Do Teleost Fishes Possess a Homolog of Mammalian Isocortex?

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Over half of the world's living vertebrates are ray-finned fishes, which are generally divided into two sister groups: the chondrosteans and the neopterygians. The chondrosteans are essentially living relicts and they consist of two genera of polypteriform fishes (bichirs and reedfishes) and six genera of acipenseroid fishes (paddlefishes and sturgeons). The neopterygian fishes consist of gars, bowfins, and teleosts, the last being one of the world's most successful radiations, with approximately 30,000 living species.

Telencephalic organization in ray-finned fishes forms a morphocline of increasing complexity, from polypteriforms, where the roof (pallium) of the telencephalon consists of a periventricularly located layer of neurons, to teleosts, where the pallium can be divided into a complex series of migrated cell groups (fig. 1).

For a number of years, all studies of the telencephalon in teleosts have used a nomenclature modified from Nieuwenhuys [1962a, b, 1963], who termed the pallium the area dorsalis (D) and the subpallium the area ventralis (V). He further divided the area dorsalis into medial (Dm), dorsal (Dd), lateral (Dl), and central (Dc) parts. Northcutt and Braford [1980] reexamined the pallium in ray-finned fishes and corroborated the nomenclature of Nieuwenhuys [1962a, b, 1963] but described a fifth pallial division which receives the bulk of the secondary olfactory projections to the

pallium. They termed this division the pars posterior (Dp), and it has been recognized in most subsequent studies (fig. 1).

Subsequently, Braford [1995] suggested that Dc is not a primary pallial division but simply the deeper, larger cells of several of the primary pallial divisions. The conclusion of Braford [1995] has been supported by several subsequent studies [Northcutt, 2006, 2008, 2011; Braford, 2009; Nieuwenhuys, 2010]. Although these large, migrated pallial cells have been described in a number of ways, the simplest definition is probably that of Nieuwenhuys [1963]: even if they do not form a primary pallial division, they nevertheless comprise the most migrated zone of the overlying pallial divisions with which they are associated and from which they are presumed to have originated during development (fig. 1).

There is considerable information on the connections of Dm, Dl, and Dp and the behavioral deficits following their ablation [Murakami et al., 1983; Portavella et al., 2002; Rodríguez et al., 2002; Folgueira et al., 2004; Yamamoto and Ito, 2005; Northcutt, 2006], and there is an emerging consensus that Dm is homologous to the pallial amygdala, Dl is homologous to the medial pallium, and Dp is homologous to the lateral pallium in terrestrial vertebrates [Northcutt, 2008; Braford, 2009; Mueller et al., 2011].

No such consensus exists for Dd and its associated migrated large cell population

Fig. 1. Schematic transverse section through one telencephalic hemisphere of a percomorph teleost. The more heavily outlined segment of the hemisphere indicates the ependymal surface, whereas the more thinly outlined segment of the hemisphere indicates the pial surface. Dc-d = Part of Dc associated with Dd; Dc-l = part of Dc associated with Dl; Dc-m = part of Dc associated with Dm; Dc-p = part of Dc associated with Dp; Dd = pars dorsalis of area dorsalis; Dl = pars lateralis of area dorsalis; Dm = pars medialis of area dorsalis; Dp = pars posterior of area dorsalis; sy = sulcus ypsilonformis; tc = tela chorioidea; V = area ventralis.

(Dc-d), however. The pallial division Dd is usually defined by its topological position between Dm and Dl and by its initial cytological description as a region of clustered, closely packed cells [Nieuwenhuys, 1959]. Not surprisingly, different cell groups in the pallium of teleosts appear to have been identified as Dd. Furthermore, there are no experimental studies reporting results of tracer injections confined to Dd or its associate, Dc-d. Both the position and connections of Dd (and Dc-d) thus remain problematic in teleosts.

For this reason, the recent study by Mueller et al. [2011] provides fascinating new data regarding Dd and its associate, Dc-d. Mueller et al. [2011] examined pallial development in the zebrafish, *Danio rerio*, and claimed that differential expression of NADPHd and parvalbumin reveals four pallial divisions: Dm, Dc, Dl, and Dp. They noted that, rostrally, all of the Dc appears to arise from a periventricular germinative zone located between that of Dm and that of Dl. Subsequently, the dorsomedial edge of Dl and the dorsolateral edge of Dm appear to overgrow the developing Dc, with Dl and Dm becoming secondarily adjacent to one another and bordered by the sulcus ypsilonformis. Furthermore, they concluded that Dd is not a primary pallial division but, rather, results from the overgrowth of Dl and Dm, as Dc is displaced centrally. Because the germinal zone from which Dc arises occupies a position comparable to that of the dorsal pallium in other vertebrates, the authors concluded that Dc in teleosts is homologous to the isocortex (neocortex) in mammals. As do many new studies, that of Mueller et al. [2011] raises as many new questions as it provides answers to old ones. In this case, these questions involve the development, organization, and phylogeny of the pallium in ray-finned fishes.

The data presented by Mueller et al. [2011] – that Dc arises from a dorsally located periventricular germinative field – are fairly convincing, but the authors' case would be greatly strengthened by cell lineage studies in zebrafish to demonstrate that the cells of Dc do, indeed, arise from this dorsal germinative zone and perhaps that the cells of Dd also arise from this zone rather than from the germinative zones that give rise to Dl and Dm. Furthermore, it is very important that similar cell lineage studies be performed in centrarchid and cichlid teleosts, where the pallia are characterized by large cell populations

that have been identified as multiple divisions of Dc [Northcutt and Braford, 1980; Demski and Beaver, 2001; Pepels et al., 2002; Burmeister et al., 2009] before the condition in teleosts can be said to be established. It is possible that the cell population recognized as Dc in cyprinids, such as the zebrafish, arises only from the dorsal germinative zone, whereas the Dc populations in more derived teleosts arise from multiple germinative zones. It is also possible that all Dc populations in more derived teleosts arise from a single germinative zone and become secondarily associated with several of the other pallial divisions.

Questions are also raised regarding the primary pallial divisions in teleosts. Although Mueller et al. [2011] argue that Dd is not a primary pallial division, based on their data from a cyprinid, a distinct pallial group has been interpreted as Dd and its associate, Dc-d, in many other teleost groups.

Unfortunately, the connections of Dd have not been experimentally determined in cyprinids or in any of the other groups. Experimental studies of Dc, however, have determined that this pallial division projects to the optic tectum in cyprinids and in several percomorph orders [reviewed by Demski and Beaver, 2001]. It is possible that new studies specifically and differentially labeling Dd and Dc-d might reveal that those connections differ from those of Dl and Dm. If this were true, it would strongly indicate that Dd and Dc-d do, in fact, constitute a primary pallial division.

There are few data on the internal organization of any primary pallial division in teleosts. A small number of Golgi studies suggest that the more periventricular zones of the pallial divisions consist of small stellate cells with widely branching thorny dendrites that receive the bulk of the ascending sensory inputs, whereas the more migrated zones consist of larger efferent neurons with more compact centrally directed dendrites that receive most of their input from the superficial stellate cells and whose axons enter the lateral forebrain bundle [Sheldon, 1912; Holmgren, 1920; Nieuwenhuys, 1959; Bannister, 1973; Schroeder, 1980; Demski and Beaver, 2001]. In this context, the Dc recognized by Mueller et al. [2011] would be the only primary pallial division that is not associated with a periventricular stellate zone. To date, experimental studies of the pallium have focused primarily on the total

connections of an individual pallial division and have not dealt with the internal organization of pallial divisions or the role of their multiple cell populations. Such studies are badly needed if we are to understand the cortical-like organization of individual pallial divisions that occurs in most teleosts.

It is possible that Dc in teleosts is homologous to the isocortex of mammals if Dc does arise from a dorsal pallial germinative zone and if Dp has been displaced caudally by the hypertrophy of Dm. This homology would only be valid, however, if Dc occurs throughout the clade of ray-finned fishes, from polypteriforms to teleosts. To date, a sulcus ypsilonformis, which is an indicator of the overgrowth of Dc by Dl and Dm, has not been recognized in nonteleost ray-finned fishes [Meek and Nieuwenhuys, 1998; Nieuwenhuys, 1998a, b]. Moreover, a cell group of clustered, closely packed cells, indicative of a putative Dd, has not been recognized in nonteleost fishes. A pallial cell group identified as a combined Dd and Dl has been recognized, but this recognition appears to have resulted from an attempt to preserve the nomenclature, rather than any new cytological or experimental data [Nieuwenhuys and Meek, 1990]. Similarly, a Dc has been recognized in many nonteleost fishes but not in polypteriforms [Nieuwenhuys, 1963, 1998a; Northcutt and Braford, 1980]. It is possible, however, that pallial cells homologous to the cells of Dc in other ray-finned fishes do not form a distinct cell group but are scattered in one or more divisions of the pallium. Extensive tracer injections into all divisions of the pallium in *Polypterus* failed to reveal projections to the optic tectum [Holmes and Northcutt, 2003], which would be expected if a Dc population existed in *Polypterus*, and DiI injections into the optic tectum of *Polypterus* reveal no labeled neurons in the pallium (unpubl. observations). Similarly, extensive tracer injections into all pallial divisions of the Siberian sturgeon, *Acipenser baeri*, failed to reveal projections to the optic tectum [Huesa et al., 2006]. Even direct injections into the putative Dc in *Acipenser* fail to label projections to the optic tectum but instead reveal connections with subpallial and diencephalic cell groups that are characteristic of Dl and Dm, which would be expected if the cells of the putative Dc in sturgeons are instead the deeper, migrated cells of Dl and Dm. Additional experiments in which the optic tectum is labeled with an

effective retrograde tracer are needed to confirm that the putative Dc does not project to the optic tectum in sturgeons, but so far all experimental data suggest that chondrosteans lack a Dc population that projects to the optic tectum. This further suggests that Dc may have arisen in neop-

terygian ray-finned fishes. If so, it must be considered a neomorphic trait of neopterygians, not a homolog of mammalian isocortex. If this is the case, it does not diminish the results presented by Mueller et al. [2011]. Rather, it raises the possibility that they may have uncovered one of the unique

traits responsible for the explosive radiation of neopterygians. Mueller et al. [2011] have opened a piscine Pandora's box of unforeseen questions whose answers can only enrich our understanding of the organization and evolution of the pallium in ray-finned fishes.

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