drawing of the rainbow trout brain. The telencephalic olfactory areas Dp and Vv are indicated in their respective sections. Below, ORN types, olfactory bulb circuitry and tracts to the telencephalon. ORN: Olfactory Receptor Neuron; OB: Olfactory bulb; Tel: Telencephalon; OT: Optic tectum; Cb: Cerebellum; R: Ruffed cell; MC: Mitral Cell; Gr: Granule Cell; CF: Centrifugal fiber; Vv: Ventral nucleus of ventral telencephalon; Dp: Dorsal posterior area; EOG, electroolfactogram. Scale bars: A: 1 mm, B: 300 μm; C: 500 μm, D: 100 μm.



