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Molecular anatomy of the alligator dorsal telencephalon

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Abstract

The evolutionary relationships of the mammalian neocortex and avian dorsal telencephalon (DT) nuclei have been debated for more than a century. Despite their central importance to this debate, non-avian reptiles remain underexplored with modern molecular techniques. Reptile studies harbor great potential for understanding the changes in DT organization that occurred in the early evolution of amniotes. They may also help clarify the specializations in the avian DT, which comprises a massive, cell-dense dorsal ventricular ridge (DVR) and a nuclear dorsal-most structure, the Wulst. Crocodilians are phylogenetically and anatomically attractive for DT comparative studies: they are the closest living relatives of birds and have a strikingly bird-like DVR, but they also possess a highly differentiated reptile cerebral cortex. We studied the DT of the American alligator, *Alligator mississippiensis*, at late embryonic stages with a panel of molecular marker genes. Gene expression and cytoarchitectonic analyses identified clear homologs of all major avian DVR subdivisions including a mesopallium, an extensive nidopallium with primary sensory input territories, and an arcopallium. The alligator medial cortex is divided into three components that resemble the mammalian dentate gyrus, CA fields, and subiculum in gene expression and topography. The alligator dorsal cortex contains putative homologs of neocortical input, output, and intratelencephalic projection neurons and, most notably, these are organized into sublayers similar to mammalian neocortical layers. Our findings on the molecular anatomy of the crocodilian DT are summarized in an atlas of the alligator telencephalon.

Keywords

dorsal ventricular ridge; cerebral cortex; neocortex; hippocampus; evolution; archosaur; RRID AB_514497

1 | INTRODUCTION

The dorsal telencephalon (DT) is broadly associated with sensory integration, execution of motor commands, and higher cognitive functions. The DT is also the most highly divergent

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of any vertebrate brain region and manifests a staggering diversity of anatomies (Butler & Hodos, 2005; Nieuwenhuys, Donkelaar, & Nicholson, 1998). It is clear that DT organization is unusually pliable to the pressures of evolution and adaptation compared with, for example, the basal ganglia, which have changed little in their essentials since the human lineage diverged from that of lampreys (Grillner & Robertson, 2016). While presumably beneficial to radiating vertebrates, this pliability has made it notoriously difficult for anatomists to resolve homologies of DT components. In particular, the questions of which DT regions, structures, or cells in reptiles and birds might have homology with similar features of the mammalian neocortex have cultivated a multigenerational debate in evolutionary neuroscience (Butler, Reiner, & Karten, 2011; Dugas-Ford & Ragsdale, 2015; Striedter, 2005). Progress has undoubtedly been hindered by a scarcity of reptile gene expression data.

Mammals, birds, and non-avian reptiles (together, the extant amniotes) share some relatively noncontroversial DT structures. In all amniotes, information from the olfactory bulbs reaches a thin, lateral territory called the piriform cortex in mammals (Figure 1, CPi of cat and mouse) and the lateral cortex in reptiles (Figure 1, LC). Birds maintain this sensory pathway despite a secondary reduction in the size of their olfactory bulbs and olfactory cortex (Figure 1, CPi of pigeon) (Atoji & Wild, 2014; Reiner & Karten, 1985). There is widespread agreement that the lateral zones are homologous as DT olfactory input centers. A medial DT territory is designated the hippocampus in mammals and birds (Figure 1, Hp of pigeon, not visible in the cat and mouse sections) and the medial cortex in reptiles (Figure 1, MC). A role for the medial DT in spatial memory is thought to be a shared feature across amniotes (Striedter, 2016). It remains unclear whether reptiles and birds have specific homologs of the dentate gyrus, CA fields, or subicular complex, which together constitute the mammalian hippocampal formation (Striedter, 2016).

The DT cell populations located between the lateral olfactory and the medial hippocampal territories are more problematic. In mammals, this space is occupied by the six-layered neocortex (Figure 1, Ncx). The neocortex varies greatly in size and folding complexity across mammalian species but its six-layered architecture, canonical columnar circuitry, and tangential organization into sensory and motor areas are highly conserved (Figure 1, Ncx is smooth in mouse and corrugated in cat) (Brodmann & Garey, 2006; Harris & Shepherd, 2015; Krubitzer, 2007; Lewitus, Kelava, Kalinka, Tomancak, & Huttner, 2014; Lorente de N, 1938).

Reptiles and birds do not have a six-layered neocortex. Instead, reptiles possess a three-layered dorsal cortex (Figure 1, DC). Interpretations of this major species difference vary, but it is widely held that the reptile dorsal cortex layers correspond to the neocortical molecular layer 1 (L1) and the deep layers L5 and L6 (Cheung, Pollen, Tavare, DeProto, & Molnar, 2007; Reiner, 1991). The upper neocortical layers L2, L3, and L4 are, in this view, mammalian innovations built upon a primitive reptilian condition. This “deep layer hypothesis” is challenged by the existence of neocortical L4-like connections and gene expression in the turtle dorsal cortex (Dugas-Ford, Rowell, & Ragsdale, 2012; Hall & Ebner, 1970). These studies indicate that the cell-type composition of the reptile dorsal cortex is more complex than previously thought, and the structural and evolutionary relationships of the dorsal cortex to the mammalian neocortex are in no sense understood.

Birds have a Wulst in place of a dorsal cortex (Figure 1, W), so named because it forms a conspicuous bulge at the top the telencephalon (Kalischer, 1905). The Wulst contains populations of clustered cells rather than cortical layers, although these closely apposed cell populations have been referred to as pseudolayers to emphasize their organizational similarities to cortex (Medina & Reiner, 2000). These cell populations include, from medial to lateral, the hyperpallium apicale (HA), the interstitial nucleus of the hyperpallium apicale (IHA), and the dorsal mesopallium (Md) (Briscoe, Albertin, Rowell, & Ragsdale, 2018; Jarvis et al., 2013). Birds likely descended from animals with a dorsal cortex, but it is unknown how the layered dorsal cortex was modified into a Wulst with distinct nuclei.

The reptilian and avian DT contains a second decidedly nonmammalian structure, the dorsal ventricular ridge (Figure 1, DVR). (Johnston, 1915; Ulinski, 1983). The DVR lies below the dorsal cortex or the Wulst, but it is still within the DT (Reiner et al., 2004). In many species, it forms a protrusion into the lateral ventricle (Figure 1, turtle). The profound challenges in comparing the DVR with the mammalian DT are well documented (Bruce & Neary, 1995; Dugas-Ford et al., 2012; Fernandez, Pieau, Reperant, Boncinelli, & Wassef, 1998; Holmgren, 1925; Kappers, Huber, & Crosby, 1936; Karten, 1969; Puelles, 2001), but the difficulty of comparing this structure across reptiles, or from reptiles to birds, is seldom acknowledged.

DVR organization differs widely across reptile groups. Pleurodiran (or side-necked) turtles have a conspicuous nuclear territory in their anterior and dorsal-most DT, a trait not apparent in Cryptodiran turtles (Riss, Halpern, & Scalia, 1969). In other groups, like crocodilians, the DVR is densely packed with cells and can be divided into multiple territories with sharp boundaries (Crosby, 1917; Riss et al., 1969). In the remarkable brain of the tuatara, the DVR contains a thin cortex-like structure that appears to be continuous with the dorsal cortex at the lateral edge of the DT (Figure 1, cortex within the DVR) (Cairney, 1926; Durward, 1930; Reiner & Northcutt, 2000). The reptile DVR is often divided into an anterior ADVR and a posterior, or basal, BDVR (Ulinski, 1983). Nonetheless, possible homologies of specific DVR subdivisions, including the ADVR and BDVR, across reptiles are poorly understood.

The bird DVR is larger and more elaborately differentiated than that of most other reptiles. The avian ADVR is divided into the mesopallium (Figure 1, M) and the nidopallium (Figure 1, N) (Reiner et al., 2004; Ulinski, 1983). The nidopallium contains primary sensory nuclei including the visual-recipient entopallium (Figure 1, E). A posterior avian DVR territory, called the arcopallium, is thought to be homologous to the reptilian BDVR (Ulinski, 1983) and contains at least four subdivisions with distinct connectional and molecular characteristics (Dugas-Ford, 2009; Herold, Paulitschek, Palomero-Gallagher, Gunturkun, & Zilles, 2017; Reiner et al., 2004). As with divisions of the avian Wulst, it is unknown whether reptiles have homologs of the mesopallium, the arcopallium and its subdivisions, or non-sensory territory of the nidopallium.

Crocodilians are phylogenetically positioned to address many of these crucial problems in comparative DT anatomy. They can serve as model reptiles, in that they possess a reptile-typical cerebral cortex with LC, DC, and MC subdivisions (Figure 1, alligator). Consistent with their position as the closest living relatives of birds (Grigg & Kirshner, 2015),

crocodilians have a large, cell-dense DVR with clear internal subdivisions. Indeed, they have the largest brain of any non-avian reptile, with an unusually large telencephalon (Northcutt, 2013). This observation, coupled with the exceptionally slow rate of crocodilian genome evolution (Green et al., 2014), suggests that the crocodilian DVR may closely resemble that of the last common ancestor of archosaurs. A molecular study of the crocodilian cerebral cortex will allow further comparisons with the avian Wulst and with the mammalian neocortex.

The value of the crocodilian brain to comparative anatomy has been appreciated by many researchers (Carr, Soares, Smolders, & Simon, 2009; Clark & Ulinski, 1984; Crosby, 1917; Herrick, 1890; Huber & Crosby, 1926; Pritz & Northcutt, 1977; Rabl-Rückhard, 1878; Reese, 1915; Riss et al., 1969; Rose, 1923; Ulinski, 1983; Unger, 1911). We advance the understanding of this important clade with a detailed molecular anatomical study of the alligator DT, the first such comprehensive effort in any non-avian reptile brain. We analyzed the DT of the late-embryonic American alligator, *Alligator mississippiensis*, by performing in situ hybridization with a panel of cell- and region-specific marker genes previously characterized in the mouse *Mus musculus* and the chicken *Gallus gallus*. These marker genes often identified differentiated subdivisions of the alligator DT with expression boundaries that coincide with cytoarchitectonic boundaries. Where possible, we propose mammalian and avian homologies to alligator DT structures. These findings are documented, in part, by an atlas of the late-embryonic alligator telencephalon.

2 | MATERIALS AND METHODS

2.1 | Animal and ethics

All animal procedures were reviewed and approved by the University of Chicago Institutional Animal Care and Use Committee (IACUC).

Fertilized alligator (*Alligator mississippiensis*) eggs were provided by Ruth Elsey and colleagues at the Rockefeller Wildlife Refuge (Grand Chenier, Louisiana). Alligator eggs were transported by car in maternally provided nesting material in moistened, perforated Styrofoam boxes. Eggs were subsequently transferred to a GQF incubator where they were incubated at 30°C and 90% relative humidity. Embryos were staged according to the criteria of Ferguson (Ferguson, 1985). The sex of the alligators was not determined.

2.2 | Brain tissue preparation

Whole embryonic alligator brains were collected by decapitation, dissection, and immersion fixation in 4°C PFA (solution of 4% paraformaldehyde in phosphate-buffered saline, or PBS). Brains were fixed for several weeks to months at 4°C. Fixed brain tissue was cryogenically protected by equilibration in 30% sucrose/PBS overnight at 4°C before sectioning. Sucrose-saturated brains were mounted on a freezing sledge microtome (Leica SM2000R) and sections were cut at a thickness of 20–24 microns. Sections were collected in PBS treated with diethyl pyrocarbonate (DEPC-PBS), mounted onto Superfrost Plus slides (Fisher Scientific), and dried at room temperature before processing for in situ hybridization.

2.3 | cDNA preparation

Dissected alligator brains were flash-frozen on dry ice and stored at -80°C , or were used immediately for RNA extraction. Tissue was homogenized with a pestle and RNA was extracted with Trizol Reagent (Invitrogen) following manufacturer instructions. RNA was either stored at -80°C in RNase-free water (Sigma) or used immediately for cDNA synthesis [SuperScript III 1st strand cDNA kit (Fisher Scientific)] following manufacturer instructions. cDNA was diluted in RNase-free water and stored at -20°C .

2.4 | Molecular cloning

Alligator mississippiensis expressed sequence tag (EST) data was retrieved from the National Center for Biotechnology Information (NCBI). Polymerase chain reaction (PCR) primers were designed with Primer3 online software (bioinfo.ut.ee/primer3-0.4.0/). PCR reaction mixtures comprised standard reagents and concentrations (1x polymerase buffer, 0.75 mM MgCl_2 , 200 μM dNTP, 0.2 μM primers, 1 μL cDNA, 1.25 units Taq DNA polymerase per 50 μL solution). PCR reactions were performed using a RoboCycler Gradient 40 (Stratagene). Reaction solutions were heated to 94°C for 5 minutes, followed by 40 cycles of 94°C for 1 minute, $50\text{--}55^{\circ}\text{C}$ for 1 minute, and 72°C for 1 minute per kilobase of predicted product length. A final elongation step was performed at 72°C for 10 minutes. When necessary, target DNA bands were gel-purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research).

PCR products were ligated into the pGEM-T Easy Vector (Promega) for 1–7 days at 4°C , then transfected into DH5 α competent cells at 42°C for 30 seconds. Transformed cells were plated on ampicillin-positive LB agar plates with X-Gal (Goldbio) for blue/white selection and incubated overnight at 37°C . Plates were stored at 4°C for 1–4 days to allow for blue-colored colonies to darken. White colonies were picked and miniprep using PureLink Quick Plasmid DNA Miniprep Kits (Invitrogen). Plasmids were sequenced with T7 and SP6 primers at the University of Chicago Comprehensive Cancer Center DNA Sequencing Facility. Plasmids were stored at -20°C .

See Table 2 for alligator primer sequences. Mouse cDNAs were isolated with the following primers: *mBcl11a* (5'-AACAAACAGAGGTAATGGAAGC, 3'-CACTCAACAACCAACTAGAGC), *mEmx1* (5'-GCCTGGCTAAGGAGTGGCC, 3'-CAGGTGAAGGTGTGGTTCC), *mId2* (5'-GTCTGCTCTACAACATGAACG, 3'-CACTGGTTGTCTGAAATAAAGC), *mSatb2* (5'-AAGAGTGGAGCGAGAGAACCTTTC, 3'-CAAACAGGGCTTGAGACACCTTG).

2.5 | Section in situ hybridization

RNA probe template preparation. 75 μg of miniprep plasmid DNA were linearized by overnight restriction digestion at 37°C . Digested DNA was treated with 5 μL 10% SDS (Bio-Rad) and 5 μL proteinase K (Roche) for 15 minutes at 37°C , phenol/chloroform (Acros Organics) extracted, and ethanol precipitated. The dried DNA pellet was resuspended in 10 μL of Sigma water.

DIG-conjugated antisense RNA probe synthesis. RNA transcription reactions comprised 4 μL water, 4 μL 10x bovine serum albumin, 2 μL 10x transcription buffer, 2 μL 10x DIG-labeling

nucleotide mix (Roche), 2 µl Protector RNase inhibitor (Roche), 2 µl DTT, 2 µl template DNA, and 2 µl of RNA polymerase T7 or SP6 (New England Biolabs). The transcription reaction was carried out overnight at 37°C. The reaction solution was treated with 4 µl of RNase-free DNase (Roche) for 1 hour at 37°C. RNA was purified with two consecutive ethanol/LiCl precipitations and suspended in 100 µl of formamide (Sigma-Aldrich). RNA in formamide was heated at 50°C for 15 minutes to remove secondary structure before being imaged on a 1% agarose gel. RNA probes were judged acceptable if one or more bright, tight bands were visible.

Pre-hybridization tissue processing. Dried slides bearing brain sections were post-fixed in PFA for 15 minutes at room temperature and rinsed three times with DEPC-PBS. Tissue was treated with proteinase K in incubation buffer (1.5 µl proteinase K per 20 ml 100 mM Tris-HCl pH8.0, 50 mM EDTA pH 8.0) for 30 minutes at 37°C. Digested tissue was fixed in PFA for 15 minutes, rinsed three times with DEPC-PBS, and transferred to 5-slot slide mailers (Evergreen Scientific) containing 15 ml hybridization solution (50% formamide, 5x SSC, 1% SDS, 0.25 grams yeast RNA [Roche], and 0.1 grams heparin sulfate [Alfa Aesar] per 500 ml).

Hybridization and antibody binding. Slides in hybridization solution were heated in a 73°C water bath for 30 minutes. Hybridization solution was then decanted into a 50 ml conical tube (Denville Scientific), rapidly mixed with 95 µl antisense RNA probe reaction, and returned to the slide mailer for an overnight incubation at 73°C. The next day, RNA solution was decanted into a 50 ml conical and stored at -20°C for up to two additional uses. Slides were washed three times for one hour each at 73°C in preheated Solution X (50% formamide, 2x SSC, 1% SDS). Following Solution X washes, slides were washed three times in TBST (A 10x TBS stock was first prepared with 250 ml Tris-HCl pH 7.5, 80 g NaCl, 2 g KCl per 1 L. This was diluted and combined with 10 ml Tween for 1 L of 1X TBST). Slides were blocked for 1 hr in 10% lamb serum (Invitrogen) in TBST at room temperature.

Hybridized complexes of antisense probe/target mRNA were detected with anti-DIG Fab fragments conjugated to the colorigenic enzyme alkaline phosphatase (AP, Roche). The antibody-enzyme conjugate was preadsorbed with chick embryo powder/1% lamb serum in TBST, and then diluted to 1:5000 in TBST/1% lamb serum. Antibody solution was poured into slide mailers and antibody binding was carried out for 2 hours at room temperature. Antibody solution was decanted and stored at 4°C for up to 2 additional uses. Unbound antibody was removed by washing slides three times for 15 min in TBST. Slides were washed once in NTMT (100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM MgCl₂, 1% Tween).

Color reaction. The antibody/nucleic acid complex was detected with phosphatase histochemistry. 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Denville Scientific) stocks were produced by diluting 50 mg powder per 1 ml 100% dimethyl formamide (DMF, Acros Organics). The formazan nitro blue tetrazolium (NBT, Denville Scientific) stock was produced by mixing 100 mg powder in 1 ml 70% DMF in water. Color reagent stocks were stored at -20°C. Slides were incubated in 4 µl BCIP and 4 µl NBT stock per 1 ml NTMT at

room temperature for 1–7 days. Color reaction solution was replaced every day for the duration of color development. Following the color reaction, slides were washed twice in Stop TE buffer (10 mM Tris-HCl pH 7.5, 10 mM EDTA pH 8.0), then once more for 2 hours to overnight. Slides were washed overnight in TBST and fixed overnight in 10% Formalin (Fisher Scientific) in PBS. Slides were rinsed in PBS, dried, dehydrated in an ethanol series, soaked in Histoclear (National Diagnostics), and coverslipped with Eukitt mounting medium (Sigma-Aldrich) and microscope cover glass (Fisher Scientific).

2.6 | Antibody Characterization

Characterization of the anti-DIG-AP antibody, including appropriate control experiments, was described previously (Rowell, Mallik, Dugas-Ford, & Ragsdale, 2010). Anti-DIG-AP binds to digoxigenin, which is not endogenous to vertebrate tissue. In control experiments, digoxigenin-labeled RNA was omitted from the hybridization mixture. No signal was detected in the absence of digoxigenin-labeled riboprobes.

2.7 | Image capture and analysis

Images were captured with a Leica Fluo III microscope or a Zeiss Axioskop 40, a mounted AxioCam HRC color camera (Zeiss), and AxioVision software (version 4.8, Zeiss). Images were cropped and corrected for brightness using Photoshop CS6 (Adobe Systems). No other changes were made to images. Illustrations were produced with Illustrator CS6 (Adobe Systems).

3 | RESULTS

3.1 | Orientation and atlas

We examined late-embryonic alligators at Ferguson's stage 25, defined as resembling "a miniature version of a hatchling, with a considerable volume of external yolk and a large umbilical region" (Ferguson, 1985). These animals appear fully formed and are within few days of hatching (Figure 2a). The external morphology of the telencephalon is typical of reptiles: elongate, slender, and without any folds (Figure 2b). The olfactory bulb is situated in the alligator snout, connected to the DT by a long olfactory tract (Figure 2b, OB). The olfactory bulb was not examined in this study.

Like other studied vertebrates, the alligator has a DT enriched in *SLC17A6*-expressing excitatory neurons (Figure 2c). The DT, as identified by *SLC17A6* staining, is equivalent to the territory sometimes referred to as "pallium," as discussed by Dugas-Ford and Ragsdale (2015). Below the DT lies a ventral telencephalon where *SLC32A1*-expressing inhibitory neurons are concentrated (Figure 2d, VT). The VT is also likely the site of origin of the inhibitory interneurons that populate the DT (Anderson, Eisenstat, Shi, & Rubenstein, 1997; Marin & Rubenstein, 2001; Martinez-de-la-Torre, Pombal, & Puelles, 2011). These interneurons are scattered across all DT subdivisions in the alligator (Figure 2d). The dorsal-most zone of the VT expresses *PPP1R1B* (*DARPP32*) and is likely the alligator striatum (Figure 2e, St) (López, Morona, & González, 2017; Ouimet, LaMantia, Goldman-Rakic, Rakic, & Greengard, 1992; Reiner, Perera, Paullus, & Medina, 1998; Reiner et al., 2004).

The alligator DT is divided into the cerebral cortex and the DVR (Table 1). The cerebral cortex is divided into a lateral cortex (LC), dorsal cortex (DC), and medial cortex (MC) (Ulinski, 1990). We do not further subdivide the LC. Our data, presented below, establish that the dorsal cortex contains lateral (DCI) and medial (DCm) fields. Furthermore, the medial cortex contains lateral (MCI), intermediate (MCI), and medial (MCM) fields. We show that the DVR is divided into the mesopallium (M), nidopallium (N), and arcopallium (A). We do not designate further mesopallium subdivisions, but we do identify an important cell population that demonstrates anatomical continuity across the mesopallium and the dorsal cortex. We name this structure the mesopallial bridge (mb). The nidopallium contains at least three primary sensory input territories identified as nucleus basorostralis (Bas), the entopallium (E), and Field L (L), but these three territories only account for a small fraction of the total nidopallium volume. We divide the arcopallium into a dorsolateral nucleus (Adl), a dorsomedial nucleus (Adm), and a ventral nucleus (Av).

The described DT subdivisions are shown in the atlas, with 12 levels of the right telencephalon placed in an anterior to posterior sequence (Figures 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). The atlas is derived from a single cerebral hemisphere sliced coronally at 20 μ m thickness. At each level, three serially adjoining sections labeled for *ELAVL4* (panel a), *NEFM* (panel b), and *SLC17A6* (panel c) are shown. A schematic illustrates our interpretation of the anatomy at each level (panel d), produced by tracing the *ELAVL4* section. Gene expression patterns used to define these divisions and to support the proposed homologies are described below. Marker genes employed in this study are listed in Table 2.

3.2 | Organization of the alligator dorsal ventricular ridge

3.2.1 | A note on dorsal ventricular ridge nomenclature—Researchers have generally employed a nomenclature for the reptile DVR that is distinct from bird nomenclature. Ulinski, for example, divided the reptile DVR into ADVR and BDVR (Ulinski, 1983). Few have ventured to use avian nomenclature for reptile anatomy, even for territories highly likely to be homologous, such as the DVR target of ascending visual information from the thalamic nucleus rotundus (see below). The alligator DVR, however, bears a compelling resemblance to the bird DVR. We break from this tradition by using several avian terms that represent explicit proposals of homology.

3.2.2 | Sensory input domains and the nidopallium—In all amniotes studied, the DT receives ascending visual, auditory, and somatosensory information from the dorsal thalamus. Sensory input reaches neocortical L4 of primary sensory cortices in mammals. In birds, sensory input targets a trio of distinct DVR nuclei: nucleus basorostralis (somatosensory, auditory, and vestibular), the entopallium (visual), and Field L (auditory). We sought to identify molecularly the targets of primary sensory thalamic axons in the alligator DT.

Harvey Karten first proposed that the primary sensory input neurons of the mammalian and avian DT are homologous at the cell-type level (Karten, 1969; Karten, 2015). The Ragsdale laboratory provided support for Karten's cell-type homology hypothesis by identifying the ion channel gene *KCNH5/EAG2* and the transcription factor *RORB* as conserved molecular

markers of DT input territories (Dugas-Ford et al., 2012). Our work adds the transcription factors *RORA* and *SATB1* to this list of conserved input neuron marker genes (Briscoe, 2017). We examined the expression patterns of these four markers in the alligator DVR.

In birds, the thalamic nucleus rotundus receives visual information from the optic tectum. Rotundus projects in turn to the avian entopallium within the DVR. Michael Pritz identified a comparable projection from nucleus rotundus to the lateral DVR in alligators (Braford, 1972; Pritz, 1975). A charting of this projection pattern appears in Figure 15a. *KCNH5*, *SATB1*, *RORA* (Figure 15b–d), and *RORB* (not shown) riboprobes label this territory densely, closely following the nuclear position and contours that Pritz described. We designate this nucleus the alligator entopallium (E).

KCNH5 and *SATB1* are expressed in an additional nucleus ventrolateral to the entopallium (Figure 15b,c, Bas). *RORA* strongly labels this structure at more anterior levels (not shown). This nucleus resembles the avian nucleus basorostralis, which in chickens expresses the same input markers and is located in a similar topographical position (Briscoe, 2017).

Auditory information in the avian brain is relayed from the midbrain to the thalamic nucleus ovoidalis before reaching Field L in the medial DVR. Alligators have a thalamic auditory relay nucleus, the nucleus reuniens, which receives auditory information from the midbrain and projects to the medial DVR (Pritz, 1974a, 1974b; Pritz & Stritzel, 1992). The DVR target of this reported projection is illustrated in Figure 16a. *KCNH5*, *SATB1*, and *RORA* are expressed in a territory likely corresponding to this site of auditory input (Figure 16b–d, L). We name this zone the alligator Field L (L). Unlike the entopallium and the avian Field L, the alligator Field L does not appear to form a clearly delineated nucleus.

In alligators, somatosensory information originating in the spinal cord and the dorsal column nuclei is relayed through the thalamic nucleus medialis and reaches central ADVR (Pritz & Northcutt, 1980; Pritz & Stritzel, 1994). This thalamic projection to the DVR likely terminates between the entopallium and Field L. Input neuron marker gene expression is consistent with the described somatosensory projections (Figure 16b–d, asterisk). No cytoarchitectonic specializations are obvious here and we do not provide a name for this territory.

Avian nucleus basorostralis, entopallium, and Field L are subdivisions of a larger DVR territory, the nidopallium (Reiner et al., 2004). The nidopallium is a complex territory of unknown homology. Non-primary input zones of the avian nidopallium form associational connections implicated in avian cognitive functions (Atoji & Wild, 2009). The transcription factor *DACH2* is the only known marker gene that specifically labels the entire avian nidopallium (Briscoe et al., 2018; Rowell & Ragsdale, 2012; Szele, Chin, Rowlson, & Cepko, 2002). We found that alligator *DACH2* labels a massive territory similar to the avian nidopallium. A series of six sections from a single cerebral hemisphere is shown to demonstrate the overall organization of the alligator nidopallium (Figure 17a–f). Alligator *DACH2* labels a contiguous DVR territory that includes the previously described input zones in addition to a rostral area (Figure 17a), areas medial to nucleus basorostralis and the entopallium (Figure 17b,c), and a large caudal territory (Figure 17e,f). We do not provide

specific names for these nidopallium territories in order to avoid implying avian homologies. Dorsal cortex expression of *DACH2* (Figure 17), *KCNH5*, and *SATB1* (Figures 15 and 16) is discussed in the section on cerebral cortex.

3.2.3 | Mesopallium—Birds have a large DT associational territory called the mesopallium (Atoji & Wild, 2012; Reiner et al., 2004). The avian mesopallium is a target for sensory information from the primary sensory nuclei basorostralis, the entopallium, Field L, and the IHA (Atoji & Wild, 2012). Clarifications to mesopallium nomenclature denote a ventral mesopallium (Mv) located in the avian DVR and a dorsal mesopallium (Md) located in the avian Wulst (Jarvis et al., 2013; Reiner et al., 2004; Sun & Reiner, 2000). Our RNA-sequencing and gene expression studies identified a collection of six transcription factors (*EMX1*, *SATB2*, *ID2*, *FOXP1*, *BCL11A*, and *NHLH2*) highly enriched in avian Mv/Md (Briscoe et al., 2018). Five of these six transcription factors (the exception being *NHLH2*) are expressed in mammalian neocortical intratelencephalic (IT) association neurons, which in placental mammals include projection neurons of the corpus callosum. We proposed that the avian mesopallium (Mv/Md) and mammalian IT neurons are homologous at the cell-type level (Briscoe et al., 2018). The common ancestor of mammals and birds also gave rise to all extant reptiles, so we predicted that these transcription factors would be expressed together in the alligator DT.

We examined the expression of five IT-specific transcription factors and the mesopallium-specific ion channel gene *GRIK3* (Jarvis et al., 2013) in the alligator DT. These six genes identified the *DACH2*(–) dorsal DVR (Figure 18a–f) (Briscoe et al., 2018). All six genes are also expressed in the dorsal cortex. There are, therefore, two distinct alligator DT territories that express conserved IT/mesopallium marker genes: a dorsal cortex domain and a DVR domain (Briscoe et al., 2018). Dorsal cortex expression patterns will be described in more detail below. These two alligator domains form a continuous cell population laterally. This continuity is most obvious in Figure 18b, where *SATB2*-expressing cells curve around the lateral ventricle to link the dorsal cortex to the mesopallium, forming the mb (see also Figures 6–10). We do not propose any specific mammalian or avian homolog for the alligator mb.

To demonstrate the relational anatomy of the mesopallium and the nidopallium more clearly, we performed in situ hybridization for *EMX1* and *DACH2* on serially adjoining sagittal sections (Figure 19). Sections are oriented with anterior to the left and dorsal at top. *EMX1*- and *DACH2*-expressing territories are complementary in the DVR, but partially overlap in the dorsal cortex. At the medial-most level (Figure 19a,d), the mesopallium extends to the anterior pole of DVR while the nidopallium occupies the posterior pole. At lateral levels (Figure 19c,f), the mesopallium is limited to the anterior DT but the nidopallium is more extensive. The alligator arcopallium (Figure 19c,f, A) is identified as the *DACH2*(–)/*EMX1*(+) domain in posterolateral DT.

3.2.4 | Arcopallium—The alligator arcopallium is a relatively small territory in the posterolateral DT, defined in part by the absence of *DACH2* staining. The *DACH2*-expressing nidopallium extends to the posterior pole of the DVR and includes nearly all of the DVR territory that bulges into the ventricle (Figure 14d and Figure 17f). We used

cytoarchitectonic criteria to define a cell-dense dorsolateral nucleus (Adl), a cell-diffuse dorsomedial nucleus (Adm), and a thin, superficial ventral arcopallium (Av) (Figures 13, 14, 20). We identified very few molecular markers for specific alligator arcopallium nuclei. The exception is Adl, which is visible in *NEFM* staining (Figure 20a) and expresses the transcription factors *EMX1*, *LHX9*, and *LHX2* (Figure 20b–d). Future studies may reveal additional molecular subdivisions in the alligator arcopallium. We do not propose specific avian homologs of alligator Adl, Adm, or Av.

3.3 | Organization of the alligator cerebral cortex

3.3.1 | A note on reptile cerebral cortex nomenclature—Previous researchers studied the cerebral cortex in a variety of reptiles with differing cerebral anatomies. Consequently, a confusing array of nomenclatures has developed (Nieuwenhuys et al., 1998). Most researchers, however, agree that the reptile cerebral cortex contains three layers and can be divided into medial, dorsal, and lateral cortices (Naumann & Laurent, 2017; Ulinski, 1990). Some confidently named the medial cortex “hippocampus” and the lateral cortex “piriform cortex” (Cairney, 1926; Crosby, 1917; Johnston, 1915). We opt for the more commonly used topographic, evolutionarily neutral terms MC, DC, and LC and explore possible homologies with gene expression analyses.

3.3.2 | Dorsal cortex—*ELAVL4* is used as a general marker of neurons in metazoans (Shigeno, Parnaik, Albertin, & Ragsdale, 2015). *ELAVL4* staining in the alligator dorsal cortex demonstrates the canonical division into three layers (Figure 21a). An upper L1, closest to the pial surface, contains only a few, scattered neuronal cell bodies. A thicker L2 forms the principal cellular layer and is densely packed with cell bodies. An inner L3, closest to the ventricle, contains loosely arranged neurons.

SLC17A6 encodes the vesicular glutamate transporter 2 (*VGLUT2*) and is expressed in glutamatergic excitatory neurons. *SLC17A6*-expressing cell bodies are confined to the principal cellular layer, L2 (Figure 21b). *SLC32A1* encodes the vesicular inhibitory amino acid transporter (*VIAAT*) and is expressed in GABAergic and glycinergic inhibitory interneurons. *SLC32A1*-expressing cell bodies are scattered from the pial surface to the ventricular surface, in all three dorsal cortex layers (Figure 21c). These staining patterns suggest that nearly all neurons in L1 and L3 are inhibitory interneurons born in the VT, while L2 contains a mixture of inhibitory interneurons and DT-derived excitatory neurons. This result is consistent with the distribution of neurons described in turtle (Blanton, Shen, & Kriegstein, 1987; Reiner, 1991; Shen & Kriegstein, 1986) and lizard cortices (Schwerdtfeger & Lorente, 1988).

The sections in Figure 21 illustrate the transition zone where the relatively thin lateral L2 broadens to a thicker L2 medially (Figure 21b, arrow). The thinner lateral territory (DCl) and the thicker medial territory (DCm) are dissociated by gene expression. *SATB2*, an avian mesopallium marker, labels the lateral dorsal cortex (DCl) and the medial dorsal cortex (DCm), with an expression cutoff point that coincides with the medial boundary of DCm (Figure 22a, arrow). *SATB1* is a conserved marker of primary sensory input neurons (Briscoe, 2017). Its expression is restricted to DCm (Figure 22b, Figure 15c, Figure 16c).

Thus, we can molecularly define DCI as *SATB1*(-)/*SATB2*(+) and DCm as *SATB1*(+)/*SATB2*(+). The transcription factor *ID2* is robustly expressed in DCI (Figure 22c). Within DCm, *ID2* is weakly expressed in an upper territory and more strongly expressed in a lower territory (Figure 22c). *SATB1* is more strongly expressed in upper DCm (Figure 22b). *SATB1* and *ID2* expression indicates that DCm, and not DCI, contains molecularly distinct sublayers. We name the upper DCm sublayer L2a and the lower DCm sublayer L2b.

NEFM encodes the middle-weight neurofilament, which, like *ELAVL4*, is used as a general neuronal marker gene (Ding et al., 2016). *NEFM* strongly labels DCI, weakly labels DCm L2a, and strongly labels DCm L2b (Figure 23a). *DACH2*, a nidopallium and IHA marker in birds, is expressed in a stripe along the anteroposterior axis of the alligator dorsal cortex (Figure 17a–f, Figure 23b). The sections shown in Figure 23a and Figure 23b are serially adjoining: *DACH2*-expressing cell bodies are located in the *NEFM*-weak L2a sublayer along its entire extent. *NEFM* and *DACH2* show opposing expression gradients, with *NEFM* expressed more strongly in medial L2a and *DACH2* expressed more strongly laterally (Figure 23a,b). *KCNH5*, a conserved marker of DT input neurons (Dugas-Ford et al., 2012), is expressed in a small domain in lateral L2a (Figure 23d, also visible in Figure 15b). The IT neuron marker *FOXP1* is expressed in a band of uniform thickness in DCI L2 and DCm L2b (Figure 23c), a pattern similar to that of *GRIK3* (Figure 18f).

The mammalian neocortex contains primary sensory input neurons in L4 and subcerebral output neurons in L5. Output neurons project to motor-related targets in the brainstem and spinal cord, most conspicuously through the pyramidal tract. Karten proposed that neocortical output neurons are homologous to the long-range projection neurons in the avian HA and arcopallium, a prediction supported by comparative gene expression studies (Dugas-Ford et al., 2012; Rowell et al., 2010). We tested whether the alligator dorsal cortex expresses conserved marker genes for DT output neurons. Two such markers, *SULF2* and *CACNA1H*, are expressed in L2b of alligator DCm (Figure 23e,f). These expression patterns raise the possibility that alligator input neurons are found in a cortical layer directly above output neurons, a pattern reminiscent of the organization in the mammalian neocortex. It is unknown whether the alligator dorsal cortex projects to brainstem motor centers, but previous studies reported projections from the turtle cortex to the visual thalamus (dorsal lateral geniculate nucleus and nucleus rotundus) and the optic tectum (Ulinski, 1990).

The five IT-neuron transcription factors expressed in the alligator mesopallium are also found in the alligator dorsal cortex (Figure 18a–e). All five genes are expressed in DCI, a region depleted of the input markers *KCNH5* and *SATB1*. These gene expression patterns suggest that DCI will be found in future studies to be an association territory similar to the avian mesopallium (Atoji & Wild, 2012). The IT-neuron markers, with the possible exception of *EMX1* (Figure 18a–f), are also expressed in the inner layer of DCm, L2b. Alligator DCm L2b may therefore contain a mixture of intratelencephalic and extratelencephalic projection neurons, which would be notably similar to the cellular composition of neocortical infragranular layers (Fame, Dehay, Kennedy, & Macklis, 2017; Sohur, Padmanabhan, Kotchetkov, Menezes, & Macklis, 2014).

DCm and the mesopallium first appear at a more anterior level than DCI. The *SATB1*(+)/*SATB2*(+) DCm is first seen as a nub-like structure in Figure 4, adjacent to the anterior mesopallium. The *SATB1*(-)/*SATB2*(+) DCI appears at a more posterior level, as shown in Figure 6. We suggest that anterior mesopallium is indistinguishable from, or equivalent to, anterior DCI (Figure 4). Posteriorly, the mesopallium separates into the DCI and the DVR mesopallium cell populations by the intervening ventricle. DCI and mesopallium continuity is reflected by the mesopallial bridge, visible in Figures 6 through 10. The mesopallial bridge likely corresponds to the primordial general cortex of Crosby (1917), a “special” part of the anterior DVR that appears to transform into the cortex.

3.3.3 | Medial cortex—The six-layered mammalian neocortex transitions medially to a three-layered hippocampal formation, which features three highly conserved subdivisions. From medial to lateral, these subdivisions are the *Prox1*(+)/*Zbtb20*(+) dentate gyrus, the *Prox1*(-)/*Zbtb20*(+) CA fields, and the *Prox1*(-)/*Zbtb20*(-) subiculum (Mitchellmore et al., 2002; Nielsen, Nielsen, Ismail, Noraberg, & Jensen, 2007; Pleasure, Collins, & Lowenstein, 2000). There is a long-standing consensus that the reptile medial cortex contains a homolog of the mammalian hippocampus (Kappers et al., 1936). However, these conclusions are based on regional topography and differ significantly as to whether reptiles have homologs of individual hippocampal subdivisions (Striedter, 2016). We examined the expression of the well-characterized marker genes *PROX1* and *ZBTB20* in the alligator medial cortex.

NEFM staining demonstrates three differentiated subdivisions in the alligator medial cortex (Figure 24a). The ventral and medial-most field (MCm) contains densely packed neurons in a tight tri-laminar configuration. The main cellular layer is broader and less densely packed in an intermediate field (MCi). A narrow and more densely packed lateral field (MCl) separates MCi from the dorsal cortex (Figure 24a). Alligator *PROX1* is expressed specifically in MCm (Figure 24b). The *ZBTB20* expression domain encompasses both MCm and MCi (Figure 24c).

The third and lateral-most division of the medial cortex (MCl) does not express either *PROX1* or *ZBTB20* (Figure 24b,c). It does, however, strongly express *ETV1* (Figure 24d), a trait shared with the rodent and ferret subiculum (Dugas-Ford et al., 2012). No unique marker genes of the mammalian subiculum or the alligator MCl are presently known. We therefore distinguish the alligator MCl as the field between the *SATB2*(+) DCm and the *ZBTB20*(+) MCi (Figure 25a, MCl between arrows). We similarly found that the mouse subiculum is a *Satb2*(-) territory separating the neocortex from the CA fields (Figure 25b, Sub between arrows). *Satb2* is also expressed in the CA fields, although not in alligator MCi, which we interpret as a species difference in light of the remaining similarities of molecular topography (Table 3).

3.3.4 | Lateral cortex—Crocodilians have a large olfactory-recipient lateral cortex (LC) (Scalia, Halpern, & Riss, 1969). In the anterior-most atlas level (Figure 3), we interpret all visible cortex as a circumferential LC. This region does not express markers of DC, including either *SATB1* or *SATB2* (not shown). Consistent with this molecular observation, olfactory tracing studies in *Caiman sklerops* demonstrated circumferential olfactory input in anterior sections (Scalia et al., 1969). From the atlas level in Figure 4 onward, the LC is

restricted to the lateral DT surface. At the levels in Figure 5 through Figure 10, the dorsal boundary of LC is discontinuous with DC. Caudal to these levels, LC appears to merge with DC and it becomes increasingly difficult to discern dorsal and ventral LC boundaries based on cytoarchitecture. Interestingly, LC and DC merge at approximately the posterior boundary of the mesopallial bridge. Scalia et al. did not observe olfactory projections to ventroposterior DT in *Caiman* (Scalia et al., 1969), suggesting that the arcopallium does not contain olfactory cortex (Figures 13 and 14).

We did not identify any LC-specific marker genes, nor did we identify any genes that label the entire LC. We identified three transcription factors expressed in the anterior alligator LC and in the mouse piriform cortex: *BCL11A* (Figure 26a,d), *ID2* (Figure 26b,e), and *EMX1* (Figure 26c,f). Expression of these markers was weaker or absent at posterior levels (e.g., Figure 18a), indicating molecular heterogeneity across the LC. While these three genes are also mesopallium/IT neuron markers, LC does not express either *SATB2* or *FOXP1* (Figure 18b,d). These gene expression data supplement existing topographic and connectational data and provide some additional support for the homology of alligator LC to mammalian piriform cortex.

4 | DISCUSSION

“The relative simplicity and the primitive character of the brain of the alligator seemed to make it a fit subject for comparison with that of [mammals]. The important position occupied by these reptiles with relation to their own class and the Sauropsida generally... and especially their resemblance to birds in very important structural points, adds importance to otherwise trivial details. It soon appeared that, entirely aside from the problems in the interest of which the investigation was made, the intrinsic importance of the subject warranted a more detailed treatment than at first expected.”

-C.L. Herrick (Herrick, 1890)

4.1 | Alligator dorsal telencephalon in relation to birds

4.1.1 | Alligator dorsal cortex and avian Wulst—The most dramatic difference between the DT of reptiles and the DT of birds is that reptiles have a prominent cerebral cortex and birds do not (Figure 1). The avian Wulst is organized into nuclei and lacks the defining characteristics of cortex including either an outer fiber-rich layer or principal neurons with layer-spanning dendrites oriented perpendicular to the brain surface (Nauta & Feirtag, 1986). Despite these differences, some classical authors speculated that the avian Wulst is a greatly modified form of reptile dorsal cortex (Huber & Crosby, 1929). Molecular data support this interpretation and suggest homologies of the alligator dorsal cortex cell populations to cells within avian Wulst nuclei.

The avian Wulst contains input, output, and IT neurons organized into three separate nuclei (Figure 27, Avian Wulst) (Briscoe et al., 2018; Dugas-Ford et al., 2012). The IHA is a thin domain intercalated between the HA medially and Md laterally. The IHA is a primary sensory input territory in receipt of somatosensory and visual information from the dorsal thalamus (Karten, Hodos, Nauta, & Revzin, 1973). Consistent with its connections, the IHA

expresses conserved marker genes of mammalian neocortical L4 including *KCNH5* and *RORB* (Dugas-Ford et al., 2012). The IHA is also labeled strongly by *DACH2* (Briscoe et al., 2018). The HA is an output population that extends axons to brainstem motor centers (Karten, 1971). The HA expresses molecular markers of the output neurons in mammalian neocortical L5 (Dugas-Ford et al., 2012). The ventrolateral Wulst division, the Md, is an intratelencephalic relay territory and expresses markers for neocortical IT neurons (Briscoe et al., 2018).

We propose that the upper layer of alligator medial dorsal cortex is homologous at the level of cell type to the avian IHA. Alligator DCm L2a expresses *KCNH5* (Figure 23d), *SATB1* (Figure 22b), and *DACH2* (Figure 23b), and runs along the anteroposterior axis in a stripe-like configuration (Figure 17a–f). These characteristics are notably similar to the three-dimensional architecture of the avian IHA. L2a is a strong candidate for a primary sensory input zone of the alligator DC, but it is not known whether these connections exist. Previous experiments have recorded evoked potentials to visual and somatic stimulation in the alligator dorsal cortex, but lesion studies failed to identify retrogradely degenerated cells in the thalamus (Kruger & Berkowitz, 1960). *KCNH5* expression identifies a small subset of L2a cells. This may indicate that the primary input zone is very small, and relatively few thalamic projection fibers would be affected in lesion studies. On the other hand, this may simply reflect immaturity of the brain at the stage we examined. In the chicken *Gallus gallus*, for example, *KCNH5* expression does not become robust in the IHA until fairly late in gestation (Rowell, 2013).

The output neuron markers *SULF2* and *CACNA1H* are both expressed in DCm L2b (Figure 23e,f). These data suggest that L2b is an output-like territory in alligator cortex. There is to date no evidence for projections from the alligator dorsal cortex to the brainstem (Ulinski & Margoliash, 1990), but this point demands further study. Similarities in gene expression and position suggest that alligator DCm L2b and the avian HA contain homologous populations of output cell types.

We propose, again at the level of cell type, that the lateral part of the alligator dorsal cortex, DCI, is homologous to the avian Md. These structures share very similar positions in the DT, as the avian Md is found at the ventrolateral margin of the Wulst while alligator DCI is in ventrolateral dorsal cortex. Alligator DCI expresses five transcription factors characteristic of the avian mesopallium (Figure 18) and forms a physically continuous cell population with the mesopallium around the lateral ventricle (Figure 18b, mb). The avian Md abuts the DVR mesopallium anteriorly and, at more posterior levels in some species, is separated from the DVR mesopallium by the ventricle (Chen, Winkler, Pfenning, & Jarvis, 2013; Jarvis et al., 2013). Connections of the alligator DCI are not known. Previous studies reported association projections running anteroposteriorly within the cortex of lizards (Lohman & Mentink, 1972). Alligator DCI may form association connections, either anteroposteriorly within DCI or laterally across cerebral cortical fields. In the anterior alligator DT, a shallow sulcus separates the dorsal cortex from the DVR (Figures 4 and 5, arrow). This sulcus could be homologous as an anatomical landmark to the avian vallecule, which separates the Wulst from the DVR. Alternatively, this indentation above the alligator olfactory cortex may instead be comparable to the rhinal sulcus (Hall & Ebner, 1970).

Despite the potential homologies of cell populations described above (L2a to IHA, L2b to HA, and DCI to Md), the dorsal cortex and the Wulst are organized in strikingly different ways (Figure 27). The IHA in many birds is not orientated parallel to the brain surface like L2a, but is instead roughly orthogonal to the brain surface. Moreover, the IHA is not superficial as is alligator L2a, but is buried beneath the HA. Alligator L2a is separated from DCI in the plane of the cortical sheet. In birds, the IHA is stacked atop the Md. The developmental mechanisms of DT morphogenesis underlying these differences are an important future research direction.

Likely in order to adapt to their behavioral and ecological niches, birds expanded total cell numbers in their DT, even mirroring primate numbers when corrected for brain size (Olkowicz et al., 2016). A simple mediolateral tangential expansion of the alligator dorsal cortex would separate DCm cell populations from relays in DCI. This effect could be countered by reorganizing dorsal cortex cell types into a Wulst-like organization. Stacking the IHA, HA, and Md may be a more efficient means to build circuitry between input, output, and IT neurons: an IHA input neuron does not need to extend an axon very far to reach Md IT neurons for associative functions. Mammals independently evolved a similar, but distinct, system in the form of the columnar neocortical circuit linking input, IT, and output neurons (Medina & Reiner, 2000). Cell-type layering such as that present in the neocortex and in the Wulst might also provide computational benefits by bringing multiple distinct cell-type populations into topographical alignment. Such an alignment may facilitate the generation of map-like representations of sensory information and the coherent propagation of maps through multiple stages of information processing (Krahe & Maler, 2014). It is unknown whether nuclear or cortical architectures are better suited for processing different types of information, or why one architecture would be favored over another across evolutionary time.

4.1.2 | Alligator DVR and avian DVR—The bird DVR is large, cell-dense, and contains many internal subdivisions. The evolutionary origin of these subdivisions, and in particular the origin of the avian mesopallium and nidopallium, has been uncertain. We report here, in conjunction with our previous work, that the alligator has clear molecular homologs of the mesopallium, the nidopallium (including the entopallium and Field L), and the arcopallium (Briscoe et al., 2018). Of particular note is the presence of a putative nucleus basorostralis in the alligator DVR.

The unusual avian nucleus basorostralis receives trigeminal somatosensory information via a direct projection from the hindbrain principal trigeminal sensory nucleus, which travels through the quinfrofronal tract without an intervening synapse in the thalamus (Dubbeldam, Brauch, & Don, 1981). It also receives auditory input from the nucleus of the lateral lemniscus, as well as vestibular input (Arends & Zeigler, 1986; Wild & Farabaugh, 1996; Wild, Kubke, & Carr, 2001). Although not formally demonstrated, previous studies provided evidence for similar projections in a turtle (Kunzle, 1985) and a lizard (Ten Donkelaar & De Boer-Van Huizen, 1981). Clark and Ulinski noted that this territory in the alligator does not receive sensory input from any thalamic nuclei studied to date, and suggested that it may correspond to the avian basorostralis (Clark & Ulinski, 1984). The available molecular data support this homology and moves the evolutionary origin of this DT sensory input nucleus

to at least the common ancestor of birds and crocodilians. Future tracing and functional studies may show that the alligator nucleus basorostralis represents trigeminal somatosensory information from the jaws (George & Holliday, 2013). Whether the sensory projections to nucleus basorostralis are homologous to any mammalian pathways remains an interesting outstanding question.

The afferent and efferent connections of the alligator mesopallium, nidopallium, and arcopallium are not known. If they accord with what is known in birds, the alligator mesopallium and nidopallium would receive secondary sensory information from basorostralis, the entopallium, and Field L; the mesopallium and nidopallium would interconnect with one another; and the mesopallium and nidopallium would send projections to the arcopallium.

The alligator arcopallium deserves special attention, as it appears considerably smaller and less differentiated than its avian homolog. The avian arcopallium can be divided into two broad divisions based on function and connectivity. A dorsal somatomotor region receives sensory information from intermediate relays in the nidopallium and mesopallium, and projects to brainstem nuclei. A ventral “limbic” division forms connections with the hypothalamus (Zeier & Karten, 1971). Connections of the reptile BDVR are generally poorly understood, and this is especially true in alligator. Without tracing studies in the alligator, it is premature to propose homologies with either the somatomotor or limbic avian arcopallium. However, connections resembling the long-distance motor output of avian arcopallium have not yet been reported in reptiles. Moreover, the output neuron markers *SULF2* and *CACNA1H* are expressed in the chicken arcopallium (Dugas-Ford et al., 2012), but we did not detect their expression in the alligator arcopallium. It is possible that a somatomotor district of the crocodilian arcopallium is small or even absent.

4.2 | Alligator dorsal telencephalon in relation to mammals

4.2.1 | Alligator medial cortex and mammalian hippocampus—The hippocampus is thought to be “functionally homologous” across amniotes despite substantial variations in architecture (Colombo & Broadbent, 2000). In mammals, the hippocampal formation comprises the dentate gyrus, CA fields, and subicular complex. There is presently no consensus on whether reptiles and birds have specific homologs of these three divisions (Reiter, Liaw, Yamawaki, Naumann, & Laurent, 2017; Striedter, 2016). In this study, we provided evidence for a highly conserved tri-partite molecular organization present in the hippocampus of the mouse and the medial cortex of a reptile. Furthermore, these data strongly suggest that the crocodilian medial cortex alone is homologous to the mammalian hippocampus, and undermine the proposal that the reptile dorsal cortex and medial cortex are together homologous to the hippocampus (Desfilis, Abellan, Sentandreu, & Medina, 2018; Medina, Abellan, & Desfilis, 2017).

4.2.2 | Alligator dorsal cortex, alligator DVR, and mammalian neocortex—Non-avian reptiles are, along with mammals, the only known vertebrates with a multilayered cerebral cortex. That the reptile three-layered dorsal cortex must in some character feature

be homologous to the mammalian six-layered neocortex has never been controversial. The nature of this homology, however, has not been resolved.

One hypothesis proposes that the reptile dorsal cortex resembles the ancestral mammalian condition. In this view, the dorsal cortex layers L1, L2, and L3 are homologous to neocortical layers L1, L5, and L6. The dorsal cortex was elaborated into the neocortex by adding the upper neocortical layers L2, L3, and L4 (Cheung et al., 2007; Ebner, 1976; Reiner, 1991). This “deep-layer hypothesis” is no longer tenable. The reptile dorsal cortex contains cellular homologs of neocortical L4 input neurons. Tracing studies demonstrated nearly 50 years ago that the turtle dorsal cortex receives visual information from the LGN of the dorsal thalamus (Hall & Ebner, 1970). Although visual projections could have terminated on the apical dendrites of L5-like neurons, it is now known that visual input zones of the turtle dorsal cortex express neocortical L4 marker genes including *KCNH5* and *RORB* (Dugas-Ford et al., 2012). In this and a previous report, we provided molecular evidence that the alligator dorsal cortex contains homologs of neocortical IT neurons, which form the greater part of neocortical layers L2 and L3 (Briscoe et al., 2018). The IT neurons in the alligator dorsal cortex could be comparable to neocortical deep layer IT neurons, rather than L2/3 IT neurons, but there is currently no evidence to suggest that these neuronal subclasses are conserved from mammals to reptiles (Briscoe et al., 2018). We instead propose a homology at the level of IT neurons generally.

The dorsal cortex and the neocortex contain a similar set of neuronal cell types, but their organizations are divergent. Alligator DCI may consist almost entirely of IT neurons, while putative input and output neurons, together with some IT neurons, are layered in the alligator DCm (Figure 27, Alligator Dorsal Cortex). DCI and DCm form longitudinal bands along the anteroposterior axis. In contrast, the cell-type composition of the neocortex is relatively homogenous across the cortical sheet. Every neocortical area is thought to contain the same fundamental set of cell types in the same basic laminar organization: deep layers L5 and L6 contain output and IT neurons, L4 contains input neurons, and L2 and L3 contain IT neurons (Figure 27, Mammalian Neocortex). The only core neocortical cell type that may be absent from the crocodilian dorsal cortex is the cortico-thalamic neuron, neurons located in L6 that project from the neocortex to the thalamus but not beyond. These have not yet been formally demonstrated in the DT of any nonmammalian amniote. Previous studies identified projections from the avian Wulst to the thalamus, although it remains unclear whether these connections are collaterals of pyramidal-tract-like brainstem projections (Miceli, Repérant, Villalobos, & Dionne, 1987; Wild & Williams, 2000).

Both the alligator dorsal cortex and the DVR contain homologs of three core neocortical cell types. It is easy to imagine how the reptile dorsal cortex could be remodeled into a six-layered mammalian neocortex, but less so for the DVR. This effect of DVR appearance on human intuition contributed to early mischaracterizations of the DVR as an elaboration of ventral telencephalon structures (Kappers et al., 1936; Reiner et al., 2004). The very question of how the dorsal cortex or DVR could be modified into the neocortex is, however, misguided. The phylogenetic distribution of DT structures implies that there is no more reason to think the dorsal cortex and DVR are ancestral to mammals than to think the neocortex is ancestral to extant reptiles. More likely, the neocortex, DVR, and dorsal cortex

were derived from a structurally simple evolutionary antecedent that did not closely resemble any of its complex modern descendants. The hypothetical ancestral structure contained input, output, and IT neurons in an unknown configuration. It may, for example, have architecturally resembled the simple cortex found in the tuatara (Figure 1) (Reiner & Northcutt, 2000).

4.3 | Alligator dorsal telencephalon in relation to other non-avian reptiles

4.3.1 | Comparative anatomy of the reptile cerebral cortex—The cerebral cortex shows marked variation across non-avian reptiles, as reflected in the diverse nomenclatures developed for medial and dorsal cortices (Nieuwenhuys et al., 1998; Striedter, 2016). It is not known whether snakes, lizards, turtles, and tuataras have homologs of the alligator MCm, MCi, MCl, DCm, or DCI. In particular, some lizards and snakes have a discontinuous cerebral cortex where ends of opposing cortices can overlap to form a short segment of “five-layered” cortex (Figure 1, Iguana) (Northcutt, 1967; Ulinski, 1990). The molecular tools presented in this report could, with the exception of the endangered tuatara, readily address these outstanding questions.

The striking conservation of molecular topography in the mouse hippocampus and the alligator medial cortex strongly suggests that the same fields, molecularly defined, are present in all amniotes. Previous authors recognized three fields in the turtle medial cortex: a medial small-celled cortex (zone “2”), an intermediate large-celled diffuse cortex (“3”), and a lateral cell-dense cortex (“4”) (Riss et al., 1969). These fields are strong candidate homologs of the MCm, MCi, and MCl, respectively. Riss et al. were convinced of this possibility and designated three medial fields in crocodiles by the same nomenclature (Riss et al., 1969). The combinatorial expression of *PROX1*, *ZBTB20*, and *SATB2* may be sufficient to test for this tripartite organization in other reptiles and birds. At least in the chicken, *PROX1* and *ETV1* pick out non-overlapping territories in the medial-most DT (Dugas-Ford et al., 2012). *ZBTB20* is expressed in the medial DT of the zebra finch (www.zebrafinchatlas.org).

The evolutionary origin of the cell-type layering in alligator DCm is an important outstanding question requiring further comparative study. Such a layering scheme has not been described in any other reptile. It also has not been described in the numerous previous studies of the adult crocodilian telencephalon. The relative thickness of DCm compared to DCI is hard to miss in the presented embryonic specimens, but the cortex appears thin and uniform in available images of adult alligators and crocodiles (Crosby, 1917; Riss et al., 1969). It is possible that the embryonic layers flatten to an unknown arrangement after hatching. Late-stage embryos and adults should therefore be examined in other reptiles. If reptiles inherited and subsequently simplified a multilayered dorsal cortex, it would be a surprising reversal of the common conception that mammals elaborated the “simple” reptile cortex.

4.3.2 | Comparative anatomy of the reptile DVR—We identified an alligator DVR mesopallium and propose it is homologous to the avian ventral mesopallium (Mv) (Briscoe et al., 2018). The alligator mesopallium and the avian Mv have similar organizations and

share expression of five transcription factor markers and an ion channel gene. This conservation across more than 240 million years of divergence (Green et al. 2014) raises the possibility that a mesopallium may exist in other reptilian species. Pleurodiran turtles, for example, have a large nuclear territory in the rostral dorsal cortex that appears to continue into rostral DVR. This territory strongly resembles the alligator mesopallium in topography and morphology. Indeed, Riss et al. designated the Pleurodiran turtle structure and the crocodilian mesopallium as zone “5” (Riss et al., 1969).

Our previous studies of the chicken nidopallium transcriptome failed to identify any specific nidopallium markers beyond the transcription factor *DACH2* (Briscoe et al., 2018). Alligator *DACH2* expression identifies a bird-like nidopallium containing multiple primary sensory input nuclei. This marker gene may be useful for identifying the nidopallium, mesopallium, and arcopallium in other reptiles. The function of *DACH2* in the developing DT is not known, but conservation of expression across archosaurs suggests that it is an important cellular determinant. Future studies of *DACH2* function in the experimentally tractable chicken and mouse systems, together with more extensive molecular surveys in reptiles and anamniotes, may help to answer the outstanding question of nidopallium origins.

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Figure 1. Amniote phylogeny and representative telencephalon anatomies

The clade Amniota includes Mammalia and Sauropsida (reptiles and birds). Snakes (not shown), lizards, and the tuatara form a major Sauropsida group called Lepidosauria. The second major Sauropsida group includes turtles and Archosauria (crocodilians and birds). Schematic tracings of telencephalon anatomy for representative amniotes are shown in coronal cross sections, with medial to the right and dorsal at top. All amniotes have a ventral telencephalon (VT) and a dorsal telencephalon. Mammalian dorsal telencephalon includes the neocortex (Ncx), piriform cortex (Cpi), hippocampus (not shown), and amygdala (not shown). Non-avian reptile dorsal telencephalon includes a cerebral cortex with medial (MC), dorsal (DC), and lateral (LC) divisions, as well as a dorsal ventricular ridge (DVR). Birds

have a DVR, but with further internal subdivisions including the mesopallium (M), nidopallium (N), entopallium (E), and arcopallium (not shown). Birds also have a dorsally located Wulst (W), a medial hippocampus (Hp), and a lateral piriform cortex (CPi). Drawings are not to scale.

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Figure 2. Overview of the *Alligator mississippiensis* telencephalon

(a) Lateral view of a stage 25 alligator embryo. This animal is a “miniature hatchling.” (b) Rostrolateral view of an alligator telencephalon. Dorsal telencephalon (DT), ventral telencephalon (VT), hypothalamus (a diencephalic structure, Hy), and olfactory bulb (OB) are labeled. (c) *SLC17A6* expression identifies excitatory neurons of the DT. Medial cortex (MC), dorsal cortex (DC), lateral cortex (LC), and dorsal ventricular ridge (DVR) are labeled. (d) *SLC32A1* expression labels the ventral telencephalon (VT) and inhibitory interneurons scattered across all DT subdivisions. (e) *PPP1R1B* expression strongly labels the striatum (St), a VT subdivision. The internal VT region that does not express *PPP1R1B* is likely homologous to the mammalian and avian globus pallidus (GP). Sections correspond approximately to atlas level 8. Photograph in (a) by Z. Y. Wang.

a S8 *ELAVL4*

b S9 *NEFM*

c S7 *SLC17A6*

d Atlas

Figure 3. Atlas: Level 1

Sections 7–9 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration based on *ELAVL4*. The sections photodocumented in each Figure of this atlas (Figures 3–14) are serially adjoining neighbors.

a S14 *ELAVL4*

b S15 *NEFM*

c S13 *SLC17A6*

d Atlas

Figure 4. Atlas: Level 2

Sections 13–15 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration. The arrow indicates a shallow sulcus between dorsal cortex and DVR.

a S20 *ELAVL4*

b S21 *NEFM*

c S19 *SLC17A6*

d Atlas

Figure 5. Atlas: Level 3

Sections 19–21 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration. The arrow indicates a shallow sulcus between dorsal cortex and DVR.

a S32 *ELAVL4*

b S33 *NEFM*

c S31 *SLC17A6*

d Atlas

Figure 6. Atlas: Level 4

Sections 31–33 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

a S38 *ELAVL4*

b S39 *NEFM*

c S37 *SLC17A6*

Figure 7. Atlas: Level 5

Sections 37–39 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

a S47 *ELAVL4* b S48 *NEFM* c S46 *SLC17A6* **Figure 8. Atlas: Level 6**

Sections 46–48 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

a S53 *ELAVL4*

b S54 *NEFM*

c S52 *SLC17A6*

Figure 9. Atlas: Level 7

Sections 52–54 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

a S59 *ELAVL4*

b S60 *NEFM*

c S58 *SLC17A6*

Figure 10. Atlas: Level 8

Sections 58–60 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

Figure 11. Atlas: Level 9

Sections 70–72 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

Figure 12. Atlas: Level 10

Sections 76–78 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

Figure 13. Atlas: Level 11

Sections 85–87 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

a S95 *ELAVL4*

b S96 *NEFM*

c S94 *SLC17A6*

d Atlas

Figure 14. Atlas: Level 12

Sections 94–96 are shown. In situ hybridizations for (a) *ELAVL4*, (b) *NEFM*, and (c) *SLC17A6*. (d) Schematic illustration.

Figure 15. Conserved input neuron markers identify the crocodilian entopallium and nucleus basorostralis

(a) A schematic illustration adapted from Pritz (1975) shows the DVR target of ascending visual information from the thalamic nucleus rotundus (Rt). Stippling represents axon terminals. (b) *KCNH5* riboprobe labels the entopallium (E) and nucleus basorostralis (Bas). (c) *SATB1* riboprobe labels E and Bas. (d) *RORA* riboprobe labels E. *RORA* labels Bas weakly at this level, but strongly in more anterior sections (not shown). Sections correspond approximately to atlas level 7.

Figure 16. Conserved input neuron markers identify the crocodilian Field L

(a) A schematic illustration adapted from Pritz (1974b) shows the DVR target of ascending auditory information from the thalamic nucleus reuniens (NR). Stippling represents axon terminals. (b) *KCNH5*, (c) *SATB1*, and (d) *RORA* riboprobes identify Field L (L). These three genes are also expressed in a central territory (asterisk) that may receive somatosensory information from the thalamic nucleus medialis. (b) *KCNH5* and (c) *SATB1* label the posterior end of Bas at this level. Sections correspond approximately to atlas level 8.

Figure 17. Alligator *DACH2* expression identifies the nidopallium and a dorsal cortex cell population

An anteroposterior series of sections from a single cerebral hemisphere labeled with *DACH2* riboprobe. Primary sensory input nuclei are contained within the nidopallium (N): Basorostalis (Bas) is present in **b**, **c**, and possibly in **d**. The entopallium (E) is present in **c** and **d**. Field L (L) is likely present in **d**, but is difficult to identify with cytoarchitectonics. Territories labeled as N may be associative parts of the nidopallium. The *DACH2*(-) territory in dorsal DVR is the mesopallium (M). The *DACH2*(-) territory in ventroposterior DVR is the arcopallium (A). A thin dorsal cortex domain is labeled by *DACH2* across much of the anteroposterior axis of the telencephalon.

Figure 18. Conserved intratelencephalic projection neuron markers are expressed in the dorsal ventricular ridge mesopallium and in cerebral cortex cell populations

In situ hybridization for five intratelencephalic (IT) neuron transcription factors, and the mesopallium-specific ion channel gene *GRIK3*. (a) *EMX1*, (b) *SATB2*, (c) *ID2*, (d) *FOXP1*, (e) *BCL11A*, and (f) *GRIK3* identify the mesopallium (M) in the dorsal DVR. All six genes are also expressed in the dorsal cortex. (b) *SATB2* labeling connects the mesopallium to the dorsal cortex around the lateral ventricle. We name the *SATB2*(+) cell population at the lateral-most excursion of the ventricle the mesopallial bridge (mb). Other IT-specific genes label mb more strongly at anterior levels (not shown). Sections correspond approximately to atlas level 6. Photodocumentation of *EMX1*, *SATB2*, and *BCL11A* expression from a different alligator brain appears in Briscoe et al. 2018.

Figure 19. *EMX1* and *DACH2* in sagittal view

Six sagittal sections from the same cerebral hemisphere labeled with (a–c) *EMX1* or (d–f) *DACH2* riboprobes. Anterior is orientated to left and dorsal at top. a/d, b/e, and c/f are serially adjoining pairs progressing from medial to lateral. The *EMX1*(+) mesopallium (M) and the *DACH2*(+) nidopallium (N) form complementary territories in the DVR. The arcopallium (A) is visible in posterolateral DT (c, f).

Figure 20. Alligator arcopallium contains Adl, Adm, and Av subdivisions

(a) *NEFM* staining demonstrates a cell-dense dorsolateral arcopallium (Adl) and a cell-diffuse dorsomedial arcopallium (Adm). Adl is labeled by riboprobes for the transcription factors (b) *EMX1*, (c) *LHX9*, and (d) *LHX2*. We did not identify molecular markers for Adm. A thin, superficial ventral arcopallium (Av) is lightly stained for *EMX1*. Av can be seen more fully in the atlas. Sections correspond approximately to atlas level 12.

Figure 21. Neuronal distribution in the alligator dorsal cortex

Magnified views of central dorsal cortex are shown with medial to the right. The general neuronal marker (a) *ELAVL4* demonstrates the three principal layers in dorsal cortex, labeled L1, L2, and L3. (b) The excitatory neuron marker *SLC17A6* is restricted to the middle layer, L2. (c) The inhibitory neuron marker *SLC32A1* labels cells in all three dorsal cortex layers. The arrow in b indicates a transition from a thin lateral L2 to a thicker medial L2. The sections in b and c are serially adjoining, a is from a different brain. Sections correspond approximately to atlas level 7.

Figure 22. Alligator dorsal cortex contains medial and lateral fields

Alligator dorsal cortex can be divided into medial dorsal cortex (DCm) and lateral dorsal cortex (DCI). DCm expresses both (a) *SATB2* and (b) *SATB1*. DCI expresses (a) *SATB2*, but not (b) *SATB1*. (c) *ID2* strongly labels DCI, and labels lower DCm more strongly than upper DCm. *SATB1* shows the opposite pattern: it is expressed most strongly in (b) upper DCm. The sections in a and b are serially adjoining, c is from a different brain. The arrow in a indicates the medial boundary of DCm, where it meets the lateral medial cortex (MCI). Sections correspond approximately to atlas level 7.

Figure 23. Alligator medial dorsal cortex (DCm) contains molecularly distinct sublayers
(a) Within DCm, *NEFM* weakly labels an upper layer (L2a) and more strongly labels a lower layer (L2b). **(b)** *DACH2* expression is specific to DCm L2a. **(c)** The IT neuron marker *FOXP1* is expressed in DCI L2 and DCm L2b. **(d)** The input neuron marker *KCNH5* is expressed in a lateral subset of L2a cells. **(e)** The output neuron marker *SULF2* is most strongly expressed in DCm L2b. **(f)** The output neuron marker *CACNA1H* is expressed in DCI L2 and DCm L2b. The sections in **a** and **b** are serially adjoining, other sections are from different telencephala. Sections correspond approximately to atlas level 7.

Figure 24. Medial cortex contains three fields and expresses conserved hippocampal marker genes

(a) *NEFM* identifies three fields within the medial cortex: medial medial cortex (MCm), intermediate medial cortex (MCi), and lateral medial cortex (MCI). (b) The dentate gyrus-specific transcription factor *PROX1* is expressed only in MCm. (c) The dentate gyrus and CA field marker *ZBTB20* is expressed in MCm and MCi. (d) *ETV1* is expressed in MCI and MCi, but is nearly absent from MCm. Four serially adjoining sections are shown. Sections correspond approximately to atlas level 6.

Figure 25. A subiculum-like domain separates alligator intermediate medial cortex and dorsal cortex

(a) A two-probe, single-color in situ hybridization experiment for alligator *SATB2* and *ZBTB20*. *SATB2* has an expression boundary at the medial edge of medial dorsal cortex (DCm, left arrow). *ZBTB20* has an expression boundary at the lateral edge of intermediate medial cortex (MCi, right arrow). We define the unlabeled zone between these expression territories as lateral medial cortex (MCI). (b) The subiculum is a *Satb2*(-) zone separating the neocortex from the CA fields in postnatal day 6 mouse (unlabeled space between arrows). Mouse *Satb2* is also expressed in the CA fields.

Figure 26. Alligator lateral cortex expresses transcription factor markers of mouse piriform cortex

Anterior alligator lateral cortex (LC) expresses the transcription factors (a) *BCL11A*, (b) *ID2*, and (c) *EMX1*. Mouse piriform cortex (Cpi) expresses the same combination of genes (d–f) at postnatal day 6. Sections correspond approximately to atlas level 4.

Figure 27. Evolution of neocortical input, output, and intratelencephalic neurons

The common ancestor of amniotes had input (green), output (red), and intratelencephalic (IT, blue) neurons in its dorsal telencephalon. Input neurons receive primary sensory information from the dorsal thalamus (dTh), while output neurons extend axons from the dorsal telencephalon to the brainstem (Bst). IT neurons serve as a relay between input and output neurons. These three principal DT cell types were reorganized into divergent architectures in extant mammals, reptiles, and birds, but nonetheless maintained their defining connections and gene expression profiles.

Table 1**Atlas abbreviations**

Cerebral cortex	
DC	dorsal cortex
DCI	lateral dorsal cortex
DCm	medial dorsal cortex
LC	lateral cortex
MC	medial cortex
MCi	intermediate medial cortex
MCI	lateral medial cortex
MCm	medial medial cortex
 Dorsal ventricular ridge (DVR)	
A	arcopallium
Adl	dorsolateral arcopallium
Adm	dorsomedial arcopallium
Av	ventral arcopallium
M	mesopallium
mb	mesopallial bridge
N	nidopallium
Bas	nucleus basorostralis
E	entopallium
L	Field L
VT	ventral telencephalon

Table 2

Molecular markers for analysis of alligator telencephalon

Alligator cDNAs	Expression Pattern	PCR Primers
<i>BCL11A / CTIP1</i>	MCm, MCl, DC, LC, M, VT	F: TATCCCACAATTCATCTTCC R: GGTGGACTAGAGGTGTTTCC
<i>CACNA1H</i>	DCm L2b, DCI	F: TATCTGGACCTGTTTCATTACG R: GAATACTGTGCTGGCTATACG
<i>DACH2</i>	DCm L2a, N	F: TCATCAACACTTTCATCTCG R: AGAGCTGGACACACTACTGC
<i>KCNH5 / EAG2</i>	DCm L2a, Bas, E, L	F: GCTTGGATCTGATATTCTTCC R: GTGACATTATTCCAGTCTTCC
<i>ELAVL4 / HUD</i>	General neuronal marker	F: ACAGTAGAACTGCCCTTCC R: CTGTCTCCTAAACGGTATCC
<i>EMX1</i>	MCm, MCl, DCm L2a, DCI, LC, M, Adl	F: ACCAGAAGAAGAAGGTTCC R: AGCTGTTGACGACCTACG
<i>ETV1 / ER81</i>	MCl, MCl, DC	F: AGTCCATTCAAGACAACAGC R: GCAGACCATAGGAGTAATGC
<i>FOXP1</i>	DCm L2b, DCI, M, VT	F: TGTATGGACATGGTGTATGC R: ATGGTTCTTCTTTGACATGC
<i>GRIK3</i>	DCm L2b, DCI, LC, M, VT	F: TTAGACCTAGAGCCATACCG R: GTGAAGAACCACCAGATACC
<i>ID2</i>	MCm, MCl, DCm L2b, DCI, LC, M, VT	F: ACCCTAAATACAGACATCAGC R: CACTAGGAACTGAACACTGG
<i>LHX2</i>	Adl	F: CCTTAACGGACTTGACTAACC R: TGCCTCTCAAATTATTCACC
<i>LHX9</i>	Adl	F: AGTGCTGTGAATGTAACTGG R: CTGGCTGATACTTCAATAAGG
<i>NEFM</i>	General neuronal marker	F: CAACCATCAGTCACAATATCC R: CTCTACCTTCTTAGTGGACACC
<i>PPP1R1B / DARPP32</i>	LC, N, St	F: AGAGAAGAGACAGAGACAGAGC R: GAAAGTCCTGGAGATACATGG
<i>PROX1</i>	MCm	F: GGTCAGATAACGAGATGTCC R: GATGAGCTGAGAGGTAATGC
<i>RORA</i>	Bas, E, L	F: ACTCCAACAGAAGATTCAGC R: AGTACCATCCAAACCTCTCC
<i>RORB</i>	Bas, E, M	F: GAATGCAAGAATTACAGTTGG R: GAGCGTGTTCACTATATCTGG
<i>SATB1</i>	MCm, DCm, Bas, E, L	F: TGAACGAGATCGAATTTACC R: TGTACTTGGGTAGGTCAAGC
<i>SATB2</i>	DCm, DCI, M	F: CATCCCTAGAGCTATCAAACC R: GTATTGCTTTCTGCGTTACC
<i>SLC17A6 / VGLUT2</i>	Excitatory neurons	F: CGTTGTAACCTGGGAGTAGC R: GGACTAAAGCAGCAATAAGG
<i>SLC32A1 / VIAAT</i>	Inhibitory neurons	F: CTGTCTTTACGAGGAGAACG R: TGCAGATACCACCTATAACG

Alligator cDNAs	Expression Pattern	PCR Primers
<i>SULF2</i>	DCm L2b	F: AGAGACCAGTCAATAGGTTCC R: ATGTAGCTGGAGAGAGAGAGG
<i>ZBTB20</i>	MCm, MCi	F: GCTACAGTGACATTGAAATCC R: CTGTCTGGCGTAAATAGAGC

Genes examined in this study are listed at left, along with commonly used abbreviations where applicable. Territories labeled by each gene are listed in the middle column. Primers used to isolate each cDNA are listed at right. F, forward primer; R, reverse primer. Other abbreviations are according to Table 1.

Table 3

A shared molecular code for hippocampal subdivisions

Gene	<i>Alligator mississippiensis</i>				<i>Mus musculus</i>			
	DCm	MCI	MCi	MCm	Ncx	Sub	CA	DG
<i>SATB2</i>	+				×		×	
<i>ETV1</i>	+	+	+		×	×	×	
<i>ZBTB20</i>			+	+			×	×
<i>PROX1</i>				+				×

The transcription factors *SATB2*, *ETV1*, *ZBTB20*, and *PROX1* are expressed in a similar relative pattern in the medial DT of alligator and mouse. Medial medial cortex (MCm) is comparable with the dentate gyrus (DG). Intermediate medial cortex (MCi) is comparable with the CA fields (CA). Lateral medial cortex (MCI) is comparable with the subiculum (Sub). *SATB2* expression shows a medial boundary in the medial dorsal cortex (DCm) and the neocortex (Ncx). *Satb2* expression in mouse CA fields is a possible group-specific deviation from this otherwise conserved molecular organization.

Antibody	Hapten	Recognizes	Manufacturer Data	Dilution Used
Anti-DIG-AP	Digoxigenin (DIG)	DIG-labeled riboprobes	Roche Diagnostics, sheep polyclonal, Fab fragments conjugated to alkaline phosphatase, catalog #11093274910, RRID: AB_514497	1:5000