Expression of cell markers and transcription factors in the avian retina compared with that in the marmoset retina

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Abstract
In the vertebrate retina, amacrine and ganglion cells represent the most diverse cell classes. They can be classified into different cell types by several features, such as morphology, light responses, and gene expression profile. Although birds possess high visual acuity (similar to primates that we used here for comparison) and tetrachromatic color vision, data on the expression of transcription factors in retinal ganglion cells of birds are largely missing. In this study, we tested various transcription factors, known to label subpopulations of cells in mammalian retinae, in two avian species: the common buzzard (Buteo buteo), a raptor with exceptional acuity, and the domestic pigeon (Columba livia domestica), a good navigator and widely used model for visual cognition. Staining for the transcription factors Foxp2, Satb1 and Satb2 labeled most ganglion cells in the avian ganglion cell layer. CtBP2 was established as marker for displaced amacrine cells, which allowed us to reliably distinguish ganglion cells from displaced amacrine cells and assess their densities in buzzard and pigeon. When we additionally compared the temporal and central fovea of the buzzard with the fovea of primates, we found that the cellular organization in the pits was different in primates and raptors. In summary, we demonstrate that the expression of transcription factors is a defining feature of cell types not only in the retina of mammals but also in the retina of birds. The markers, which we have established, may provide useful tools for more detailed studies on the retinal circuitry of these highly visual animals.

KEYWORDS
amacrine cells, cell type, fovea, ganglion cells, marmoset retina, retina, starburst amacrine cells, transcription factor

1 INTRODUCTION

In the vertebrate retina, each type of retinal ganglion cell (RGC) encodes distinct aspects of the visual input and sends this information to higher brain areas (Baden et al., 2016). Their somata reside predominantly in the most proximal nuclear layer of the retina, the ganglion cell layer (GCL). However, some (displaced) RGCs also have their somata in the proximal inner nuclear layer (INL), where the somata of most amacrine cells are located. Amacrine cells shape the output of ganglion cells; in some amacrine cells or cell types, the soma...
is located in the GCL (displaced amacrine cells). Together, amacrine and ganglion cells represent the most diverse cell classes in the vertebrate retina (Masland, 2012), for example, more than 60 types of amacrine cells (Yan et al., 2020) and ~30–46 types of RGCs (Baden et al., 2016; Bae et al., 2018; Rheaume et al., 2018; Tran et al., 2019) have been reported for the mouse retina. Individual cell types differ in their anatomy (e.g., dendritic tree diameter, branch density, stratification level, reviewed in the study by Sanes & Masland, 2015), intrinsic properties (Wong et al., 2012), connections (Helmstaedter et al., 2013), and physiological responses (Baden et al., 2016). Recently, evidence has accumulated that different cell types also differ genetically and express distinct marker proteins, such as neurofilament 200 (NF200, α-RGCs; Krieger et al., 2017; Peichl et al., 1987), and transcription factors (Rheaume et al., 2018, Roussou et al., 2016). AP2, for example, was shown to be exclusively expressed in amacrine cells but not in RGCs of the mouse retina (Bassett et al., 2012). Another example is ON–OFF direction-selective RGCs, which express the transcription factors Satb1 (Peng et al., 2017; Sweeney et al., 2019) and Satb2 (mouse and rabbit; Sweeney et al., 2019; Dhande et al., 2019). While the differential expression of various transcription factors is well established for some mammalian retinæ (mostly mouse and primate), much less is known for avian retinæ and only very recently, a cell atlas of the chick retina based on transcriptome analysis was provided (Yamagata et al., 2021). So far, most studies focused on the developmental role of transcription factors in the chick retina (Edqvist et al., 2006; Fischer et al., 2008) and not on their potential as markers for distinct cell types in the adult retina. Yet, the adult bird retina is interesting, not only for comparative reasons but also because most birds are highly dependent upon vision, similar to humans. Birds possess tetrachromatic color vision and often have high visual acuity, thanks to high photoreceptor and ganglion cell densities and fovea-like regions.

Foveae are retinal areas with localized and often sharp increases in both photoreceptor and ganglion cell density (Fite & Rosenfield-Wessels, 1975). Among mammals, primates are the only animals with a fovea for high acuity vision, whereas among birds, foveae are common retinal specializations (reviewed in the study by Bringmann, 2019). Various diurnal bird species (e.g., birds of prey, such as eagles, buzzards, falcons) even possess two foveae, a deep fovea in the central region, and a more shallow fovea in the temporal region of the retina (Mitkus et al., 2017; Reymond, 1985, 1987).

Here, we characterized the cells in the GCL of the common buzzard (Buteo buteo), a diurnal raptor, and the domestic pigeon (Columba livia domestica), a model for visual learning and cognition (Qadri & Cook, 2015; Wasserman & Young, 2010). We identified subpopulations of RGCs and amacrine cells by analyzing the expression of various transcription factors (Satb1, Satb2, Foxp1, Foxp2, Islet1, AP2) and common markers, for example, ChAT and NF200. Using some of these markers, we also determined the topographic distribution of cells along the temporal-nasal axis because reported numbers vary widely for the pigeon retina: Binggeli and Paule (1969) reported 10,000–40,000 cells/mm², whereas Querubin et al. (2009) reported 9000–100,000 cells/mm². We also compared the two foveae of the buzzard to the fovea of macaque and marmoset and show that the cellular organization differs between raptors and primates.

In summary, our data reveal some interesting differences and commonalities between mammalian and avian retinæ and may provide a starting point for further functional studies on cells in the avian GCL.

2 | METHODS

2.1 | Animals and tissue preparation

All experiments were performed in accordance with the institutional guidelines for animal welfare and the laws on animal experimentation issued by the EU and the German government. The sacrificing of birds for tissue analysis is registered with the local authorities (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit) and reported on a regular basis as demanded by law.

Pigeons from both sexes (age > 2 months) were killed with 1–2 ml Narcoren injected into one of the wing veins close to the humerus and decapitated. For immunohistochemistry, eyes were enucleated and the cornea, lens, and the vitreous body were removed in carboxygenated (95% O₂, 5% CO₂) extracellular solution (in mM: 100 NaCl, 6 KCl, 1 CaCl₂, 2 MgSO₄, 1 Na₂HPO₄, 30 NaHCO₃, 50 glucose).

Buzzards were injured animals with a low probability of recovery obtained from animal rescue centers in the Czech Republic. The eyes became available when the animals were killed for a study of their brains. The animals received a lethal dose of halothane and were then transcardially perfused with heparinized, warmed phosphate-buffered saline (pH 7.4) followed by cold phosphate-buffered 4% paraformaldehyde solution. All procedures were approved by Institutional Animal Care and Use Committee at Charles University in Prague and Ministry of Culture of the Czech Republic (permission number 47987/2013). The eyes were dissected, postfixed by immersion for 1 h in the same fixative, rinsed in PB, incubated in 30% sucrose solution for 24 h, transferred into antifreeze (30% glycerol, 30% ethylene glycol, and 40% phosphate buffer) and frozen for further processing.

Retinal marmoset tissue used in this study was obtained from adult common marmosets. The animals were sacrificed within a broad aging study at the German Primate Center in Göttingen (Mietsch et al., 2020). All procedures were approved by the local animal welfare committee and by the Lower Saxony State Office for Consumer Protection and Food Safety (reference number 33.19-42502-04-17/2496). Housing conditions were in accordance with the law for animal experiments issued by the German government (Tierschutzgesetz) and complied with the European Union guidelines on the welfare of nonhuman primates used in Research and the European Union (EU directive 2010/63/EU). The animals were anesthetized intramuscularly with a combination of ketamine (50 mg/kg, Ketamin 10%, WDT), xylazine (10 mg/kg, Xylariem 2%, Ecuphar) and atropine (1 mg/kg, Atropinsulfat, Dr. Franz Koehler Chemie GmbH) and killed by an overdose of...
pentobarbital (150–200 mg/kg) intraperitoneal. The eyes were enucleated, the right eye was immersion fixed in 4% paraformaldehyde (PFA) for 60 min, and the left eye was used for physiological experiments unrelated to this study. Following fixation, the eyes were stored at 4°C in PBS and 0.02% sodium azide. For immunohistochemistry, the retinas were dissected from the eyecup and retinal pieces of defined eccentricities were used as a whole mount or sectioned vertically (60 μm) with a vibratome (Leica VT 1200 S).

The foveal macaque section was obtained from a 12-year-old rhesus macaque female (Macaca mulatta). The animal was sacrificed in an experiment not related to ocular tissue with an overdose of sodium pentobarbital and was subsequently perfused with phosphate-buffered saline (PBS, 0.1 M, pH 7.4), followed by fixation with 4% paraformaldehyde. Once fixation was complete, the eyes were harvested and taken to the histology lab. The retinae were dissected from the eyecup and retinal pieces of defined eccentricities were used as a whole mount or sectioned vertically (60 μm thick) using a cryostat. Foveal sections were collected on Superfrost slides and stored at −20°C.

### 2.2 Immunohistochemistry

For immunohistochemistry on retinal pigeon whole-mounts, the retinae were carefully removed from the eyecup and placed on a black filter paper (Millipore) in one piece, with the photoreceptor layer downward. To flatten the tissue, the pecten was removed, leaving a wedge-shaped cut at the ventral part of the tissue. This served as a landmark during the microscopy scanning. The tissue was fixed in 2%–4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) or PBS for 20–30 min. The fixed and cryoprotected retinae of buzzards were rinsed in PB, dissected and flattened as described for the pigeon retina above. After several washing steps, retinal whole-mounts were blocked overnight with 5% donkey serum + 0.02% NaN3 in PB containing 0.5% Triton X-100. Primary antibodies (Table 1) were applied in the same solution for 3–5 days at 4°C. After rinsing, 0.5 μM DAPI and the secondary antibodies

### Table 1 Primary antibodies used in this study

| Antibody | Antigen | Host and type | Dilution | Source, cat#, RRID |
|----------|---------|---------------|----------|-------------------|
| AP2      | AP2α sequence (aa 166–197) | Mouse monoclonal | 1:250 | DSHB, 3B5; developed by Dr. T. Williams RRID:AB_528084 |
| Calretinin | Recombinant protein corresponding to AA 1 to 271 from mouse Calretinin | Guinea pig polyclonal | 1:4000 | Synaptic Systems 214,104 RRID:AB_10635160 |
| CD15     | U-937 histiocytic cell line | Mouse monoclonal | 1:100 | BD Biosciences, 559,045 RRID:AB_397181 |
| ChAT     | Purified human placental choline acetyltransferase enzyme | Goat polyclonal | 1:200 | Merck-Millipore, AB144P RRID:AB_2079751 |
| CtBP2    | Synthetic peptide corresponding to aa 974 to 988 from rat Ribeye | Rabbit polyclonal | 1:1000 | Synaptic System, 193003 |
| Foxp1    | Synthetic peptide conjugated to KLH derived from within residues 650 to the C-terminus of human Foxp1. | Rabbit polyclonal | 1:4000 | Abcam, ab16645 RRID:AB_732428 |
| Foxp2    | Synthetic peptide corresponding to human Foxp2 aa 703–715 | Goat polyclonal | 1:250 | Abcam, ab1307 RRID:AB_1268914 |
| Islet1   | E. coli-derived recombinant human Islet-1 (aa 4–349) | Goat polyclonal | 1:250 | R&D Systems AF1837 RRID:AB_2126324 |
| NF200    | C-terminal segment of enzymatically dephosphorylated pig neurofilament 200 | Mouse monoclonal | 1:500 | Sigma Aldrich, N0142 RRID:AB_477257 |
| NF200    | Purified neurofilament 200 from bovine spinal cord | Rabbit polyclonal | 1:2000 | Sigma Aldrich, N4142 RRID:AB_477272 |
| RBPMS    | KLH-conjugated linear peptide corresponding to a sequence from the N-terminal region of human RBPMS | Guinea pig polyclonal | 1:500 | Merck-Millipore, ABN1376 RRID:AB_2687403 |
| Satb1 (C-6) | Raised against aa 241–310, mapping within an internal region of Satb1 of human origin. | Mouse monoclonal | 1:250 | Santa Cruz, sc-376096 RRID:AB_10986003 |
| Satb2    | Synthetic peptide conjugated to KLH derived from within residues 700 to the C-terminus of Mouse SATB2 | Rabbit polyclonal | 1:1000 | Abcam, ab34735 RRID:AB_2301417 |
| Sox2 (E-4) | Raised against aa 170–201 within an internal region of Sox-2 of human origin | Mouse monoclonal | 1:200 | Santa Cruz, sc-365823 RRID:AB_10842165 |

Abbreviation: aa, amino acids.
conjugated to either Alexa Fluor 488, 568 or 647 (1:500; Thermo Fisher Scientific, Waltham, MA) were applied in blocking solution for 24–48 h. Whole mounts were washed extensively and finally mounted in Vectashield or Aqua-Poly/Mount. Retinal cryosections were labeled similarly, but primary antibodies were applied overnight at 4°C and secondary antibodies were applied for 2–3 h at room temperature without Na3. The specificity of the secondary antibodies was tested by omitting the primary antibodies. No unspecific staining was detected.

2.3 | Antibody characterization

All primary antibodies used in this study are listed in Table 1. The mouse antibody against AP2α was shown to specifically label horizontal and amacrine cells in the chicken retina (Fischer et al., 2007). This labeling was consistent with a previous study using different AP2α antibodies (Bisgrove & Godbout, 1999).

Many antibodies against calretinin have been used to label amacrine and ganglion cells in the mammalian retina. Consistent with the results of previous studies on chicken retina (Fischer et al., 2007), we found calretinin immunoreactivity in ganglion, amacrine, and horizontal cells in the buzzard and pigeon retina.

Antibodies against the carbohydrate epitope CD15 label flat (OFF) midget bipolar cells in marmoset. This staining pattern has been well established in previous studies (Chan et al., 2001; Puller et al., 2007).

The antibody against choline acetyl transferase (ChAT) has been characterized by the manufacturer (Merck-Millipore) in Western blots of mouse brain lysates; it detects a band of ~68–70 kDa.

The antibody against CtBP2 was tested on retina homogenates and recognized a double band of ribeye (the retina-specific variant of CtBP2). It also recognized over-expressed EGFP-CtBP2 fusion construct but did not cross-react with EGFP-CtBP1 (Hübner et al., 2012).

The Foxp1 antibody has been tested by the manufacturer (Abcam) in Western blots. It detects a band of approximately 75 kDa (predicted molecular weight: 75 kDa) and recognizes the different isoforms of Foxp1 in Jurkat cell lysates.

The Foxp2 antibody has been characterized by the manufacturer (Abcam) in Western blots of whole cell lysates from human cerebellum. It recognizes a single band of ~80 kDa, corresponding to the predicted size of 79.9 kDa.

According to the manufacturer (R&D Systems), the antibody against human Isl1 was controlled by Western blot and immunocytochemistry. It recognized a single band of ~42 kDa in lysates of human induced pluripotent stem cells differentiated into motoneurons. Undifferentiated cells were used as control and did not yield a band. The antibody was also reported to detect Isl1 in human-induced pluripotent stem cells differentiated to endocrine progenitor cells with immunocytochemistry.

The monoclonal antibody against NF200 detected phosphorylated and nonphosphorylated neurofilament with a molecular weight of 200 kDa in Western blot of rat brain extracts. The anti-NF200 produced in rabbit localized the 200 kDa neurofilament polypeptide in immunoblotting.

RBPM was found to be exclusively expressed in RGCs and displaced RGCs. A representative blot detected endogenous RBPMs in mouse and rat retina extracts, as well as exogenously expressed human RBPM in transfected HEK293T cells (Rodriguez et al., 2014).

Specificity of Satb1 antibodies was assessed by the manufacturer (Santa Cruz). Whole cell lysates from HEK293T cells transfected with mouse Satb1 showed a prominent band at 115 kDa (predicted weight 89 kDa), whereas lysates from nontransfected cells did not. We obtained similar results with this antibody as described in a previous study using two different Satb1 antibodies (Peng et al., 2017).

The Satb2 antibody has been characterized by the manufacturer (Abcam) in Western blots of mouse brain tissue lysate and recognized a band of 85 kDa, close to the predicted size of 83 kDa.

Specificity of Sox2 antibodies was assessed by the manufacturer (Santa Cruz) in Western blots of whole cell lysates with a prominent band of ~34 kDa.

2.4 | Image acquisition

Images were acquired with a confocal laser scanning microscope (Leica TCS SP8). Retinal whole-mounts were scanned with HC PL APO 20×/0.70 or HC PL APO 40×/1.3 oil immersion objectives. Voxel size was adjusted with respect to the experimental question. During data analyses with Fiji (Schindelin et al., 2012), the sum, average, or maximum projections of each stack have been used. Intensities were normalized using the Fiji plugin Contrast Enhancement with 0–0.1 saturation. For cell counting, we used the Cell Counter plugin of Fiji. Unless stated otherwise, projections of confocal stacks are shown. Images were adjusted in brightness and contrast and occasionally filtered for presentation purposes.

3 | RESULTS

Here, we compared the buzzard and pigeon retina and searched for cell markers special for RGCs and amacrine cells. The buzzard retina is similar in size as the human retina but contains two foveae (in the central and temporal region) instead of one. The pigeon retina is much smaller (similar in size as the marmoset retina) and contains one central fovea (Figure 1(a)) and a second region for high acuity vision in the center of the red field. The retinas of the birds also differ in GCL thickness: The central buzzard retina contains at least four rows of cells in the GCL, whereas the central pigeon retina contains two. In both species, retina thickness decreases with eccentricity, as demonstrated by calretinin labeling (Figure 1(b,c)) present in avian horizontal, amacrine, and ganglion cells.

3.1 | Labeling for transcription factors can be used to label distinct cell populations in the avian retina

To screen for transcription factors that are expressed in the avian retina, we performed immunohistochemistry with antibodies directed
against Foxp1, Foxp2, Islet1, Satb1, Satb2, and AP2 in buzzard and pigeon (Figure 2). As expected from studies on mouse and macaque retina (Rousso et al., 2016), Foxp1 and Foxp2 labeled many cells in the GCL. These cells were always positive for both markers in the buzzard retina (Figure 3(a)), whereas in pigeon, the GCL contained cells that were only Foxp2+, in addition to many Foxp1+/Foxp2+ cells (Figure 3(b)). In the INL, Foxp1+ and Foxp2+ cells formed separate populations in both avian species. For comparison, we also stained marmoset retina and found, in addition to Foxp2+ and Foxp1+/Foxp2+ RGCs, a few Foxp1+ and Foxp2+ amacrine cells in the GCL and a few Foxp2+ amacrine cells in the INL (Figure 3(c–f)).

Islet1 labeled putative horizontal and bipolar cells in the distal INL (Figure 2) and additionally, a prominent row of cells at both borders of the inner plexiform layer (IPL) in buzzard and pigeon retina (Figure 2, arrows). Double labeling with ChAT showed that these cells represent ChAT+ putative starburst amacrine cells (Figure 4). Some cells in the proximal INL and many cells in the GCL were Islet1+ but ChAT−.

Satb1 and Satb2 labeled almost exclusively cells in the GCL, suggesting that Satb1+ and Satb2+ cells are expressed in various RGC types, as was shown in the mouse (Peng et al., 2017; Sweeney et al., 2019) and primate retina (Nasir-Ahmad et al., 2021).
FIGURE 2  Legend on next page.
Different transcription factors label distinct cell populations in the avian retina. (a) In the buzzard, staining for Foxp1 and Foxp2 revealed cells in the ganglion cell layer (GCL) and the proximal half of the inner nuclear layer (INL). Islet1 was broadly expressed in the GCL and prominently in the proximal INL, where it likely occurs in cholinergic amacrine cells (arrows). Satb1 and Satb2 labeled many cells in the GCL. AP2+ cells were ChAT− (Figure 5(c)), indicating that also in the marmoset retina, AP2 is not a reliable marker for displaced amacrine cells.

To test how reliably AP2 labels displaced amacrine cells in the pigeon retina, we compared the staining for AP2, CtBP2, and ChAT in retinal whole mounts (Figure 5(d)). None of the markers was perfect. AP2 labeled all ChAT+ cells and some CtBP2− cells in the GCL (long arrows). However, CtBP2 not only labeled several cells that were not detected by AP2 and ChAT (short arrows) but also the cells that were AP2+/ChAT+, suggesting that it may be the most reliable amacrine cell marker for the pigeon retina.

We also tested CtBP2 labeling in the buzzard retina and compared it to ChAT labeling in vertical sections and whole-mount pieces. As in the pigeon, CtBP2 labeled putative amacrine cells in the INL and GCL. In the central retina (1 mm temporal and 3 mm nasal, Bu1T, Bu3N, Figure 6(a,b)), most displaced CtBP2+ cells were also ChAT+ (>90%, Table 2). In the peripheral buzzard retina (Bu9T, Bu20N, Figure 6(a,b)), the density of ChAT+ cells was much lower and many CtBP2− cells were ChAT− (>50%, Table 2). When we labeled the marmoset retina with AP2 and ChAT, two major differences became apparent: (a) the density of displaced amacrine cells was much lower in the central marmoset retina than in the buzzard (~3500/mm² vs. 11,500/mm²; Table 2) and (b) the number of ChAT− displaced amacrine cells (AP2−) was much higher than in the buzzard (Figure 6(b)) (>70%; Table 2). As Sox2 was reported as a reliable marker for starburst amacrine cells, Müller glia cells and astrocytes in the retina of various species (Fischer et al., 2010; Whitney et al., 2014; Zhang et al., 2019), we also labeled the buzzard and marmoset retina for this transcription factor (Figure 6(c,d)). Sox2 labeled all strongly labeled ChAT+ cells in both species, indicating that it reliably recognizes starburst amacrine cells. Weakly labeled ChAT+ cells were Sox2−. We quantified the amount of Sox2+/ChAT− starburst amacrine cells both in the GCL and INL and estimated a ratio of 43:57 and 48:52 for ON and OFF starburst amacrine cells in buzzard and marmoset, respectively.

In summary, analysis of amacrine cell markers showed that CtBP2 labeled almost all amacrine cells in buzzard and pigeon retina, whereas AP2 failed to label (all) displaced amacrine cells in the buzzard, pigeon and marmoset retina. We also demonstrate that Sox2 can be used as a marker for starburst amacrine cells in avian and primate retinae.

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FIGURE 3

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mm²) and the temporal fovea (45,000 cells/mm²); densities dropped sharply toward the temporal (7400 cells/mm²) edge and less sharply toward the nasal edge (4800 cells/mm²). A substantial fraction of the cells was Satb1+ or/and Foxp2+. Labeling with ChAT and CtBP2 confirmed our finding that most displaced amacrine cells in the central retina are ChAT+ but that this fraction becomes smaller towards the edges (Figure 6(b)). In retinal areas with high RGC numbers (calculated by subtracting the CtBP2+ cells, representing displaced amacrine cells, from the DAPI counts), displaced amacrine cells represent only 20% of all cells in the GCL of the buzzard. Toward the periphery, however, they represent the majority (>60%) of the cells.

The results from the pigeon retina showed some interesting differences (Figure 7(c,d)). As the pigeon retina contains only one central fovea, peak cell densities were found in this area, with

**Figure 3** FoXP1 and FoxP2 double labeling in buzzard, pigeon, and marmoset retina. (a) In the buzzard retina, FoXP1 and FoxP2 labeled separate populations of amacrine cells in the inner nuclear layer (INL) but colocalized in the ganglion cell layer (GCL). (b) In the pigeon retina, FoXP1+ and FoxP2+ amacrine cells belong to separated populations in the INL. In the GCL, a few cells (arrows) occur that are only FoxP2+. (c–f) In the marmoset GCL, a few cells are only FoXP1+ (c, arrows) or FoxP2+ (d, thin arrows). These cells represent displaced amacrine cells because they are RBPM5− (e, f, short and long thin arrows). In addition, there are FoXP1+/FoxP2+ retinal ganglion cells (RGCs) discernible (c–f, asterisks and fat arrows) as well as FoxP2+ RGCs (d–f, arrowheads). Scale: 20 μm [Color figure can be viewed at wileyonlinelibrary.com]

**Figure 4** Islet1 is expressed in ChAT+ amacrine cells in the pigeon and buzzard retina. (a) In pigeon, antibodies against ChAT labeled putative ON and OFF starburst amacrine cells and one population of amacrine cells with their cell bodies toward the middle of the inner nuclear layer (INL). All ChAT+ putative starburst cells were also Islet1+. (b) In the buzzard retina, Islet1 labeled all ChAT+ putative starburst amacrine cells in the ganglion cell layer (GCL) and INL. IPL, inner plexiform layer; ONL, outer nuclear layer. Scale: 20 μm [Color figure can be viewed at wileyonlinelibrary.com]
38,000 cells/mm². Within the first millimeter from the fovea, cell density steeply declined to 18,000 cells/mm² but gradually flattened toward the nasal and temporal edge (5000 and 8000 cells/mm², respectively), unlike in the buzzard. Satb1+ only, Foxp1+ only and Satb1+/Foxp1+ cells followed the same trend as DAPI-labeled cells. In contrast, CtBP2+ displaced amacrine cells were distributed more evenly with peak cell densities occurring not near the fovea but in the nasal retina (5 mm nasal, 6000 cells/mm²). Notably, the number of displaced amacrine cells was lower than the number of RGCs along the entire temporal-nasal axis, with the highest fraction toward the nasal edge (11 mm nasal, 47%) and the lowest fraction close to the fovea (11%).

**FIGURE 5** AP2 is not a reliable marker for amacrine cells in bird and marmoset retina. (a) In buzzard, AP2 is not expressed in the GCL and all ChAT+ cells in the INL are AP2−. (b) In marmoset, all ChAT+ cells are AP2+. In the GCL, several additional AP2+ displaced amacrine cells are labeled. (c) Marmoset whole-mount retina at 4 mm temporal labeled for AP2, RBPMS and DAPI: several DAPI+ displaced amacrine cells (RBPMS−) are AP2− (green arrows). (d) In pigeon, all ChAT+ cells in the GCL are AP2+ and CtBP2+. Long arrows point to cells that are AP2+ but ChAT− and CtBP2−. The arrowhead marks a cell that is AP2+ and CtBP2+ but ChAT−. Small arrows point to cells that are only CtBP2−. Scale: 50 μm [Color figure can be viewed at wileyonlinelibrary.com]
NF200 labels distinct populations of cells in the buzzard and pigeon retina

Our quantification showed that in the central buzzard retina, 75–80% of all cells in the GCL were putative RGCs and many of these can be labeled by Satb1 and Foxp2 (Figure 7). However, transcriptomic data from Yamagata et al. (2021) on the chicken retina suggest that there are at least 41 different types of RGCs. As staining for neurofilament labels α-RGCs in many mammalian retinae (Krieger et al., 2017; Peichl et al., 1987) and α-RGCs are conserved across many species, we analyzed NF200 labeling in the buzzard (Figure 8) and pigeon retina (Figure 9).

In the buzzard retina, NF200 was present in the nerve fiber layer and labeled many larger and smaller RGCs. The large RGCs were either Satb2+ or Islet1+ (Figure 8a, arrows). In the INL, a distinct population of large Islet1+ RGCs was labeled, too. As these RGCs seem to form a mosaic, co-stratify with the ChAT+ band in the distal IPL and send down axons to the nerve fiber layer (Figure 8a,b), they may be candidates for OFF direction-selective RGCs. Large displaced RGCs in pigeon and chicken were shown to project specifically to the contralateral nucleus of the basal optic root, the major component of the avian accessory optic system (Karten et al., 1977; Reiner et al., 1979). Interestingly, NF200 antibodies produced in rabbits gave a different staining than the mouse NF antibodies: both labeled a population of...
smaller displaced RGCs with dendrites stratifying in the most distal IPL; however, the large displaced RGCs were only detectable with the mouse NF200 antibodies (Figure 8(b)).

In the pigeon retina, NF200 (mouse) labeling largely resembled NF200 labeling in the buzzard retina, with prominent staining of the nerve fiber layer and GCL. In vertical sections, we discerned large displaced RGCs that co-stratified with both ChAT-immunoreactive bands in the IPL and may represent ON–OFF direction-selective RGCs (Figure 9(a)). In addition, one amacrine cell type was labeled, which stratified in the middle of the IPL (Figure 9(a), short arrow). Labeling in retinal whole-mounts confirmed that this amacrine cell type formed a mosaic (Figure 9(b)). It was Islet1⁻ (Figure 9(b), short arrow). The large NF200⁺ ganglion cell somata we found displaced in the INL were mostly Islet1⁺ (Figure 9(b), arrowhead), suggesting that large displaced RGCs in the pigeon may comprise at least two different types.

3.6 Satb1, Satb2, and Foxp2 are suitable markers to distinguish different types of retinal ganglion cells in the buzzard retina

RGC types are defined by several features, one of which is the expression of different transcription factors (Peng et al., 2017; Rousso et al., 2016; Tran et al., 2019). As Satb2 was reported to be expressed in ON–OFF and OFF direction-selective and OFF-sustained RGCs in the mouse retina (Dhande et al., 2019) and labeled RGCs in the buzzard retina (Figures 2 and 8), we combined Satb2 staining with labeling for Foxp2 and Satb1 to further differentiate between RGC types. Based on size differences in labeled nuclei and staining intensities, we identified seven different RGC candidates in the buzzard retina (Figure 10(a)); (1) strongly Satb2⁺ cells with small nuclei (4–5 μm in diameter), (2) strongly Satb2⁺ cells with medium-sized nuclei (6–7 μm), (3) medium to pale Satb2⁺ cells with medium-sized nuclei (6–7 μm), (4) large Satb2⁺ nuclei (8–9 μm), (5) Satb1⁺/Satb2⁺ cells with

### Table 2 Fraction of displaced amacrine cells that are ChAT⁺ in buzzard and marmoset retina

| Buzzard eccentricity (mm) | CtBP2⁺/dACs | ChAT⁺ | ChAT⁺ in % | Marmoset eccentricity (mm) | AP2⁺/dACs | ChAT⁺ | ChAT in % |
|---------------------------|-------------|-------|------------|-----------------------------|-----------|-------|-----------|
| 22 (nasal)                | 4750        | 2010  | 42         | 1488                        | 331       | 22    |
| 20                        | 5300        | 2790  | 53         |                             |           |       |           |
| 18                        | 5800        | 3475  | 60         |                             |           |       |           |
| 16                        | 6900        | 4400  | 64         | 10 (nasal)                  | 1664      | 368   | 22        |
| 14                        | 7500        | 5765  | 77         | 1788                        | 535       | 30    |
| 12                        | 7600        | 6700  | 88         | 2462                        | 621       | 25    |
| 10                        | 8950        | 8465  | 95         | 2525                        | 754       | 30    |
| 8                         | 9800        | 9365  | 95         | 3225                        | 850       | 26    |
| 6                         | 10,300      | 9735  | 95         |                             |           |       |           |
| 4                         | 11,150      | 10,535| 95         |                             | 11,400    | 98    |
| 3                         | 11,100      | 10,500| 95         |                             | 11,600    | 98    |
| 2                         | 11,150      | 11,335| 98         |                             | 14,400    | 98    |
| 1                         | 11,600      | 11,400| 98         |                             | 15,000    | 98    |
| −1                        | 11,500      | 11,335| 98         | −1                          | 3550      | 1018  |
| −2                        | 9900        | 9535  | 96         | −2                          | 3617      | 958   |
| −3                        | 9200        | 8600  | 93         | −3                          | 2658      | 655   |
| −4                        | 8600        | 8065  | 94         | −4                          | 2537      | 607   |
| −5                        | 8700        | 8100  | 93         | −5                          | 2165      | 502   |
| −6                        | 8700        | 8100  | 93         | −6 (temporal)               | 1776      | 415   |
| −7                        | 8200        | 4385  | 71         |                             |           |       |           |
| −8                        | 3900        | 1730  | 44         |                             |           |       |           |
| −9                        | 4600        | 1000  | 22         |                             |           |       |           |

Note: Data were obtained from N = 1–3 individuals (marmoset); N = 2–3 individuals (buzzard). Abbreviation: ONH, optic nerve head.
medium-sized nuclei (6–7 μm), (6) Satb1+/Satb2+ cells with large nuclei (8–9 μm), and (7) Foxp2+/Satb2+ cells with medium-sized nuclei (6–7 μm). Figure 10(b) reveals the mosaic of strongly SatB2+ cells with medium-sized nuclei (No. 2, circles), thus confirming the notion that the different expression levels and nuclei sizes may correspond to different cell types.
3.7 | Comparison of the buzzard foveae with the primate fovea

One of the specializations of the avian retina is the presence of areas with a high density of photoreceptors (Meyer, 1977; Mitkus et al., 2017). Here, we compared the central and temporal fovea of the buzzard to the fovea of the macaque and marmoset (Figures 11 and 12). In vertical sections, the center of the deep buzzard fovea shows a centrifugal displacement of all the ganglion and amacrine cells (Figure 11(a,b)) as well as most of the bipolar cell and some of the photoreceptor nuclei. In the central primate fovea, all inner layers are absent. However, a few dispersed ganglion cells, interneurons (Figure 11(c); cf. figure 5 in the study by Grünert & Martin, 2020) and cone pedicles (Figure 12(c); cf. figure 1B in the study by O’Brien et al., 2012) are present. The triple labeling of RBPMS with Foxp2 and Satb1 revealed a few Foxp2+ and SatB1+ RGCs in the central marmoset fovea (Figure 11(c), arrows) and some Foxp2+ amacrine cells (Figure 11(c), arrowheads). Surprisingly, immunolabeling with

![Figure 8](image-url)
calretinin and CtBP2 showed that horizontal cells and cone pedicles are densely packed in the central buzzard fovea (Figure 12(a,b)). We did not stain for horizontal cells in marmoset; however, we know from parvalbumin staining in the macaque retina that only a few horizontal cells are located in the central primate fovea (Grünert & Martin, 2020; Röhrenbeck et al., 1989). Here, we show that each of the rare cone pedicles (labeled with CtBP2, Figure 10(c)) in the central marmoset retina is innervated by a CD15+ midget bipolar cells (Figure 12(c)).

**DISCUSSION**

Transcriptomic and physiological studies revealed that amacrine and ganglion cells likely form >100 different cell types in the mouse retina (Baden et al., 2016; Rheaume et al., 2018; Yan et al., 2020) and recently, also for the chicken retina, ~100 amacrine and ganglion cells were reported (Yamagata et al., 2021). To characterize the inner retina of birds, we analyzed the expression of transcription factors and cellular markers (Table 3), determined cell densities in the GCL along the

**FIGURE 9** Neurofilament 200 staining labeled distinct populations of ganglion cells in the pigeon retina. (a) Labeling with the Neurofilament 200 (NF200) antibody from mouse revealed displaced ganglion cells, which co-stratified with both ChAT-immunoreactive bands in the inner plexiform layer (IPL, long arrows) and amacrine cells, which stratified in the middle of the IPL (short arrows). (b) Projections from the displaced ganglion cells in the proximal inner nuclear layer (INL) are Islet1+ (long arrows); occasionally, we also found a displaced ganglion cell which was Islet1− (arrowhead). NF200+ amacrine cells form a mosaic in the middle of the IPL. Their cell bodies were Islet1− (short arrows). IPL, inner plexiform layer; ONL, outer nuclear layer. Scale: 40 μm, 10 μm (a); 50 μm (b) [Color figure can be viewed at wileyonlinelibrary.com]
temporal-nasal axis and compared the high-acuity retinal regions of the buzzard with the primate.

4.1 | The avian GCL

The avian GCL contains RGCs, displaced amacrine cells and putative glial cells (Hayes, 1984). The latter were not analyzed here and excluded from counts of DAPI-labeled nuclei based on their elongated nucleus shape. In both avian species (buzzard and pigeon), many neurons of the GCL (pigeon: mean 68%, range 36%–81%; buzzard: mean: 80%, range 72%–89%) were labeled by Foxp2, Satb1, and/or CtBP2. Likely, CtBP2+ cells represent almost exclusively displaced amacrine cells (Figure 5). They were almost always (>97%) Foxp2− and Satb1− and make up ~28% (range 11%–47%) of all cells in the GCL in the pigeon and ~34% (range 20%–67%) in the buzzard. This is well in line with earlier studies from chick (30%–35%, Chen & Naito, 1999; Ehrlich, 1981) and pigeon retina (43% total average, Binggeli & Paule, 1969). However, Hayes (1984) found fewer displaced amacrine cells in the pigeon retina (23% in the yellow field, and only 2% in the red field).

Satb1 and Foxp2 are two transcription factors known to be expressed in various RGC types in mammalian retinas (Peng et al., 2017, 2019; Rheame et al., 2018; Rousso et al., 2016). As almost all Satb1+ and/or Foxp2+ cells were CtBP2−, we conclude that also in the avian retina, Satb1 and Foxp2 label distinct populations of RGCs. In pigeon, these Satb1+ and/or Foxp2+ cells make up almost 77% of all cells in the GCL of the central retina and 36% in the GCL of the peripheral retina. Of the Foxp2+ cells, only ~22% also expressed Satb1; conversely, only ~13% of all Satb1+ cells also expressed Foxp2.

In the mouse retina, Satb1 was shown to be strongly expressed in ON–OFF direction-selective RGCs and is essential for their bistratified arborization (Peng et al., 2017). Whether it labels an orthologous RGC type in the avian retina remains to be seen. Only very recently, Satb1 was shown to be expressed by many different types of RGCs in the chicken retina (Yamagata et al., 2021). In contrast, Satb2 showed a more restricted expression (Yamagata et al., 2021). It was detected in monostratified RGCs of mice and primates (Dhande et al., 2019; Nasir-Ahmad et al., 2021), in addition to bistratified ON–OFF direction-selective RGCs of mice (Peng et al., 2017) and rabbits (Dhande et al., 2019), and bistratified RGCs of primates (Peng et al., 2019). In marmoset, the majority of Satb2 expressing cells were broad thorny cells, with lower proportions of recursive bistratified, large bistratified, and outer stratifying narrow thorny cells (Nasir-Ahmad et al., 2021). Combining Satb1, Foxp2 and Satb2, we identified seven different types of putative RGCs in the buzzard retina, based on staining intensity and soma size. This supports the notion that cell types are defined not only by the expression of a certain transcription factor but also by the level of expression (Rousso et al., 2016; Yamagata et al., 2021).

Interestingly, we did not find a differential expression for Foxp1 and Foxp2 in the GCL of the avian retina, both transcription factors seemed to completely colocalized, with some exceptions in the pigeon GCL (Figure 3(b)). This is in contrast to the mouse retina where Foxp2 is expressed in all four types of F-RGCs (hence their name), whereas Foxp1 is only expressed in the two OFF variants and not the two ON variants of F-RGCs (Rousso et al., 2016). Peng et al. (2019) suggested that the evolutionary conversation of retinal cell types is weakest for RGCs after comparing transcriptomic data from primate and mouse. FIGURE 10 Satb2 expression in several ganglion cell types in the buzzard retina. Triple labeling with Foxp2 (green), Satb1 (red) and Satb2 (gray) revealed seven ganglion cell candidates distinguishable by expression level and size, 1: strongly Satb2+ cells with small nuclei (4−5 μm in diameter), 2: strongly Satb2+ cells with medium-sized nuclei (6−7 μm), 3: medium to pale Satb2+ cells with medium-sized nuclei (6−7 μm), 4: large Satb2+ nuclei (8−9 μm), 5: Satb1+/Satb2+ cells with medium-sized nuclei (6−7 μm), 6: Satb1+/Satb2+ cells with large nuclei (8−9 μm), and 7: Foxp2+/Satb2+ cells with medium-sized nuclei (6−7 μm). Mosaic of the strong, medium sized Satb2+ cells is shown in (b). Scale: 50 μm (a), 200 μm (b) [Color figure can be viewed at wileyonlinelibrary.com]
retinae. The differences we find here between RGCs of avian and mammalian species may just reflect this fact.

4.2 Cellular organization is different between raptor and primate foveae

The lifestyle of primates and birds seems to share the demand for high acuity vision. Primates have a central fovea and all bird retinae contain a region (or often two) dedicated to high visual acuity. These regions may occur as an area centralis and/or a shallow or deep foveal pit. Diurnal raptors, like the buzzard, have a bifoveal retina with a central deep foveal pit for detecting distant objects and a temporal shallow foveal pit for detecting closer objects (Bringmann, 2019). Bird foveae are not only implicated in high acuity vision but also image enlargement and motion detection. When we compared the foveal regions of the raptor and primate retina, we saw striking differences between the two groups. The deep central buzzard fovea completely lacked amacrine and ganglion cells; many bipolar cell and photoreceptor nuclei were centrifugally displaced. However, horizontal cells and...

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**FIGURE 11** Comparing the buzzard fovea with the primate fovea. (a) Vertical section through the central (cf) and temporal fovea (tf) of the buzzard and the macaque fovea reveals that the cellular organization differs between buzzard and primate: The central fovea of the buzzard is void of cells in the ganglion cell layer (GCL), in contrast to the primate where some cells are dispersed. (b) Labeling buzzard retinal whole-mounts for DAPI and Foxp2 and Satb1 confirmed that the central fovea does not contain any ganglion cells, whereas the temporal fovea does. (c) RBPMS labeling combined with Foxp2 and Satb1 labeling revealed that the dispersed cells in the innermost layer of the marmoset retina contain both RGCs (arrows, RBPMS⁺ and Foxp2⁺ or Satb1⁺) and displaced amacrine cells (arrowheads, RBPMS⁻, Foxp2⁻). HF, Henle fibers; INL, inner nuclear layer; ONL, outer nuclear layer. Scale: 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
cone pedicles were not displaced from the foveal pit. In contrast, in the central primate, fovea photoreceptors are densely packed, whereas all inner layers are absent. We detected only a few dispersed RGCs, some amacrine and bipolar cells and very few cone pedicles. Most foveal RGCs represent midget ganglion cells, which hardly receive any inhibitory input (Sinha et al., 2017). They presumably represent most of the RBPMS^+ only cells we found.

The foveal development in primates is well understood (reviewed in the study by Bringmann, 2019). In both monkey and humans, at 26%–30% gestation, no foveal pit has yet appeared (Hendrickson, 1992), but in the position where the fovea will form, all three layers of the retina, and probably many of the synaptic connections, are already present. As the fovea forms (by 63%–65% gestation in monkey and human, Hendrickson, 1992), the cone inner segments move toward each other and their density increases substantially. However, the horizontal cells and bipolar cells to which the cones are connected move radially outwards and the cone pedicles go with them by lengthening the Henle fibers. The cone to midget bipolar cell connection is adult-like by mid-gestation (Zhang et al., 2019). Foveal development in birds is not fully understood yet. For black kites, Potier, Mitkus, Lisney, et al. (2020) reported that the shape of the fovea is directly linked to eye size and the physical structure of the fovea may develop during the entire life. Sugiyama et al. (2020) used the zebra finch as a model and identified Arhgef33 as a candidate
protein possibly involved in foveal development. However, for the buzzard fovea, we can only speculate that the photoreceptor density increases only slightly so that the photoreceptors are not forced to move radially or may not be able to elongate their axons.

### 4.3 Cell densities

Previously reported densities of RGCs in the pigeon retina vary widely (Binggeli & Paule, 1969; Hayes, 1984; Querubin et al., 2009), presumably because different authors applied different methods for quantification and could not reliably differentiate between displaced amacrine cells and RGCs. Here, we introduce CtBP2 as a marker for displaced amacrine cells in the bird retina and with its help, quantified the cell densities in the pigeon GCL along the temporal-nasal axis in retinal whole-mounts. We found peak densities of RGCs close to the pigeon fovea of 32,000 cells/mm², which is close to the number from the study by Binggeli and Paule (1969) derived by axon counts in the optic nerve but very different from Querubin et al. (2009) who reported densities as high as 100,000 cells/mm² using quantifications from retinal sections and a correction factor derived from Hayes (1984). However, as Hayes (1984) found only 2%–23% of the cells in the GCL to be displaced amacrine cells (based on soma size in Nissl staining), data from Querubin et al. (2009) may represent an overestimate of pigeon RGCs. In addition, comparisons are complicated by different fixation and staining techniques, which may lead to tissue shrinkage or swelling and thereby affect area counts. However, pigeon RGC numbers relate well to buzzard RGC numbers as comparing the vertical sections from both central retinae (Figure 1(b,c)) already implied that the RGC numbers for the buzzard would be higher than for the pigeon. One striking finding from our cell density counts was that displaced amacrine cells do not show an increased density in the fovea or near it. This also seems to be the case for the primate retina; at least our counts in the marmoset retina show only a slight increase in density (Figure 13).

Compared with vultures and eagles, the common buzzard has a lower spatial acuity (Potier, Mitkus, & Kelber, 2020). Still, the common buzzard retina contains a multilayered GCL and RGC densities reach 50,000 cells/mm² close to the central fovea. Our data are comparable with an earlier report (Coli et al., 2018), which analyzed the buzzard retina in four retinal regions and found the highest density with 42,000 RGCs/mm² in the nasal part. The RGC density in Chilean eagles and sparrow hawks was highest in the central fovea with 62,000 and 65,000 cells/mm², respectively (Inzunza et al., 1991).

The differences in cell densities between the pigeon and buzzard likely reflect their different lifestyles. Pigeon retina has one fovea and an area dorsalis with increased RGC density, which allow them to peck food from the ground while simultaneously being able to detect predators (Hodos & Erichsen, 1990). Buzzards in contrast are diurnal raptors which hunt their prey during the day from the air. The high RGC density in the central fovea provides the highest visual acuity in the lateral field of view, which is presumably used for hunting prey.
from large distances while the temporal fovea enables sharp vision in the frontal field of view (Potier, Mitkus, & Kelber, 2020).

Compared to buzzard and marmoset, the retinal organization of the lateral-eyed pigeon is different because nasal and temporal side do not differ. The fovea is located in the center and cell densities decrease symmetrically toward the retinal edges; all RGCs project to the contralateral side of the brain (Saleh & Ehrlich, 1984). In contrast, the “central” fovea of the buzzard is shifted to the temporal side. This is similar in the marmoset although the asymmetry is less pronounced due to more frontalized eyes (compare Figures 7(b) and 13). In predatory birds and primates, not all RGCs project to the contralateral side, the temporal fibers are uncrossed (ten Tusscher, 2014). This organization and foveal shift to the temporal side seems to be corollary of natural selection for enlarged binocular fields which has evolved convergently in frontal-eyed species. Primates and diurnal raptors rely very much on the accurate positioning of their hands and feet, respectively, and binocular vision has recently been discussed to be important not only for depth perception but also for visually guided limb and beak movements and coordination (Larsson, 2011; Martin, 2009).

4.4 Amacrine cell markers

In buzzard, most of the displaced amacrine cells (i.e., CtBP2+ cells) were ChAT+ in the central retina and therefore likely represent ON starburst cells; in the periphery, many CtBP2+ cells were ChAT−. In contrast, in both the central and peripheral marmoset retina, the fraction of ChAT+ cells comprised less than 35% of all displaced amacrine cells (Table 2) and somewhat resembled the peripheral buzzard retina. We also compared the densities of the two most intense ChAT+ cell populations in the INL (putative OFF starburst cells) and GCL (putative ON starburst cells) and found that the density of ON starburst cells was on average slightly lower than that of OFF starburst cells (marmoset: 655 vs. 685 cells/mm2, buzzard: 2500 vs. 3250 cells/mm2, from areas of 2–10 mm in marmoset and 8–24 mm in buzzard). This is in line with the density of ON and OFF ChAT cells in mouse retina (945 vs. 1100 cells/mm2, Jeon et al., 1998) but differs from that in human and macaque retina where ON ChAT cells are more frequent than OFF ChAT cells (ratio of 70:30; Chandra et al., 2019; Rodieck & Marshak, 1992). The reported ON to OFF ratio of 30:70 in marmoset (Chandra et al., 2019; Moritoh et al., 2013; Weltzien et al., 2015) can be explained with the additional ChAT+/Sox2− cells in the INL.

Antibodies against AP2 are commonly used as amacrine cell marker. The one used here (Table 1) is supposed to only recognize the AP2α subunit but not the AP2β subunit. Very recent data from chicken suggested that AP2α expression is restricted to amacrine cells in the INL, whereas the AP2β isoform is expressed in both INL and GCL (Yamagata et al., 2021). Here, we found AP2 expression in both layers of the pigeon but only in the INL of the buzzard retina, suggesting that chicken and buzzard are more similar in AP2 isoform distribution than the pigeon. As AP2α antibodies failed to detect displaced amacrine cells in the buzzard, we established CtBP2 as a marker for avian amacrine cells; it does not only label synaptic ribbons but also amacrine cell somata as evidenced by its coexpression with ChAT and Sox2 (like Islet1, also expressed in ChAT+ putative starburst amacrine cells).

Foxp1 and Foxp2 were both expressed in nonoverlapping, putative amacrine cell populations in the INL of pigeon and buzzard. Although the same markers were shown to almost exclusively label RGCs in the mouse and macaque retina (Rousso et al., 2016), stainings in marmoset retinae revealed that a few displaced amacrine cells in the GCL are Foxp1+ or Foxp2+ and even a few Foxp2+ amacrine cells occur in the INL (Figure 3). This suggests that even the closely related marmoset and macaque retina can differ in transcription factor expression.

In summary, our data provide a first step in unraveling the diversity of amacrine and ganglion cell types in the avian retina.

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CONFLICT OF INTEREST

The authors declare that there are no potential sources of conflict of interest.

AUTHOR CONTRIBUTION

Karin Dedek and Silke Haverkamp designed the project; all authors performed experiments; Laszlo Albert, Vaishnavi Balaji, Karin Dedek, and Silke Haverkamp analyzed data; Silke Haverkamp, Karin Dedek and Laszlo Albert prepared figures; Karin Dedek and Silke Haverkamp wrote the manuscript; all authors commented on the manuscript.

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