Organization of radial glia reveals growth pattern in the telencephalon of a percomorph fish Astatotilapia burtoni

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Abstract
In the brain of teleost fish, radial glial cells are the main astroglial cell type. To understand how radial glia structures are adapting to continuous growth of the brain, we studied the astroglial cells in the telencephalon of the cichlid fish Astatotilapia burtoni in small fry to large specimens. These animals grow to a standard length of 10–12 cm in this fish species, corresponding to a more than 100-fold increase in brain volume. Focusing on the telencephalon where glial cells are arranged radially in the everted (dorsal) pallium, immunocytochemistry for glial markers revealed an aberrant pattern of radial glial fibers in the central division of the dorsal pallium (DC, i.e., DC4 and DC5). The main glial processes curved around these nuclei, especially in the posterior part of the telencephalon. This was verified in tissue-cleared brains stained for glial markers. We further analyzed the growth of radial glia by immunocytochemically applied stem cell (proliferating cell nuclear antigen [PCNA], Sox2) and differentiation marker (doublecortin) and found that these markers were expressed at the ventricular surface consistent with a stacking growth pattern. In addition, we detected doublecortin and Sox2 positive cells in deeper nuclei of DC areas. Our data suggest that radial glial cells give rise to migrating cells providing new neurons and glia to deeper pallial regions. This results in expansion of the central pallial areas and displacement of existing radial glia. In summary, we show that radial glial cells can adapt to morphological growth processes in the adult fish brain and contribute to this growth.

KEYWORDS
astrocyte, cell proliferation, doublecortin, pallium, radial glia, Sox2, telencephalon, tissue clearing

1 | INTRODUCTION

Most teleost fish continue to grow substantially after reaching sexual maturity which includes size increase and cell addition to the brain. Cell addition has been well documented in the adult teleost brain (Ganz & Brand, 2016; Maruska et al., 2012; Rahmann, 1968; Zupanc et al., 2005) and investigated from different angles and aspects such as circadian regulation (Akle et al., 2017), sex differences (Ampatzis et al., 2012), or environmental conditions (von Krogh et al., 2010). There are at least two different modes how neurons and glia are added to the existing brain: (i) adding neurons from a distinct proliferation zone like in the optic tectum and in the diencephalon and (ii) the generation of new cells from radial glia (Grupp et al., 2010; März, Chapouton, et al., 2010; März, Schmidt, et al., 2010).
The telencephalon of teleost fish generally does not show a layered organization like the mammalian cortex but rather is formed by various cell groups and nuclei. The ventral part of the telencephalon is formed by the subpallium and the dorsal part by the pallium, which is subdivided in at least two rostral, two posterior, and two central divisions (Northcutt, 2008).

The teleost dorsal telencephalon is formed by an eversion process during development (Butler & Hodos, 2005; Nieuwenhuys, 1969) with the ventricular surface extending dorsally and laterally. Consequently, the cell bodies of radial glial cells in the dorsal pallium are located on the brain surface surrounded by ventricular space and the Tela choroidea, the remnant of the roof plate. As a result of eversion, radial glial cells run in a spoke-like pattern toward the taeniae of the Tela choroidea as a hub. Besides the ventricular zone, there are only few if any proliferating cells within the dorsal pallium.

Radial glial cells are defined as the non-neuronal cells with cell bodies located in the ventricular zone and bipolar processes extending to reach the pial and ventricular surfaces (Campbell & Götz, 2002). They occur in the brains of all vertebrates, are derived from neuroepithelial cells, and give rise to neurons and glia (Campbell & Götz, 2002; Rakic, 1974). In human brain development, ventricular and outer radial glial cells have been characterized (Nowakowski et al., 2016). In the mammalian brain, most radial glial cells differentiate into astrocytes toward the end of neuro- and gliogenesis, whereas in teleost fish, they retain a radial morphology (Allen & Lyons, 2018). Thus, in teleost fish, radial glial cells are the dominant astroglial type in the mature brain. They maintain proliferative capacity and at the same time, serve astroglial functions (Jurisch-Yaksi et al., 2020).

Although the everted structure of the actinopterygian telencephalon has been documented many times, it still remained unclear how this radial glial scaffold is compatible with brain growth and how the glial cells adapt to it. To address this question, we studied the astroglial cells in the telencephalon of the cichlid fish Astatotilapia burtoni. This fish species grows to a standard length of 10–12 cm with a corresponding manifold increase in brain volume. A. burtoni has been used as a model fish for growth and the neural basis of sexual behavior (Davis & Fernald, 1990; DeOliveira-Mello et al., 2019; Desjardins et al., 2010; Johns & Fernald, 1981; Maruska & Fernald, 2018; Nikonov & Maruska, 2019).

In the present study, we used immunocytochemistry for glial markers in conventional brain sections and cleared whole brain preparations. We found that the regular pattern of radial glial fibers was distorted in the central division of the dorsal pallium (DC): the main glial processes did not run through the central nuclei but rather curved around them. The presence of doublecortin- and Sox2-positive cells in these areas suggested cell growth and expansion in the central telencephalon by a different mechanism than previously thought.

2 | MATERIALS AND METHODS

2.1 | Animals

We investigated the telencephalon of the cichlid fish A. burtoni which were raised in our own fish facility. Fish were kept at 27°C in aerated tanks in a 12/12 h light/dark cycle and fed commercial cichlid food.

Fish were anesthetized in MS222 and killed by cervical section. In this study, we used fish of both sexes and various sizes with standard lengths (SL) of 8, 14, 50, and 120 mm which corresponds to approximate ages of 1 day (about 14 days post fertilization), 14 days, 6 months and >2 years, respectively. A. burtoni can easily live for 5 years and older in our fish facility. We investigated five animals in each of the 1 day and 6 months group and at least two animals in the other age groups. The brains were dissected out and fixed in 4% (wt/v) paraformaldehyde in PBS (pH 7.4 at 4°C) overnight. For small specimens, the entire head was fixed.

2.2 | Histology

After incubation in 30% sucrose (wt/v) and freezing in TissueTek, cryostat sections were prepared at a thickness of 16 μm. Following our standard procedures, immunostains were performed with antibodies listed in Table 1 and described in Subsection 2.3. After blocking serum, primary antibodies were applied on the sections overnight at 4°C, either separately or in combination in phosphate buffered saline containing 0.3% TritonX. Next, following washes, sections were incubated with secondary fluorescence conjugated antibodies (Table 1) for 90 min at room temperature. Cell nuclei were stained with DRAQ5 (1:1000; Thermo Fisher) or in the case of a triple immunostain with DAPI (1:1000). After washes, the sections were coverslipped with Mowiol.

2.3 | Antibody characterization

The antibodies listed in Table 1 have all been used in fish nervous tissue before, in cichlids or other related species. In particular, the doublecortin (C-18) sc-8066 antibody is an affinity purified antibody raised against a peptide at the C-terminus of the human doublecortin protein. It has been shown to label differentiating neurons next to the peripheral growth zone in the retina of A. burtoni (Garcia-Pradas et al., 2018). The islet1 antibody is known to bind to a subset of neurons in the zebrafish CNS (Johnson et al., 2016). Sox2 antibodies were generated against mouse Sox2 peptide and label stem cells and glia in the fish retina (DeOliveira-Mello et al., 2019). Glial Fibrillary acidic protein (GFAP), glutamine synthetase (GS), and PCNA antibodies have all been characterized and used on teleost brain (Grupp et al., 2010).

2.4 | Tissue clearing

For passive tissue clearing, we followed our adjusted protocol (Neckel et al., 2016). Briefly, brains designated for tissue clearing were fixed in hydrogel monomer solution composed of 4% (wt/v) acrylamide, 4% (wt/v) paraformaldehyde, and 0.25% (wt/v)
VA-044 Initiator (WAKO, cat. No 017-19362) in PBS (pH 7.4) for 2 days. Afterward, the acrylamide gel was polymerized in a vacuum oven at 42–45°C for 3 h. Embedded brains were cleared in an 8% (wt/v) sodium dodecyl sulfate solution in PBS at pH 7.4 at 55–60°C on a rotator for 1–2 weeks (depending on specimen size and clearing success), with the clearing solution exchanged every 3–5 days. After washes, the preparations were incubated in anti-GFAP primary antibody for 7 days, followed by secondary antibody and DRAQ5 for 5 days. This was followed by washes and incubation in 80% glycerol for refractive index matching.

2.5 Image acquisition

Images of cryosections were acquired on a confocal LSM510 Meta with laser lines at 488, 543, and 633 nm for excitation and appropriate filter sets, on an Axio Imager Z.1 fluorescence microscope, or an LSM 900 Airyscan (all Carl Zeiss, Jena, Germany). Stacks of images of cleared brains were scanned on a confocal LSM Exciter or on a Lightsheet Z.1 (both Carl Zeiss). Brightness and contrast were only linearly adjusted. For further analysis and 3D reconstruction, images and stacks were processed using the system-implemented software ZEN (Zeiss).

| TABLE 1 List of antibodies used |
|------------------------------|
| **Primary antibody** | **Source** | **Catalog number** | **Host** | **Dilution** |
| Anti-Doublecortin (DCX) | Santa Cruz Biotechnology | sc-8066 | Goat | 1:100 |
| Anti-Glial Fibrillary acidic protein (GFAP) | Dako | Z 0334 | Rabbit | 1:300 |
| Anti-Glutamine Synthetase (GS) | Millipore | mab302 | Mouse | 1:500 |
| Anti-Proliferating cell nuclear antigen (PCNA) | DAKO | M0879 | Mouse | 1:300 |
| Anti-Sox2 | Seven Hills | WRAB-1236 | Rabbit | 1:400 |
| Anti-Islet-1 | DSHB, Iowa | 394D5 | Mouse | 1:100 |

**Secondary antibody**

| **Secondary antibody** | **Source** | **Host** | **Dilution** |
|------------------------|------------|---------|-------------|
| Anti-Rabbit-Alexa546 | Molecular Probes/Thermo Fisher | Goat | 1:400 |
| Anti-Mouse-Alexa488 | Molecular Probes/Thermo Fisher | Goat | 1:400 |
| Anti-Goat Alexa488 | Molecular Probes/Thermo Fisher | Donkey | 1:400 |
| Anti-Rabbit Alexa647 | Molecular Probes/Thermo Fisher | Donkey | 1:400 |
| Anti-Mouse Alexa555 | Molecular Probes/Thermo Fisher | Donkey | 1:400 |

**FIGURE 1** Brain growth in A. burtoni. (a) Transverse sections through the mid-telencephalon of a large fish (left, SL 12 cm), and a small fry (right, top of the figure, SL 1 mm) at the same magnification, immunostained for glial fibrillary acidic protein (GFAP) and islet-1, indicating radial glia and cells in the subpallium, respectively. The arrows indicate the location of the external sulcus, the insert shows the size of the telencephalon with increasing body length. (b) Dorsal view of brains from different sized fish (standard length, SL of the fish as indicated). The brain length of small fry measured approx. 1 mm, whereas in the 12 cm long fish the brain was about 10 mm long, about the length of the entire young fish (shown on the right); * indicates the telencephalon [Color figure can be viewed at wileyonlinelibrary.com]
The telencephalon of *A. burtoni* has been described previously in cresyl violet stained paraffin sections (Burmeister et al., 2009), a study we used for anatomical orientation and structure identification.

### RESULTS

#### 3.1 Radial glia in the telencephalon of cichlid fish

Astroglial cells in the telencephalon of *A. burtoni* occur predominantly in radial morphology with their cell bodies forming the ventricular surface. An exception is the olfactory bulb comprising astroglia cell bodies and long processes within the bulb's cell mass. In this article, we use the term “radial glia” for ventricularly located glial cells, and use “astroglia” when cells of the astroglia family were found in the brain parenchyma. The telencephalic ventricular surface is enlarged by the eversion process and ranges from the medial ventral aspects of the subpallium to the lateral areas of the pallium. The *Tela choroidea*, derived from the roof plate and covering the dorso-lateral pallium, is not easily identified in cryostat sections. Yet the shape and location of the radial glial cells labeled by GFAP immunoreactivity clearly delineates the transition from the ventricular surface of the dorsal pallium to the meningeal surface. The ventral subpallium region was identified.

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**FIGURE 2** Displacement of radial glia fibers in the central pallial regions in growing fish. (a–c) Frontal sections and (e–h) parasagittal sections through the telencephalon of young fish of 8 mm SL (a, e, g), 14 mm SL (b) and adults with a 50 mm SL (c, f, h) stained for glial fibrillary acidic protein (GFAP) (green), islet-1 (in a–c red), and Draq5 (blue). (d) Schematic redrawn to scale from coronal sections of a large and small fish with the course of radial glia fibers in the dorsal pallium (P). The arrows in (a–c) and the green arrow in (d) indicate the location of the external sulcus and the curved arrows in (d) indicate the relocation of the lateral pallium and central sulcus during growth. In the large brains, few if any radial astroglial fibers run through the deeper areas of the central division (DC4 and DC5) of the dorsal pallium. Approximate orientation of the parasagittal sections in e–h is indicated by the inserts. Note the different magnifications, scale bars = 200 μm in each figure. OB olfactory bulb, SP subpallium [Color figure can be viewed at wileyonlinelibrary.com]
by the presence of islet-1 positive cells (Figure 1(a)). Islet-1 has been shown to be expressed in the zebrafish ventral telencephalon by immunocytochemistry (Ganz et al., 2012) and a transgenic line (Baeuml et al., 2019).

### 3.2 Growth of the telencephalon

In this study, we took advantage of the fact that the standard body length of the model organism *A. burtoni* increases more than 10-fold.
during the animal's lifetime, and this growth includes increasing brain size. Brain length range from 1.5 mm in small fry (standard length \([SL] = 0.8\) cm) to 9 mm in large animals (SL = 12 cm; Figure 1(b) and supporting online material [SOM] Figure S1). This translates into a more than 100-fold volume increase for the brain. The increase includes the telencephalon where each hemisphere can reach a length

**FIGURE 5** Differentiating neurons positive for doublecortin (DCX) were located subventricularly to the radial glial cell layer at the ventricular surface, positive for glutamine synthetase (GS). (b1–b3) are detailed views of the boxed area in (a). In (c1-3), a triple immunostain showed that all GS-positive radial glial but none of the DCX positive differentiating neurons expressed Sox2. This indicated a layered cell addition to the telencephalon. (a) and (b) are from a large fish (SL = 70 mm), (c) is from a small fish (SL = 10 mm), nuclei in (a) and (b) were stained with DRAQ5, in (c) with DAPI, scale bars = 50 μm in (a), 20 μm in (b), and 10 μM in (c) [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 6** Triple immunostains for doublecortin (DCX), glutamine synthetase (GS), and Sox2 in a transverse section at the level of the anterior commissure. (b) Overview indicating the locations shown in detail in (a1) and (c2), individual channels in (a2–a5) and (c2–c4). GS, Sox2, and DCX positive cells were found in the ventricular surface layers (see also Figure 5) and also in cell groups in central areas of the telencephalon. (a1–a5) and (c1–c5) all GS positive cells also expressed Sox2 (arrows), whereas DCX cells were never co-labeled with neither GS nor Sox2. D Dorsal (pallial) division of the telencephalon, Dm medial division of D, Dl-g granular zone of lateral D, Dc central division of D, subdivisions 2 and 4, Vc central nucleus of the ventral division, * sulcus ypsiloniformis, scale bars = 10 μm in (a) and (c), and 200 μm in (b) [Color figure can be viewed at wileyonlinelibrary.com]
of 2.5 mm in large fish compared to less than 0.2 mm in the small fish (insert Figure 1(a)). As a consequence, the radial glial cells in the dorsal telencephalon become substantially longer. According to the brain sizes, we measured the length of radial glia to be up to 200 μm in small fish and reaching more than 1 mm in large fish (Figure 1(a)). In addition, we found that the lateral pallium (part of the dorsal telencephalon) extends further ventrally in larger brains, indicating an ongoing eversion process during brain growth. Thus, in transverse sections through the telencephalon in large fish, the lateral pallium reaches the ventral surface of the brain, whereas in small fish, the ventricular-meningeal surface transition is located at about two-third of the dorso-ventral extent of the brain (Figures 1(a) and 2(a–c), arrows).

In brain sections of large fish, we discovered that the long glial processes followed the expected radial spoke-like pattern only in the rostral telencephalon but not in the middle to posterior part of the pallium where they curved around cell groups of the central division (DC4 and DC5; Figure 2(a–c)). To find out whether or not this phenomenon is a consequence of growth processes, or a peculiarity of cichlid fish brain we compared telencephalic sections from small and large fish. A central area in the dorsal telencephalon devoid of but surrounded by GFAP positive main glial processes was clearly identified in brain sections of middle and large sized-fish (Figure 2(b,c)), yet in small, newly born fry, only some irregularities in the radial course of glial fibers was observed (Figure 2(a)). This was also found in sagittal sections (Figure 2(e–h)), and even more strikingly, it was verified when we applied glial stains to cleared brains: Radial glia fibers could be followed from the (ventricular) surface to deeper center of the telencephalon for more than 1 mm, clearly indicating that the main process of the radial glia “curve” around the areas DC4 and DC5 (Figure 3, and SOM movies S1–S3). This was distinctly more pronounced in larger brains and implies that the distorted path of radial glia fibers is linked to growth processes.

### 3.3 Glial cell addition to the telencephalon

To investigate locations of cell proliferation and differentiation in the telencephalon, we stained transverse sections with the proliferation marker PCNA, the transcription factor Sox2 expressed in stem cells and fish glial cells (Taboada et al., 2018), and doublecortin (DCX), a marker for neurogenesis and neuronal differentiation (Couillard-Despres et al., 2005). PCNA stainings clearly confirmed proliferating cells in the outer surface layer of the telencephalon where the cell bodies of radial glia cells are located. A dense layer of proliferating cells was found on the medial surface of the telencephalon with the strongest clustering in distinct proliferation zones in the subpallium. This was clearly more pronounced in the brains of small fish but PCNA-positive cells were also present in the telencephalon of large fish (Figure 4). Radial glia cell bodies were located at the (outer) ventricular surface layer of the ventro-medial subpallium, and medial and lateral aspects of the dorsal pallium. Their glial function was indicated by their immunoreactivity for the enzyme GS, their proliferative and stem cell properties by the immunoreactivity for PCNA and Sox2 (Figure 4(b,c)). A row of subventricular cells was positive for DCX indicating a population of differentiating neurons, especially in the pallial areas (dorsal telencephalon; Figure 5). The DCX-positive cells directly adjacent to this layer did not co-label with neither Sox2 nor GS. Thus, a germinal/glial surface layer can be distinguished from a subventricular layer of differentiating neurons. In addition, we found several groups of DCX positive cells in deeper nuclei of the telencephalon (see below). However, Sox2-positive cells were not

![Figure 7](image-url)
restricted to the ventricular surface layer, nor did they all appear to be glial cells.

3.4 | Enlargement of central areas of the dorsal telencephalon

The finding that the GFAP-positive radial glial fibers curve around the central areas of the mid-posterior telencephalon raised the question how these areas with a dense neuropil are supplied by astroglial processes and function. Scrutinizing stains for GS in the dorsal pallium, we discovered few fine GS positive processes entering the DC4/5 nuclei in a pattern aberrant from the general radial orientation (SOM Figure S2). Furthermore, we found some GS positive perikarya with nonradial GFAP/GS positive processes in DC nuclei (Figure 6). Some of these GS-positive cells also expressed Sox2. Interestingly, at least some groups of these glial cells were located in areas where we also found DCX-positive cells (Figure 6(a,c)), but these cells did not colabel with neither GS nor Sox2. This indicates that astroglial cells and differentiating neurons were present in these regions. However, we found only few PCNA-labeled cell in these central nuclei.

In addition to the identified deeper DC areas, we detected GS/Sox2-labeled cells with radial processes in the posterior dorsal telencephalon ventral of the sulcus ypsiloniformis (SY in Figures 6(b) and 7(b)) a recess between the lateral and central-medial regions of the pallium (i.e., DC2 and Dl-g, see Burmeister et al. 2009). These GS/Sox2 cells appeared to be migrating assessed by their shape and location (Figure 7). This suggests that the DC regions, disproportionately enlarging during postembryonic growth of the telencephalon, are supplied with new cells by astroglial cells migrating away from the ventricular surface to these deeper central areas.

4 | DISCUSSION

Adult neurogenesis in the mammalian telencephalon is largely restricted to the subventricular zone and the hippocampus (Zhao et al., 2008) although the significance in adult humans remains unclear (Sorrells et al., 2018). In contrast, substantial brain growth and neurogenesis has been demonstrated in the telencephalon of various adult fish species long after reaching sexual maturity (Chapouton et al., 2007; Diotel et al., 2015; Ganz & Brand, 2016; Jurisch-Yaksi et al., 2020; Kaslin et al., 2008; Lange et al., 2020; Lindsey et al., 2012; März, Schmidt, et al., 2010; Olivera-Pasilio et al., 2017; Than-Trong & Bally-Cuif, 2015). In this study, we focused on the growth adaptations of the unique glial pattern in the everted telencephalon in a cichlid fish. Our data confirm the radial organization of glial processes and the localization of glial cell bodies on the ventricular surface of the pallium, yet we also show that the glial processes get contorted in the central division of the telencephalon with increasing age. Moreover, our data suggest that at least some nuclei in DC4 and DC5 region receive new cells apparently migrating from the ventricular surface. In addition, some fine, branching glial processes, positive for GS might provide functional glial supply to the expanding central pallium.

In mammals, the main telencephalon cell mass enlarges by thickening of the ventricular walls, thereby increasing the meningeal surface—a process called evagination. In contrast, it has been well documented and is generally agreed on that in actinopterygians which includes teleosts, the ventricular wall of the pallium expands laterally, thereby increasing the ventricular surface (Butler & Hodos, 2005). The relatively extensive size increase of the telencephalic cell mass involves the ongoing eversion process, that is, increase of the ventricular surface with the consequence that the lateral pallium extends ventrally, and, as our data suggest, the enlarging central cell masses (DC4 and DC5) apparently displace some of the radial fibers in the medial to posterior pallium.

Based on developmental origins, the telencephalon of vertebrates is subdivided in two main parts, the ventral area or subpallium, and the dorsal area or pallium. GABAergic neurons originate from the subpallium and can later migrate into the pallium, recently reviewed by Briscoe and Ragsdale (Briscoe & Ragsdale, 2019) and shown previously for zebrafish (Mueller et al., 2006; Mueller et al., 2008; Mueller & Wullimann, 2016). Thus, GABAergic expression dominates nuclei of the subpallial ventral telencephalon, while glutamatergic expression dominates nuclei of the pallial dorsal telencephalon. This general pattern has been confirmed for A. burtoni in a recent study (Maruska et al., 2017).

The degree of eversion varies among fish groups, with a moderate eversion in cladistians and a high degree in perciform fish, and determines the proportions of ventricular and meningeal surfaces of the telencephalon (Nieuwenhuys, 2011). In a recent study using birthdating approaches in transgenic zebrafish (Furlan et al., 2017), it was shown that the dorsal pallium is generated in an inside-out stacked architecture consistent with the notion that the surface ventricular zone gives rise to new neurons throughout life. Our triple stain for DCX, Sox2, and GS confirms this idea of a radial glia layer followed by a DCX subventricular layer of differentiating neurons. In addition, the radial spoke-like radial glia in young fish also supports this view. However, three observations indicate other concurrent events: (1) The external sulcus marking the pallial-subpallial border and the transition from the ventricular to the meningeal surface was more pronounced and located more ventrally in large fish than in juveniles. (2) The distortion or displacement of radial fibers increased with brain size. (3) Groups of DCX-positive and Sox2/GS-positive cells were found in central DC areas. Thus, our data suggest that in addition to the layered growth on the ventricular surface, expansion and cell addition in the central pallial areas reshape the telencephalon. We would like to point out that the degree of eversion in the adult zebrafish brain corresponds to the juvenile brain in A. burtoni, as judged by the transition point from the ventricular to meningeal surface. A comparative stain for glial markers did not reveal a contorted course of the radial glia fibers in the zebrafish brain (SOM Figure S2). Unfortunately, the DCX antibody does not show reactivity in the zebrafish brain, possibly due to the expression of doublecortin-like protein kinase but not DCX in zebrafish (Shimomura et al., 2007).
although DCX has been reported in other fish groups including the telencephalon of sharks (Docampo-Seara et al., 2020) and the retina of cichlids (Garcia-Pradas et al., 2018).

The continuous stacked layer model would imply that the central areas in the cichlid brain enlarge simply by hypertrophy or cell proliferation. Indeed, cells in the DC4/5 are relatively large and certainly contribute to the size increase of these areas, yet cannot account for the tremendous size increase of the central regions.

The cell bodies of the radial glia cells were positive for Sox2, a transcription factor suggesting stem cell characteristics of these cells. This is along the lines of previous studies (Gorsuch et al., 2017; März, Chapouton, et al., 2010; März, Schmidt, et al., 2010). At the same time, and consistent with our data, Sox2 has been shown previously to be expressed in mature radial glia in the retina (DeOliveira-Mello et al., 2019; Surzenko et al., 2013).

The fact that we found such cells (GS/Sox2 positive) in deeper regions suggests that these regions receive or maintain cells with stem cell characteristics and glial function analogous to the surface radial glia. One possibility would be that a central stem cell niche continues to produce cells in the central pallial areas. Although we cannot exclude this completely, the only occasionally found cells marked with proliferation markers in this and other studies (Maruska et al., 2012; Zupanc et al., 2005) do not support this notion. We did however find Sox2/GS positive astroglial cells well away from the ventricular surface in the region of the sulcus ypsilonformis separating the area DC2 from Dl-g (Burmeister et al., 2009) where the ventricular surface showed a high level of proliferation (Maruska et al., 2012). Judging by their morphology and location, we suggest that these are migrating cells. We therefore propose the following scenario for cell addition to the central pallial areas (mostly DC4 and DC5): An astroglial cell population in the region between the DC2 and Dl-g (and dorsal of the anterior commissure) leaves the ventricular surface, migrates ventrally and joins cells in deeper nuclei. Since these cells loose contact to the ventricular surface and change morphology, we refer to them as astroglia rather than radial glia. In the DC region, they can give rise to new neurons (DCX positive cells), or function as glial cells (Sox2/GS positive). The differentiation into neurons is supported by the fact that we did not observe an accumulation of glia cells in DC4/5 region, which one would expect over time, nor did we see any potentially DCX-positive migrating cells. Clearly, more experiments will be necessary to verify this hypothesis. Whether the astroglial cells have the progenitor potential like the radial glia on the ventricular surface appears possible but remains to be shown.

The contribution of migration to the development of the central pallium in fish has been discussed controversially (Demski, 2013). Cells of the DC area (DC4 and DC5 of Burmeister et al. (2009)) are believed to have migrated from various neighboring regions (Braford, 2009). Interestingly, Mueller et al. (2011) proposed an invagination model for the generation of the DC area in zebrafish, based on parvalbumin staining pattern. In addition, they showed by BrdU pulse chasing experiments in 3–8 day-old zebrafish that the dorsal posterior zones receive migrated cells from the medial zone of the pallium. In contrast, Furlan et al. (2017) found no evidence for migration in a genetic birthdating study in zebrafish. We want to emphasize that the central DC area in percomorph fish such as A. burtoni is much more differentiated and has more cell groups than in cyprinids (Demski, 2013).

Our study was motivated by the structural changes occurring in radial glial cells during growth and was not intended to contribute to the debate on the origin and homology of the different pallial areas across different vertebrate groups (Aboliz & Montiel, 2019; Briscoe & Ragsdale, 2019). We can conclude, however, that radial glial cells can adapt to morphological changes during growth in the adult fish brain and contribute themselves to these growth processes. Whether the radial glial cells in the telencephalon represent a heterogeneous population as suggested by some studies in zebrafish (Lange et al., 2020; März, Chapouton, et al., 2010; März, Schmidt, et al., 2010) and/or show a high degree of plasticity remains to be shown for cichlids. In the light of the recent finding that radial astroglia in the zebrafish brainstem contribute to information processing and behavior (Mu et al., 2019), one can speculate that in central pallial areas lacking radial glia fibers such a contribution does not occur, possibly changing during growth. The mechanisms of the differential expanding growth, and how areas rendered devoid of astroglial support might attract astroglial cells and branches from surrounding radial glia remain to be elucidated.

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CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
Andreas F. Mack: Study concept, design, and intellectual content; acquisition of data; analysis and interpretation of data; drafting and writing of the manuscript. Laura DeOliveira-Mello: Preparation and acquisition of some of the data; discussion and critical revision of the manuscript. Ulrich Matthews: Preparation and acquisition of some of the data (histology and tissue clearing); critical revision of the manuscript. Peter H. Neckel: Preparation and acquisition of some of the data (tissue clearing) interpretation, discussion, and critical revision of the manuscript.

PEER REVIEW
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DATA AVAILABILITY STATEMENT
The original image data and quantitative data that support the findings of this study are available from the corresponding author upon reasonable request.
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