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Área de Psicobiología

Identificación y caracterización funcional y anatómica de las áreas del palio del telencéfalo de los peces teleósteos implicadas en procesos emocionales

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Identification and functional and anatomical characterization of the teleost telencephalic pallium areas involved in emotional processes

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Área de Psicobiología**Identificación y caracterización funcional y anatómica de las áreas del palio del telencéfalo de los peces teleósteos implicadas en procesos emocionales**

Trabajo presentado por Manuel Reiriz Rojas para optar al grado de Doctor por la Universidad de Sevilla

Los Directores,

Dr. Cosme Salas García **Dr. Emilio Durán García**

1. INTRODUCTION

1.1. The evolution of the forebrain of vertebrates.

Traditional theories about vertebrate forebrain evolution have described the evolution of this neural structure as a linear series of increasing complexity. These theories placed the fishes in the first step of the 'evolutionary leader', as the most primitive and less 'evolved' vertebrate group, followed by the amphibians, reptilians and birds, to end with the superior cerebral and cognitive stage of mammals, and specially humans, who posses new and more complex neural mechanisms and circuits which have been acquired along the successive evolutionary stages (Ariëns, et al 1936; MacLean, 1990; Noback, 1983; Papez, 1929). According with these traditional theories, the telencephalon of fishes consisted on a structure dominated by olfactory inputs, with simple circuits sustaining elemental ´instinctive´ or ´reflexive´ forms of behavior and with limited capabilities for learning and plasticity processes. Consequently, fishes should exhibit rigid and fixed behaviors rather than the flexible ones that characterize mammals and that are generally associated with the expansion of the six-layered neocortex (Ariëns et al., 1936; MacLean, 1990; Noback, 1983; Papez, 1929). However, these old theories seem to be inadequate and anthropocentric in the light of the recent data coming from the different fields of Comparative Neurosciences and Evolutionary and Developmental Biology. Thus, increasing neurobiological and psychobiological evidence challenges these traditional notions on brain and cognition evolution, showing that fishes, as land vertebrates, also show complex learning and

memory capabilities based on comparable neural mechanisms and brain systems.

The telencephalon of vertebrates is considered one of the most variable structures throughout the phylogeny (Figure 1.1). In fact, the telencephalon of vertebrates is more variable in shape, size or organization than other brain regions like the cerebellum, the mesencephalon or the spinal cord (Butler & Hodos, 2005; Nieuwenhuys, Donkelaar, & Nicholson, 1998; Northcutt, 1995) Nevertheless, recent neuroanatomical, embryological, and genetic studies show that the telencephalons of all vertebrates are comparable and that they retain a huge number of primitive features.

These studies have shown that one of the primitive features that have been preserved throughout the vertebrate forebrain evolution is the division of the telencephalon in a pallium, located in a dorsal position in the telencephalic hemispheres, and a ventrally located sub-pallium. Although in some cases the exact delimitation between the pallial and the sub-pallial boundaries is under discussion, both regions can be easily distinguished in each vertebrate group. Morphogenetic studies have been proved to be particularly relevant to determine the boundaries between the pallium and the sub-pallium in vertebrates. For example, the technique of immediate early genes expression has notably contributed to the description of the boundaries between these two domains in amphibians, reptiles, birds and mammals (Bachy, Berthon, & Rétaux, 2002; Brox, Puelles, Ferreiro, & Medina, 2003, 2004; Bulfone et al., 1999; Garda, Puelles, Rubenstein, & Medina, 2002; Smith, 1968).

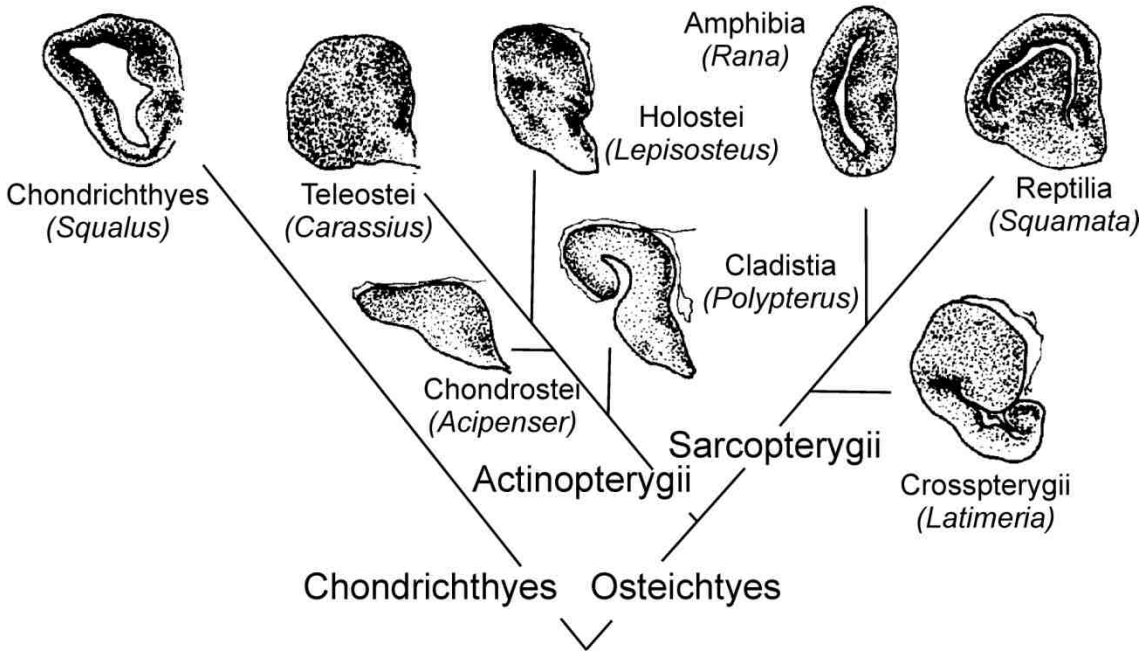


Fig 1.1. Cladogram representing transversal sections of the telencephalon of the main vertebrate radiations. Note the morphological and cytoarchitectonical variation existing among the different groups. Modified from *Northcutt, 1995; Northcutt and Braford, 1980*.

Multiple data indicate that the telencephalon of teleost and tetrapods share a basic pattern of organization with equivalent pallial and sub-pallial divisions, and even more, comparable pallial subdivisions, some of which considered homologous to the hippocampus and the amygdale of the land vertebrates (Braford, 2009; Broglio et al., 2005; Broglio, Rodríguez, Gómez, Arias, & Salas, 2010; Butler & Hodos, 2005; Nieuwenhuys et al., 1998; Nieuwenhuys, 2011; Northcutt, 2008; Salas, Broglio, & Rodríguez, 2003; Wullimann & Mueller, 2004; Yamamoto et al., 2007).

1.2. The telencephalon of the teleost fishes

Teleosts represent an extremely species-rich and diversified clade within the actinopterygian radiation, and given the large number of species and their long phylogenetic history, it is not surprising that their brain exhibit a remarkable range of variation. However, the telencephalon of actinopterygian fishes presents a unique morphological feature that makes it equal among them but quite different compared to the telencephalon of the remaining vertebrate groups. In this sense, the telencephalon of actinopterygian fishes consist of two main solid hemispheres separates by a single ventricular cavity (Braford, 1995; Nieuwenhuys, 1963; Northcutt & Kaas, 1995; Northcutt, 2006, 2008; Northcutt & Kicliter, 1980; Striedter & Northcutt, 2006), instead of the telencephalon with paired intra-hemispheric ventricular cavities that characterize non-actinopterygian vertebrates. This variation is caused by the different processes underlying the forebrain development in actinopterygians and non-actinopterygian vertebrates during the embryogenesis. Thus, whereas the forebrain of actinopterygian fishes undergoes a process of eversion or outward folding of the walls of the ispheres, the forebrain of the non-actinopterygian vertebrates develops by a process of evagination or inward bending (Figure 1.2) (Braford, 1995; Nieuwenhuys, 1963; Northcutt, 1995; Northcutt, 2006, 2008; Northcutt & Kicliter, 1980; Striedter & Northcutt, 2006).

These developmental peculiarities have traditionally hindered the aim of identifying homologies among vertebrates. However and despite of the differences between the telencephalon of actinopterygians and non-actinpterygian vertebrates, the process of differentiation of the pallial regions

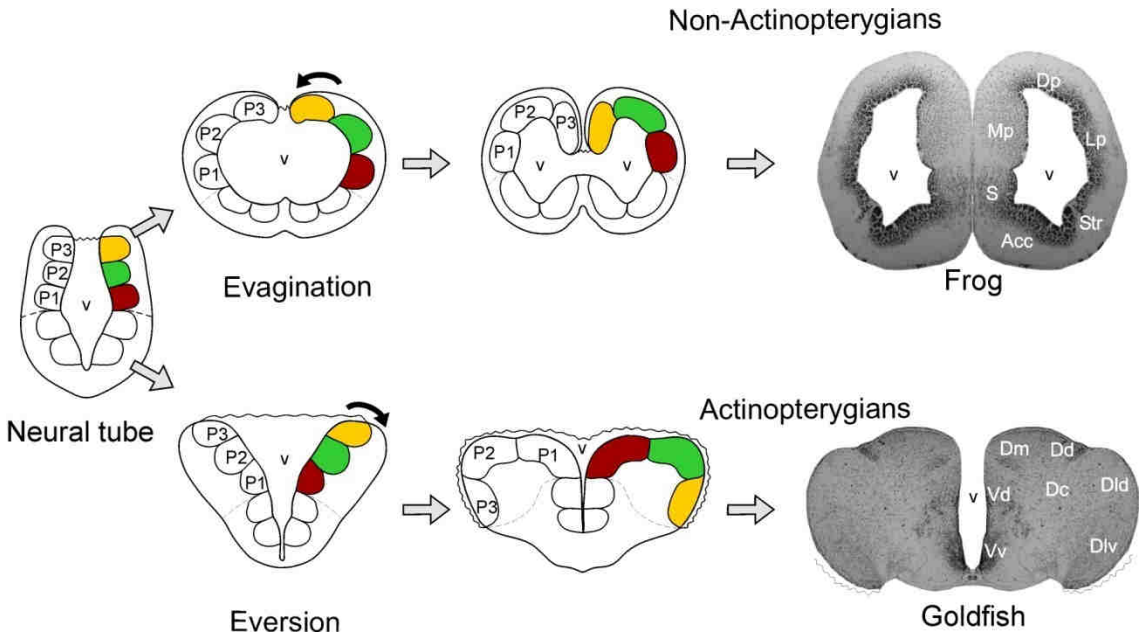
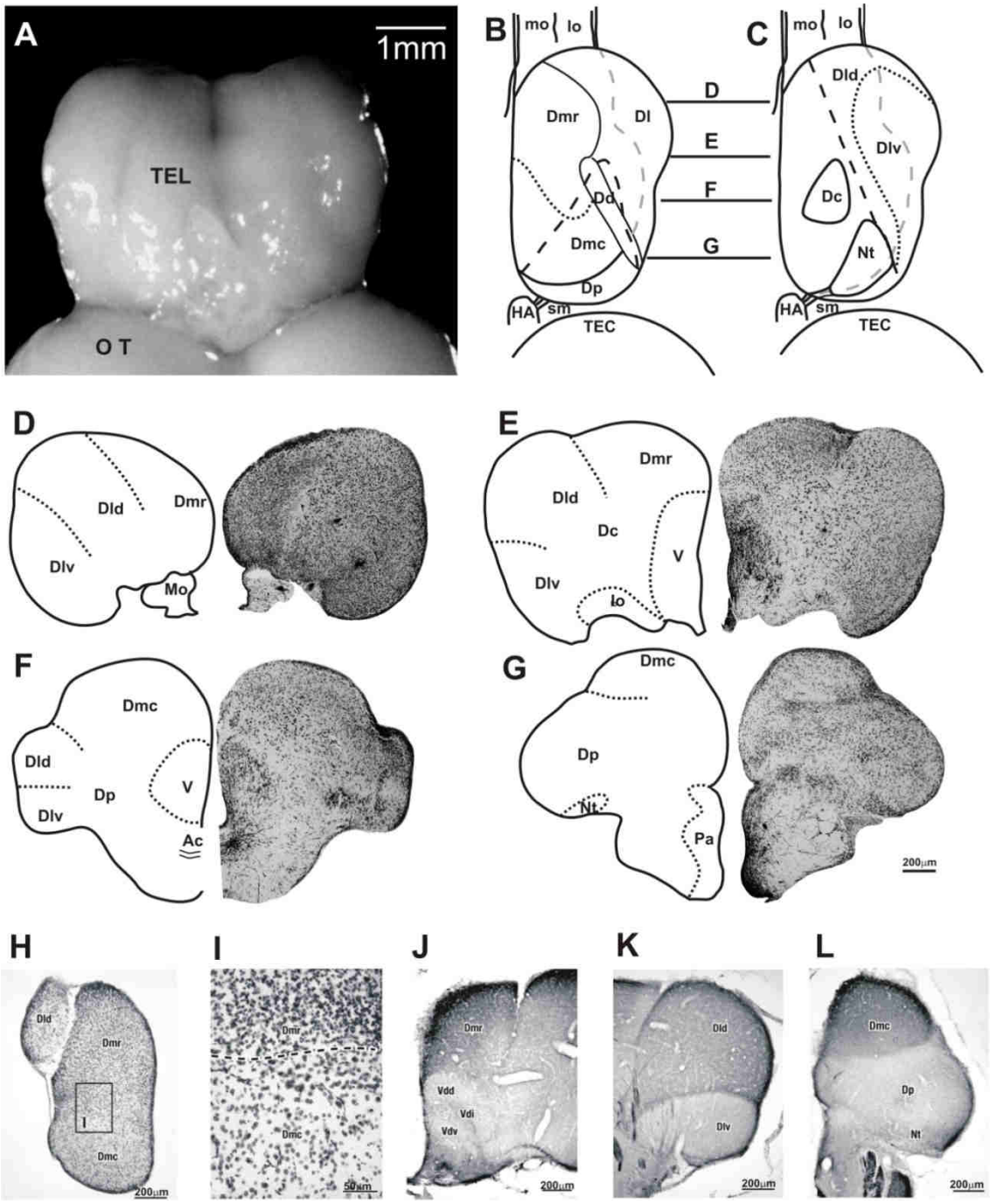


Fig 1.2. Comparison of the embryonary development of the telencephalon in non-actinopterygian and actinopterygian vertebrates. An eversion process occurs during the embryonary development of the telencephalon in actinopterygians. Due to this eversion, the telencephalon of actinopterygian fishes consist of two main solid hemispheres separated by a single ventricular cavity. The result of the evagination process in non-actinopterygian vertebrates are two ventricular cavities, each of them localized within one hemisphere. Abbreviations: Acc, nucleus accumbens; Dc, central division of the area dorsalis; Dlv, ventral subdivision of the lateral division; Dld, dorsal subdivision of the area doralis; Dm, medial division of the area dorsalis; Dp, posterior division of the area dorsalis; Lp, lateral pallium; Mp, medial pallium; P1, P2, and P3, Three main pallial subdivision; S, septal nuclei; Str, striatum; V, ventricle; Vd, dorsal nucleus of area ventralis; Vv, ventral nucleus of area ventralis. *Modified from Nieuwenhuys et al, 1998.*

occurring during the development of the vertebrate telencephalon seems to be re conservative that it was initially thought, in such a way that it is seems to be plausible that a basic organizational pattern of the telencephalon have remained constant along the vertebrate phylogeny (Wullimann & Mueller, 2004; Wullimann & Rink, 2002). It is thought that the major implication of the eversion process relative to evagination is the reversal of the topography of the telencephalic pallium. Even so, it should be to take into account that in both, actinopterygian and non- actinopterygian vertebrates, the topology of the

telencephalic pallium remains preserved. For example, it has been shown that some immediate early genes existing in both amniotes and teleost, have equal expression telencephalic domains, delimiting therefore, comparable regions in topological terms (Akimenko et al., 1994; Ganz et al., 2012, 2015; Hauptmann & Gerster, 2000; Püschel et al., 1992) Particularly, these studies have indicated that the dorsal and the ventral subdivision of the telencephalon of teleost fishes correspond to the pallium and the subpallium of land vertebrates, respectively. More precisely, the pallium of teleost fish has been traditionally subdivided in medial, dorsal and lateral regions (Butler & Hodos, 2005; Karten, 1997; Medina & Reiner, 2000; Nieuwenhuys et al., 1998; Northcutt, 1981, 1995; Northcutt & Kaas, 1995) which, in turn, have been considered homologous to the three main pallial divisions of the tetrapod telencephalon (Braford, 1995; Butler & Hodos, 2005; Northcutt, 1995; Wullimann & Rink, 2002) .

Fig 1.3. Cytoarchitectonics of the goldfish (*Crassius auratus)* telencephalic pallium. **A.** Photography of a dorsal view of the goldfish telencephalon. **B-C.** Reconstruction of the dorsal surface of the telencephalic pallium. In **B**, all the pallial division can be identified from a dorsal view, but only the rostral (Dmr) and caudal (Dmc) subdivision of the medial division can be seen in their entirely. The boundary between these two Dm region is marked by the dot line. From the surface, only the caudal pole of Dp can be seen. This structure lies beneath Dm and its boundary is marked by a dashed line. The dorsal division of the dorsal pallium in goldfish (Dd) separates Dm from Dl along the rostrocaudal axis. The dashed gray line marks the attachments of the tela choroidea. The full extent of the other pallial divisions can be visualized in a reconstruction that does not include Dm, Dd or Dp (**C**). In this reconstruction, the boundary of the large-celled subdivision of caudal Dm (Dc) can be indentified medially and a dashed oblique line marks the dorsomedial border of Dl. The lateral pallial division in goldfish can be subdivided in a ventral (Dlv) and dorsal (Dld) subdivisions. The dotted line marks the boundary between these two structures. As in the reconstruction before, the attachment of the tela choroidea is indicated by the dashed gray line. **D-G.** Photomicrographs of cresyl violet-stained transverse sections that correspond with the lines drawings in figures B and C. **H-I.** Photomicrography of horizontal sections where differences in cell density and size are shown between the rostral and caudal subdivisions of Dm. **J-L.** Photomicrography of coronal sections showing the calretinin distribution in Dmr (**J**), Dl (**K**) and Dmc (**L**). Abbreviations: Dc, large-celled subdivision of Dm; Dd, dorsal division of area dorsalis; Dld, dorsal subdivision of lateral division of area dorsalis; Dlv, ventral subdivision of lateral division of area dorsalis; Dmc, caudal part of medial subdivision of area dorsalis; Dmr, rostral part of medial subdivision of area dorsalis; Dp, posterior division of area dorsalis; Ha, habenula; Io, lateral olfactory tract; mo, medial olfactory tract; Nt, nucleus taeniae; Pa, preoptic area; sm, stria medullaris; OT, optic tectum; TEL; telencephalon; V, area ventralis; ac, anterior commisure. *Modified from Northcutt, 2006.*



1.3. Cytoarchitectonics of the goldfish telencephalic pallium

The telencephalic pallium or area dorsalis telencephali in goldfish can be divided into five regions: medial (Dm), dorsal (Dd), central (Dc), lateral (Dl) and

posterior (Dp) (Braford, 2009; Mueller & Wullimann, 2009). Figure 1.3 illustrates the pallial subdivisions in goldfish.

The medial area of the dorsal pallium (Dm) in goldfish is the closest area to the subpallium and is continued with the Vs at the commissural level (Braford, 1995; Braford, 2009). According to Northcutt (2006), Dm is cytologically characterized by a superficial layer of packed granule-like cells overlying a core of larger cells whose size gradually increases with depth. Nevertheless, the area where deeper cells are located in Dm has traditionally been designated as a separate, central pallial division (Dc). Dm in goldfish can be divided in two main regions: rostral (Dmr) and caudal (Dmc). Although both regions have a moderate concentration of calretinin in its neuropil, a superficial layer of cells that are much larger in Dmc than the superficial granulate -like cells of the rostral Dm easily distinguishes these two regions.

The pallial division Dl can be easily separated from Dm by the sulcus ypsiloniformis and citoarchitectonically by a marked increase in the cell size that characterizes its medial border. More caudally, Dl is distinguished from Dm due to the presence of an area composed by densely packed medium sized cells, which forms Dd (Northcutt, 2006). Thus, Dl is found along the entire dorso-ventral axis and in media-lateral axis Dl comes to occupy about 2/3 of the telencephalon from the most lateral border until the ypsiloniformis sulcus. Therefore Dl is, as in other teleosts, one of the largest areas of the pallium of the goldfish and it is easy to distinguish due to it is close proximity to ypsiloniformis sulcus.

Unlike most pallial divisions, Dl has the largest cells in a medial segment, not in the core (Northcutt, 2006) and can be divided in two main regions, dorsal (Dld) and ventral (Dlv). Unlike the ventral subdivision, the dorsal subdivision is characterized by a moderate concentration of calretinin (Northcutt, 2006), as well as by large cells that decrease in size with depth. It is easy to distinguish the border between Dld and Dlv in a caudal position due to a shallow sulcus between the two regions although this border is hard to distinguish in the rostral pole. In addition, the ventral subdivision of Dl can be divided in a dorsal and ventral part. A large region of scattered cells forms the dorsal part of the ventral subdivision of Dl, whereas the ventral part is formed by a smaller region of densely packed granule-like cells.

In transverse sections, the two parts of the ventral subdivision of Dl, as well as the dorsal subdivision of Dl are easily distinguished at the level of the anterior commissure. Dld and Dlv drop off rapidly in size as they are traced caudally to the anterior commissure. At this level, only the outer layers of the ventral subdivision of Dl can be distinguished. At a caudal telencephalic level any subdivision of Dl can be fully appreciate, and the ventral caudal pole is dominated by a superficial ring of medium sized cells surrounding a core of large and densely packed neurons that characterized Dp. The border between Dlv and Dp could be the harder border to distinguish in the goldfish pallium, but the transition from Dlv to Dp is much more evident in the horizontal plane. In this plane, the caudal pole of Dlv is marked by different sulcus, and there is actually a narrow, cell-free zone between Dlv and Dp (Northcutt, 2006).

1.4. Recent hypothesis on the pallial homologies between teleost and land vertebrates

The telencephalic pallium of teleost shows a notable structural variety. In fact, different subdivisions of its five main divisions have been described in different teleost species (Burmeister, Munshi, & Fernald, 2009; Giassi, Harvey-Girard, Valsamis, & Maler, 2012; Nieuwenhuys & Meek, 1990; Northcutt, 2006), which makes difficult the identification of homologies among these areas and the pallial nuclei in other vertebrate species. In addition, the different interpretations of the eversion process (See Nieuwenhuys, 2011) and the neurochemical and connectional data of the implied regions (Braford, 1995; Folgueira, Anadón, & Yáñez, 2004a; Kaslin & Panula, 2001; Mueller, Dong, Berberoglu, & Guo, 2011; Nieuwenhuys & Meek, 1990; Northcutt, 2006, 2008; Wullimann & Mueller, 2004; Yamamoto et al., 2007) have lead to the proposal of different models of homologies, most of them proposing conflicting or partially conflicting ideas (Figure 1.4).

The “partial pallial eversion model” (Mueller, Dong, Berberoglu, & Guo, 2011; Mueller & Wullimann, 2009; Wullimann & Mueller, 2004) based on connectional and gene expression data, proposes a partial eversion followed by a cellular migration. The “eversion-rearrangement theory” by Northcutt and Bradford suggests that differential expansion of the ventricular surface of some pallial zones and the differential proliferation and migration of neuroblasts from the different ventricular zones might result in displacement or shifting of the different pallial subdivisions (Braford, 1995, 2009; Northcutt & Kicliter, 1980). In the “new eversion model”, the eversion was suggested to occur in a

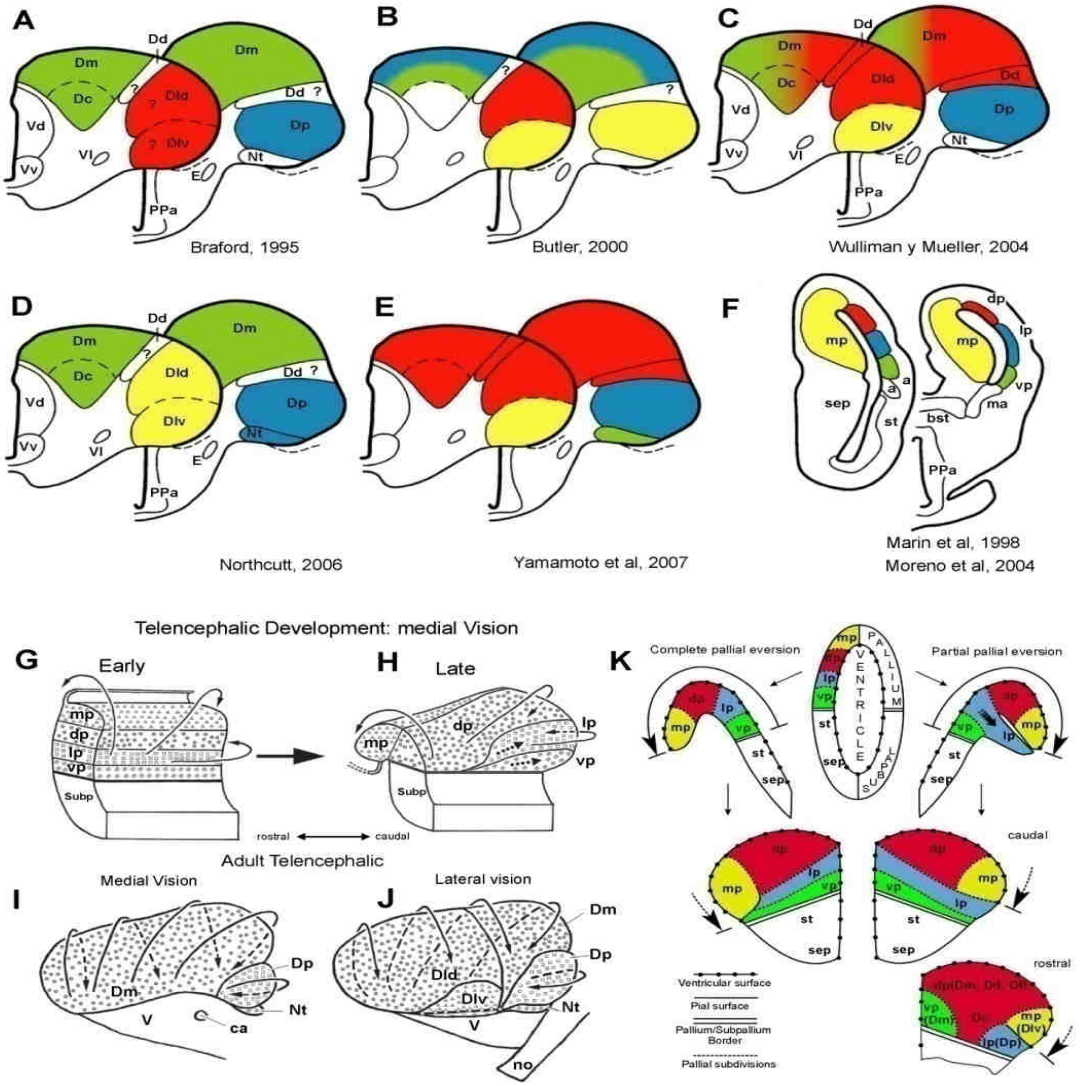


Fig 1.4. Recent hypothesis about pallial homologies in teleosts. Each hypothesis is illustrated using a rostral and a caudal section of the right telencephalic hemisphere of goldfish (**A-E**) using a color code which represents the possible homologous regions with a representative amphibian (*Rana*, **F**). Question mark indicate uncertainty regarding the homology. (**G-J**) Schematic representations showing the eversion hypothesis proposed by yamamoto et al (2007). (**G**) Early development stage when the pallial organization is still similar to other vertebrates. (**H**) Late stage of telencephalic organization if the pallial development followed the direction of the arrows. (**I-J**) Medial (**I**) and lateral (**J**) view of adult goldfish telencephalon. (**K**) Schematic comparison of the complete pallial eversion versus the incomplete eversion model proposed by Wulliman and Muller, 2004. Abbreviations: aa, anterior amygdaloid area; ac anterior commissure; Bst, bed nucleus of the stria medullaris; Dc, large-celled subdivision of Dm; Dd, dorsal division of area dorsalis; Dld, dorsal subdivision of lateral division of area dorsalis; Dlv, ventral subdivision of lateral division of area dorsalis; Dm, medial subdivision of area dorsalis; dp, dorsal pallium; Dp, posterior division of area dorsalis; E, emtopeduncular nucleus; lp, lateral pallium; ma medial amygdala; mp, medial pallium; Nt, nucleus teniae; on, optic nerve; PPa, anterior parvocellular preoptic nucleus; Subpsubpallial region; sep, septum; st, striatum; V, area ventralis telencephali; Vd, dorsal nucleus of area ventralis; vp, ventral pallium (lateral amygdala); Vv, ventral nucleus of area ventralis. *Modified from Northcutt, (2008); Yamamoto et al, (2007) and Wullimann and Muller, (2004).*

caudolateral direction leading to a shift of the arrangement of the different pallial subdivisions(Yamamoto et al., 2007). Yamamoto and colleagues propose a complex eversion that implies cell reorganization in the pallial areas, meaning that ventral pallial and lateral pallial homologous are not present in the rostral but only in the caudal pallium. Finally, (Butler, 2000) and (Nieuwenhuys, 2011) claim that the teleost telencephalon develops by a simple eversion process.

Although differences exist among these hypotheses in the interpretation on the pallial development, there is a general consensus that at least some pallial structures in the telencephalon of actinopterygians are homologous to specific regions in the cortex of tetrapods. Thus, Dlv, the ventral part of the lateral pallium subdivision (Dl) is considered homologous to the medial or hippocampal pallium of amphibians and other tetrapods (Northcutt, 2006; Portavella, Vargas, Torres, & Salas, 2002; Rodríguez et al., 2002; Yamamoto et al., 2007). What it is still under discussion is whether the entire Dl, i.e., Dld plus Dlv, or by contrast Dlv exclusively, are comparable to the hippocampal pallium. In this regard, Northcutt suggests that the hippocampal pallium in actinopterygians also involves the dorsal part of the lateral pallium subdivision (Dld). However, Butler, Wullimann and Mueller (2000), and Yamamoto and colleagues (2007) claim that Dld is homologous to the isocortex and therefore, that only Dlv might be considered homologous to the hippocampal pallium of actinopterygians. In addition, some authors (Butler, 2000; Nieuwenhuys, 2011) have proposed that the posterior region of pallium (Dp), a region commonly considered homologous to the piriform cortex on the basis of it massive olfactory inputs (Mueller and wulliman, 2009; Northcutt, 2006; Yamamoto et al.,

2007), is actually a specialized part of the medial pallium or hippocampus.

Although with the exception of Yamamoto and co-workers, there is also a general consensus that at least part, if not all, of Dm of teleosts is homologous to the pallial amygdala of amphibians and other tetrapods. This hypothesis is based primarily on topological, genetic, and connectional data and assumes that Dm or at least part of it, is derived from the ventral pallium and therefore, that constitutes a limbic area lying between the subpallium and the olfactory pallium (Braford, 1995; Northcutt & Kicliter, 1980; Puelles & Rubenstein, 1993; Wullimann & Rink, 2002). However, Yamamoto and co-workers (Ito & Yamamoto, 2009; Yamamoto et al., 2007; Yamamoto & Ito, 2008. See figure 1.4) propose that Dm, Dd and the dorsal part of Dl should be homologous to the dorsal pallium of amphibians and the isocortex of tetrapods. This assumption is based on the evidence that Dm and Dl receive their major inputs from the preglomerular complex, which has been proposed as homologous to part of the dorsal thalamus of tetrapods (Yamamoto et al., 2007). Nevertheless, other authors suggest that the preglomerular complex has multiple embryonic origins and remain skeptical about that homology (Northcutt, 2008). Finally, Nieuwenhuys (2009) proposed that Dm is homologous to the lateral pallium based on topology.

1.5. Anatomical and functional organization of Dm of teleost fishes

As we previous mentioned, there is a wide consensus between the comparative neuroanatomists that at least part of Dm of teleost fish is

homologous to the amygdala of tetrapods, although some discrepant views has been suggested. For example, based in the pattern of connectivity, Yamamoto and colleagues (2007) have proposed that Dm, together with the dorsal part of Dl, must be considered homologous to the isocortex of tetrapods.

The amniote amygdala is an structure located in the anterior region of the temporal lobe that is critical for emotional learning and memory (LeDoux, 2000; Maren, 2001; McGaugh, 2004). From an anatomical point of view, the amygdala is formed by set of nuclei that include the basolateral complex, subdivided into the lateral, basolateral and basomedial nucleus; the extended amygdala, which include the central and medial amygdala and the bed nucleus of the stria terminalis; and the intercalate cell masses (Duvarci & Pare, 2014; Janak & Tye, 2015; Sah, Faber, Armentia, & Power, 2003). Functionally, the amygdaloid complex in amniotes can be subdivided in olfactory, frontotemporal, and autonomic systems (Swanson & Petrovich, 1998). The olfactory system can be divided in two different regions. Firstly, an accessory olfactory (or vomeronasal) component dominated by the medial amygdala (MeA). This region is a key for the perception of pheromonal stimuli involve in recognition (Newman, 1999) and detection the odors of predators (Canteras, 2008). The second region of the olfactory system is the main olfactory component, dominated in mammals by the posterior basolateral amygdala, the basomedial amygdala, and the posterior amygdala (Swanson & Petrovich, 1998). The origins of these two regions are different. Thus, the first subdivision has a subpallial origin whereas the second subdivision has a ventropallial origin (Brox

et al., 2004; Bupesh, Legaz, Abellán, & Medina, 2011; García-López et al., 2008; Waclaw, Ehrman, Pierani, & Campbell, 2010).

The second amygdala complex component suggested by Swanson and Petrovich (1998) is the frontotemporal amygdaloid system. This subdivision has a ventropallial origin (Brox et al., 2004; Waclaw Ehrman, Pierani, & Campbell, 2010). This system is composed by the basolateral amygdala (BLA) and the lateral amygdala (LA). The third component of the amygdaloid complex is the autonomic amygdala. This component is formed by the central extended amygdala (CEXA), composed of central amygdala (CeA) and bed nucleus of the stria teminalis (BNST) which has a subpallial in origin.

Numerous studies demonstrated that the amygdala of amniotes shares its basic developmental, hodological and neurobiological features (Moreno & Gonzalez 2007). In addition, homologous territories of all the main amygdaloid subdivisions have been recognized in amniotes characterized by the expression of common patterns of developmental genes (Medina., et al 2002; Moreno & González 2006). In addition the organization of the anamniote amygdaloid complex is also conserved in its main essential features, supporting the idea that the basic plan of organization is shared in mammals, birds, reptiles, and also in amphibians (Moreno & Gonzalez, 2007).

1.5.1. Hypothesis regarding the homology of Dm

As result of the eversion process, the medial region of the area dorsalis telencephali (Dm), topographically correspond to the lateroventral pallium of non-everted brains has it is located at the boundary between the dorsal and the

ventral telencephalon (Maximino, Lima, Oliveira, Batista, & Herculano, 2013; O'Connell & Hofmann, 2011; von Trotha, Vernier, & Bally-Cuif, 2014). Accordingly, several studies support the idea that Dm correspond to the amniote pallial amygdala based on the conexion pattern, gene expression, developmental and comparative evidences, neurochemical distribution, and behavior (Braford, 2009; Desjardins & Fernald, 2010; Mueller & Wullimann, 2009; Nieuwenhuys, 2009; Northcutt, 2006, 2008; Portavella, Torres, & Salas, 2004; Wullimann & Mueller, 2004).

Although a complete vomeronasal system is not present in bony fishes (Døving & Trotier, 1998), they are able to detect social cues, food odors, sex pheromones and skin extract from conspecifics (Ubeda-Bañon et al., 2011). In this sense, it has been described that the ventral part of the precommisural Dm of the trout receives direct inputs from the olfactory bulb (Folgueira et al., 2004a). This cells projecting to this subregion of Dm from the medial portions of the olfactory bulb are calretinin-positive (Gayoso, Castro, Anadón, & Manso, 2011). In crucian carps (*Carassius carassius*) this subregion of Dm has neurons selective and sensitive for skin extract (Hamdani & Døving, 2003; Lastein, Hamdani, & Doving, 2008). These hodological and functional data suggest that the ventral subdivision of the precommisural Dm is part of a putative fish vomeronasal system (Maximino et al., 2013). In fish (and amphibians) the differences between the frontotemporal and the olfactory amygdaloid system has not been yet described and it seems to be more adequate to consider the pallial amygdala as a single multisensorial region which receives olfactory information as well as polymodal inputs from brainstem and thalamus (Davies,

Martínez-García, Lanuza, & Novejarque, 2002; Martínez-García, Novejarque, & Lanuza, 2007; Medina, Bupesh, & Abellán, 2011; Moreno & González, 2006, 2007).

In addition the characterization of the distribution of markers in the telencephalon of teleost fish, as for example the cannabinoid receptor gene Cb1, a marker of basolateral amygdala in mammals (Mailleux & Vanderhaeghen, 1992; Matsuda, Bonner, & Lolait, 1993) support also the homology between the amniote pallial amygdala and Dm (Harvey-Girard, Giassi, Ellis, & Maler, 2013; Lam, Rastegar, & Strähle, 2006; Ruhl, Moesbauer, Oellers, & Emde, 2015). On the other hand, different markers distribution support the idea that Vs and Vp of Zebrafish larvae could be homologous to the centromedial amygdala and bed nucleus of the stria terminalis (Mueller, Wullimann, & Guo, 2008). The pattern of distribution of other markers molecules supports also the hypothesis of homology of Dm with the pallial amygdala. For example, while CRF expression is lacking in Dm (as occur in frontotemporal amygdala), urotensin I expression is moderate in the rostral but no in the caudal area of the zebrafish Dm (Alderman & Bernier, 2007). Moreover, in goldfish vasotocin inmunoreactive fibers terminate in Dm just rostral to the anterior commissure (Thompson & Walton, 2009). In addition, in the catfish, low levels of cocaine and amphetamine regulated transcript peptide (CART), a peptide enriched in the medial amygdala of rodents (Olmos et al., 2004) are found in Dm (Subhedar, Barsagade, Singru, Thim, & Clausen, 2011). These peptides are expressed or secreted at neurons of the vomeronasal amygdala of

mammals, but are also present in the BLA (Koylu, Couceyro, Lambert, & Kuhar, 1998; Sofroniew, 1980).

1.5.2. Hodological data

Hodological data regarding Dm in teleost fish has been obtained in trout (Folgueira et al., 2004a) and goldfish (Northcutt, 2006; Yamamoto et al., 2007). In mammals, the amygdala receives primary sensory information. The lateral nucleus of the amygdala receives visual, auditory, somatosensory, olfactory and gustatory inputs from the cortex and thalamus (LeDoux, 2007; Swanson & Petrovich, 1998). Similarly, it has been demonstrated in different teleost fishes that Dm received olfactory, gustatory, auditory, visual and mechanosensory information (Folgueira, Anadón, & Yáñez, 2004b; Northcutt, 2006; Yamamoto et al., 2007). Moreover, as occur in mammals, the putative homologue region of the hippocampus in fish, Dl, projects to Dm (Northcutt, 2006). The projections from the anterior part of Dm extend to pre- and postcommissural portions of Dm (Maximino, Silva, Gouveia, & Herculano, 2011). This area could be thus considered the interface between the putative basolateral and central amygdala and expresses transcription factors that are markers of the intercalated cell masses (Shah, Medina-Martinez, Chu, Samaco, & Jamrich, 2006; Waclaw et al., 2010; Zerucha & Prince, 2001). Finally, the pattern of afferences of Dm suggests that there are no differences between accessory and olfactory amygdaloid system in teleosts (Maximino et al., 2013; Swanson & Petrovich, 1998).

1.5.3. Behavioral data

Dm receive stimulus from different sensory modalities, including nociceptive information. Thus, galvanic stimulation of the tail at noxious intensities produces potentials in Dm in salmons (Nordgreen, Horsberg, Ranheim, & Chen, 2007). Some studies suggest the existence of a spino-parabrachial-amygdalar pathway in teleost involved in aspects of nociception (Bernard et al., 1996). Moreover, a C-fos (a marker of stimulus induced neural activity) inmunoreactivity is increased in Dm in Chinese mudskippers (*Periophtalmus cantonensis*) when these animals are agitated by stirring the water (Wai, Lorque, Webb, & Yew, 2006), and increased of C-fos mRNA in Dm has been described in zebrafish after the animal exposure to a light/dark box, a procedure that is considered anxiogenic for this species (Lau, Mathur, Gould, & Guo, 2011).

Lesion studies suggest that Dm is essential for the acquisition of fear conditioning. Thus, lesions in caudal Dm produce deficits in the habituation of the startle response in *Rutilus rutilus* (Laming & McKee, 1981) and goldfish (Rooney & Laming, 1986). Nevertheless, an opposite results was observed in *Betta splendens* (Marino-Neto & Sabbatini, 1983). Lesions in Dm but not in Dl impair the acquisition of active conditioned avoidance response in goldfish (Portavella, Salas, Vargas, & Papini, 2003; Portavella, Torres, Salas, & Papini, 2004). These results are similar than those obtained in mammals after amygdalar lesions (Aggleton, Kentridge, & Sembi, 1992; Davis, 1992) and suggest that Dm could have an important role in emotional learning and

memory as it occurs with the amygdala of mammals (Kim & Jung, 2006; Maren, 2001; Medina, Repa, Mauk, & LeDoux, 2002; Parkes & Westbrook, 2010).

Taste aversion learning in goldfish is also dependent of Dm. In an experiment from our laboratory (Martín, Gómez, Salas, Puerto, & Rodríguez, 2011) goldfish learn to avoid the ingestion of a flavor paired with visceral discomfort, when trained in a delayed procedure consisting of the presentation of two flavors on different days, one followed by lithium chloride (associated with the discomfort) and other by saline, both after 10 minutes delay. Animal with lesions in Dm were no able to learn the task. In contrast, when the damage was in Dl (the region homologous to the hippocampus) there was not effect in the acquisition of the taste aversion procedure. Thus, the Dm lesion in goldfish produces a similar effect on the taste aversion procedure than that produced by the lesion of the amygdala in mammals (Bermúdez-Rattoni et al., 2004 Bernstein et al., 1999; Lamprecht and Dudai, 2000; Yamamoto et al., 1994). In this sense, neuroanatomical data show that gustatory and visceral inputs converge in the Dm, supporting the taste aversion learning (Folgueira, Anadón, & Yáñez, 2003; Folgueira et al., 2004a; Northcutt, 2006; Yoshimoto & Yamamoto, 2010) and suggesting that this region, like the amygdala of mammals, could be a site for the taste malaise integration in teleosts.

Altogether, these functional data support the hypothesis that Dm in teleost fish, as the amygdala in mammals, is essential for emotional learning and memory system, and provide further support concerning the hypothesis of homology between these both structures (Davis & Whalen, 2001; Fanselow &

Ledoux, 1999; Kim & Jung, 2006; LaBar et al., 1998; Lavond et al., 1993; LeDoux, 1993; 2000; Maren, 2001; Medina et al., 2002; Parkes and Westbrook, 2010; Phelps et al., 2004).

Thus, multiple anatomical, hodological, genetic and functional data support the idea that Dm is homologous to the amygdala in tetrapods, and only Yamamoto and colleagues (2007) disagree with this hypothesis. Nevertheless, it is not clear if the entire Dm can be considered homologous to the amygdala or only a specific subregion of it should be considered as such. In this sense, according to Northcutt (2006) Dm is a homogeneous region of the area dorsalis telencephali that could be subdivided in two main subregions: Dmr and Dmc. Both subregions have similar calretinin concentration and projections. Both, Dmr and Dmc received projections from the suprachiasmatic nucleus, all preglomerular nuclei and the posterior thalamic nucleus. On the other hand, both structures project to the anterior tuber, hypothalamus and nucleus diffusus. These similarities support the idea that Dm, as a unitary entity (Dmr + Dmc), is homologous to the amygdala.

In contrast, Butler (2000), and Wulliman and Muller (2004) suggest that not all the Dm region would be homologous to the ventral pallium of the amniotes but only a part of it. Supporting this proposal, a recent study in our lab (Rodriguez-Expósito, 2014) suggest that only the medial and precommisural region of the medial pallium in goldfish is critically involved in the acquisition of fear classical conditioning and plays a role similar to that of the amygdala in mammals (Blanchard, 1972; Cousens and Otto, 1998; Iwata et al., 1986;

LeDoux, 1993; Maren et al., 1996; Sananes & Davis, 1992). Thus, this study suggests the possibility that not the entire Dm, but only a restricted subregion of it, would be considered homologous to the amniote pallial amygdala. In this sense, based on calretinin-like immunoreactivity, Castro et al (2003) have identified at least four zones in Dm of trouts along the rostrocaudal axis. The zebrafish telencephalon is also heterogeneous in the dorso-ventral axis and shows different markers which clearly distinguish a dorsal and ventral region (castro et al.,2006).

1.6. General objectives

The main objective of the present work was to further investigate the functional identity of the dorsomedial subdivision of the area dorsalis telencephali (Dm) of goldfish. Particularly, we analyzed the possible involvement of this region in the nociceptive somatosensory processing and in the generation of affective and emotional states associated with noxious stimulation and pain. Special emphasis was done in the identification of differentiated functional subregions within the Dm area. The implication of the functional results obtained in this work will be discussed in the light of the different hypotheses of homology about the telencephalic pallial areas of teleost fish in order to clarify the bases of the evolution of the telencephalic pallium in vertebrates.

Four experiments were included in the present doctoral dissertation manuscript. In Experiment 1, we used voltage-sensitive dye imaging to map the areas of goldfish telencephalic pallium involved in the processing of nociceptive information. In Experiment 2, we used intracerebral electrical microstimulation to map systematically the surface of the telencephalic pallium in order to identify the areas involved in the generation of motor and visceral components of escape, fearful-like, emotional responses. In Experiment 3, we used a conditioned place aversion procedure (CPA) to behaviorally study whether the activation by means of intracerebral electrical stimulation of the pallial areas identified in the Experiment 2 produced unpleasant, emotionally negative, or fearful states in goldfish. Finally, in Experiment 4, we tested, by means of

selective pallial lesions in goldfish trained in a conditioned place aversion procedure, if the pallial areas identified in the Experiments 1, 2 and 3 are critical for the generation of the affective and fear components of the nociceptive stimulation involved in fear conditioning.