Organizational Conservation and Flexibility in the Evolution of Birdsong and Avian Motor Control

Bradley M. Colquitt

Howard Hughes Medical Institute, Chevy Chase, MD, USA; Department of Physiology, University of California-San Francisco, San Francisco, CA, USA

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Abstract
Birds and mammals have independently evolved complex behavioral and cognitive capabilities yet have markedly different brain structures. An open question is to what extent, despite these differences in anatomy, birds and mammals have evolved similar neural solutions to complex motor control and at what level of organization these similarities might lie. Courtship song in songbirds, a learned motor skill that is similar to the fine motor skills of many mammals including human speech, provides a powerful system in which to study the links connecting the development and evolution of cells, circuits, and behavior. Until recently, obtaining cellular-resolution views of the specialized neural circuitry that subserves birdsong was impossible due to a lack of molecular tools for songbirds. However, the ongoing revolution in cellular profiling and genomics offers unprecedented opportunities for molecular analysis in organisms that lack a traditional genetic infrastructure but have tractable, well-defined behaviors. Here, I describe recent efforts to understand the evolutionary relationships between birdsong control circuitry and mammalian neocortical circuitry using new approaches to measure gene expression in single cells. These results, combined with foundational work relating avian and mammalian brains at a range of biological levels, present an emerging view that amniote pallium evolution is a story of diverse neural circuit architectures employing conserved neuronal elements within a conserved topological framework. This view suggests that one locus of pallial neural circuit evolution lies at the intersection between the gene regulatory programs that regulate regional patterning and those that specify functional identity. Modifications to this intersection may underlie the evolution of pallial motor control in birds in general and to the evolutionary and developmental relationships of these circuits to the avian pallial amygdala.

Introduction
From elaborate avian courtship dance and whale song to intricate spider webs and cephalopod camouflage, nature abounds with complex behaviors generated by brains with diverse architectures and evolutionary histories. Understanding the organizational principles and innova-
tions that connect brain and behavior across species is a fundamental challenge in neurobiology and one that has tremendous importance for deciphering how nervous systems function and evolve. Within the vertebrate lineage, birds and mammals have independently evolved complex behavioral and cognitive abilities, such as highly developed food-caching, imitative tool use, and sensorimotor skill learning [Güntürkün and Bugnyar, 2016]. These abilities are matched in the brains of both birds and mammals by an expanded dorsal telencephalon, or pallium, which houses much of the neural circuitry that supports multimodal sensory integration, memory, and complex motor control. However, the organization of the pallium is markedly different between mammals and sauropsids (birds and reptiles), which has complicated efforts to understand the evolutionary relationships between regions that have similar functional properties yet have highly divergent structures. This difficulty has been particularly acute in understanding the relationships between the two largest structures in the mammalian and avian pallium – the neocortex and the dorsal ventricular ridge (DVR), respectively – each of which support forms of complex behavior and cognition seen in both lineages [Güntürkün et al., 2021]. In songbirds, the DVR contains the specialized neural circuitry that is dedicated to courtship song, a learned behavior with parallels to sensorimotor skills in mammals whose pallial circuitry resides in the neocortex, most notably speech learning in humans.

Complementary Perspectives on Pallial Homology

Two viewpoints currently dominate the conversation on the evolutionary relationships between the diverse pallial structures of amniotes [Striedter and Northcutt, 2019; Tosches, 2021]. The first, the cell type/circuit homology model, holds that amniote brains across different lineages contain conserved neuronal types and neural circuit architectures that were present in the pallium of the last common amniote ancestor [Dugas-Ford et al., 2012; Briscoe et al., 2018; Briscoe and Ragsdale, 2018; Stacho et al., 2020]. After the separation of the sauropsid and mammalian lineages, these archetypal components diversified into distinct structural arrangements – e.g., layered neocortex in mammals and nuclear dorsal ventricular ridge in sauropsids – yet retained their ancestral cellular identities and connectivity patterns. The second, the field homology model, stems from topological models of brain organization in which the pallium (and the neocortex generally) is divided into distinct sectors with defined relative positions [Medina et al., 2011; Puelles et al., 2017]. This regional map is argued to be strongly conserved across amniotes such that two structures found in two different species are said to be homologous “as a field” if they are located in the same spatially and transcriptionally defined pallial region.

In comparisons of the brains of birds and mammals, these broad classes of models have taken different forms over the years, with links drawn between different structures and connectivity patterns across species [Karten, 1991; Striedter, 1997; Jarvis et al., 2005; Reiner et al., 2005]. Under the current dominant framing, the cell/circuit view has been described under a “nuclear-to-layered” model, in which the different nuclei and anatomical subdivisions of the avian pallium are homologous to different layers of the neocortex [Karten, 1991; Reiner et al., 2005; Wang et al., 2010]. Similarly, the “field” view has taken the specific form of the “nuclear-to-claustrum/amygdalar nuclei,” which refers to the relationship between the dorsal ventricular ridge and its hypothesized field homology with the claustrum and pallial amygdala of mammals [Medina et al., 2011; Puelles et al., 2017; Medina et al., 2021].

Each perspective focuses on different biological features to marshal support for its case. The cell type/circuit model brings together similarities in connectivity properties between avian and mammalian neural circuits as well as common patterns of gene expression in differentiated, mature neurons between birds and mammals. In contrast, the field model is supported by shared expression patterns of key regional transcription factors during embryonic brain development and the spatial relationships between these transcriptionally and morphologically defined regions. Although the two models can be seen as in conflict, the distinct evidential emphases of each perspective suggest that each has merit and both models represent valid approaches to understanding pallial homology through a comparative lens.

Birdsong and Its Neural Circuitry as a Window into Pallial Evolution

The courtship song of songbirds, or birdsong, has served for several decades as an excellent model to understand the neural mechanisms that support the learning and performance of motor skills [Brainard and Doupe, 2013]. Birdsong is a complex vocal motor behavior that is learned by memorizing then imitating the songs of adult
con specifics. Birdsong begins as babbling-like, poorly structured vocalizations then gradually transforms through sensorimotor learning into a stable, ordered sequence of spectrally complex sounds. This course of developmental vocal learning is strikingly similar at the behavioral level to speech learning in humans [Doupe and Kuhl, 1999], making birdsong one of the few experimentally accessible models for studying the neural mechanisms supporting our own species’ adept abilities at vocal imitation. Beyond this developmental period of skill acquisition, the songs of adult songbirds exhibit a range of other forms of motor modulation and plasticity, including altered performance in different social contexts [Hessler and Doupe, 1999; Kao and Brainard, 2006] and adaptive modification of song features in response to environmental stimuli [Tumer and Brainard, 2007; Andalman and Fee, 2009; Charlesworth et al., 2011; Warren et al., 2011], making it an excellent general model for understanding motor skill learning.

A defining feature of birdsong is the specialized neural circuitry that subserves its performance and learning. This circuit, termed the “song system,” consists of a collection of interconnected nuclei that are located in disparate parts of the forebrain and are anatomically distinct from their surrounding regions [Brainard and Doupe, 2013]. The song system is divided into two pathways with different roles in motor performance and plasticity. The “song motor pathway” (or “vocal motor pathway”) is required for the performance of birdsong, in particular the ordering, tempo, amplitude, and spectral features of song elements. This pathway projects out of the forebrain to motor regions in the medulla that control the syrinx, the vocal organ of birdsong, as well respiratory centers that orchestrate the complex patterns of inspiration and expiration required for song production. The second pathway, termed the “anterior forebrain pathway,” is required for both song acquisition during juvenile development and adaptive song plasticity in adults. Its structure consists of a cortical (pallial)-basal ganglia-thalamic loop, a canonical neural circuit motif also found in the mammalian brain.

The behavioral likenesses between birdsong and mammalian motor skills combined with similarities in the functional and connectivity properties of the song system and mammalian motor circuits have made birdsong and its neural circuitry a focal model system for attempts to understand the evolutionary relationships between avian and mammalian brains [Konopka and Roberts, 2016; Jarvis, 2019]. Molecular analyses, in particular transcriptomic and in situ hybridization methods, have been instrumental in identifying compelling similarities between the song system and neocortical circuits. In particular, the song motor output nucleus RA (and adjacent regions of the arcopallium in which it is embedded) is transcriptionally similar to layer 5 of the neocortex, where extratelencephalic projection neurons reside [Dugas-Ford et al., 2012; Pfenning et al., 2014; Mello et al., 2019; Nevue et al., 2020]. Likewise, HVC, which projects to various targets in the telencephalon, bears some transcriptional similarity to layer 2/3 of the neocortex, which contains intratelencephalic projection neurons [Pfenning et al., 2014]. Combined, these correspondences between individual song system regions and neocortical layers are consistent with the “nucleus-to-layer” hypothesis.

However, the similarities to cortical circuitry are at odds with field arguments that emphasize that the song system is embedded in the dorsal ventricular ridge [Bruce and Neary, 1995; Puelles et al., 2000, 2017]. Instead, under this framework, these structures derive from distinct pallial fields – the song system within the ventral pallium and neocortical circuits within the dorsal pallium – with distinct regional patterning and developmental histories, and cannot therefore be considered homologous.

**Comparative Cellular Transcriptomics of Birdsong Control Circuitry**

Much of the difficulty in reconciling these views stems from differences in developmental time points assessed and the types of data that are emphasized. Methodological limitations also have restricted researchers to either looking at the expression of a small handful of genes across regions or thousands of genes in bulk microdissected tissue. In each approach, regions, and not individual cells, have been the primary units of comparison, which may be difficult to interpret given how highly divergent forebrain structures are across amniotes and the heterogeneous cellular composition of neural tissue.

Recent years have seen an explosion of new assays to profile the molecular properties of individual cells, opening up exciting avenues to generate cell-resolved molecular profiles across species that have historically been difficult to study at the cellular and molecular level. Such approaches allow treating cell types as the primary unit of comparison across neural domains and species, potentially providing greater clarity to questions of homology between highly diverged species that nonetheless have similar neural circuit and behavioral features. The song system, with its extensively characterized connectivity...
and roles in motor learning and performance, provides an excellent framework to make such cell-to-cell comparisons to similar neural circuits in the mammalian brain.

**Regional and Cellular Identities in the Song Motor Pathway**

To generate an initial picture of the cell types present in the song system, my co-authors and I generated single-cell and single-nucleus transcriptomic datasets for the two regions of the song motor pathway, HVC (proper name) and RA (robust nucleus of the arcopallium), from two closely related Estrildid finches, the Bengalese finch (*Lonchura striata domestica*) and zebra finch (*Taeniopygia guttata*) [Colquitt et al., 2021]. These regions are embedded in two distinct neuroanatomical domains, the nidopallium and arcopallium respectively, that are part of the dorsal ventricular ridge. We found a diversity of neural and non-neural types, ranging from neurons, astrocytes, and oligodendrocytes to endothelial, ependymal, and mural cells. We extensively characterized the number, regional distributions, and molecular profiles of the different neuronal classes found in each song system region. Glutamatergic neurons differed strongly between HVC and RA, forming distinct transcriptional clusters. GABAergic neuron classes, in contrast, were similar in both relative number and transcriptional profiles between each region, suggesting that these two song system components with distinct roles in song control have similar inhibitory networks.

With these neuronal profiles in hand, we performed a comparative transcriptional analysis of song system neuronal classes to neurons in the forebrains of mammals and non-avian reptiles, using previously generated cellular transcriptomic datasets from mouse (*Mus musculus*) [Saunders et al., 2018; Tasic et al., 2018; Zeisel et al., 2018] and the red-eared slider turtle (*Trachemys scripta elegans*) [Tosches et al., 2018]. Across these datasets, we separately compared glutamatergic and GABAergic neuronal classes, which gave different perspectives on the relationships between field and cellular identity. As in mammals, glutamatergic neurons in birds radially migrate out from ventricular neurogenic zones during embryogenesis to populate the pallium [Striedter et al., 1998; García-Moreno et al., 2018], thus forming regional territories that reflect their developmental origins. In contrast, GABAergic neurons migrate long distances from subpallial structures and spread throughout the pallium [Cobos et al., 2001; Marín and Rubenstein, 2001], such that the cellular identities of pallial inhibitory neurons are more strongly reflective of their distant subpallial origins and not their final pallial address.

We first examined field and cell type homology models by comparing the transcriptional profiles of songbird glutamatergic neurons with several transcriptional datasets obtained from the mouse pallium, including spatially resolved expression from the Allen Brain adult mouse in situ hybridization atlas and several single-cell RNA-seq datasets, encompassing the neocortex and some non-neocortical structures like the piriform cortex and pallial amygdala. We divided our comparisons into two broad sets of genes: transcription factors (representative of gene regulatory networks and positional information) and non-transcription factors (encompassing effector genes more closely related to cellular function). Comparisons using these two sets of genes yielded different associations between songbird glutamatergic neurons and mouse pallial regions. In particular, we found that glutamatergic neuron transcription factor profiles showed the strongest similarity to mouse ventral pallial regions, such as the piriform cortex, endopiriform nuclei, and pallial amygdala. In contrast, non-transcription factor profiles were most similar to regions in the dorsal pallium, including the neocortex and the cingulate cortex. A closer inspection of these neocortical similarities through comparisons with a mouse neocortical dataset that is categorized by layer and projection class [Tasic et al., 2018] indicated that each component of the song motor pathway was similar to distinct glutamatergic projection classes: HVC neurons were most similar to intratelencephalic (corticocortical and corticostriatial) projection neurons in multiple neocortical layers, while RA neurons were strongly similar to extratelencephalic (subcerebral) projection neurons in layer 5 (Fig. 1a).

The molecular profiles of GABAergic neurons in the song motor pathway clustered into three primary groups that had strong transcriptional similarity to the three major classes of pallial interneurons in mammals, which correspond to the different embryonic subpallial regions from which they are born – the medial ganglionic eminence (MGE), caudal GE (CGE), and lateral GE (LGE). We found that most songbird GABAergic neurons had close mappings to defined interneuron populations in the mouse neocortex, such as *Sst*, *Pvalb*, and *Vip*-class interneurons, as well as similar GABAergic neurons in the turtle pallium. However, the most abundant class of interneurons in the songbird song system is most strongly similar to various LGE-class neurons located outside of the neocortex, including medium spiny neurons in the striatum and granule neurons in the olfactory bulb. In-
Indeed, we found that these putative LGE-neurons are transcriptionally similar to medium spiny neurons in Area X, the striatal component of the song system. Lastly, we found that these neurons are not restricted to the song motor pathway but are broadly distributed throughout the songbird pallium. Hence, the similarity of GABAergic neurons across songbirds, turtles, and mice suggests that these are conserved populations that were present in the last common amniote ancestor. However, the abundance and wide distribution of LGE-class neurons in the songbird pallium and the absence of these neurons in the mammalian neocortex suggests that the patterns of LGE interneuron migration differ between each amniote branch, which could produce distinct local inhibitory circuit architectures. An open question is whether this LGE-class interneuron population plays a distinct computational role in avian neural circuits that is not found in mammalian neocortical circuits, and how these neurons influence birdsong performance and learning.

**Organizational Flexibility of the Pallium**

Our results are in agreement with a view that there is considerable evolutionary flexibility in the development and organization of pallial neural circuits. The presence of neuronal types in the ventral pallium with connectivity patterns and transcriptional profiles that are strongly similar to mammalian neocortical neuronal types indicates that regional location and/or developmental origin does not fully restrict terminal cell identity (Fig. 1b). Instead, similar, and perhaps homologous neuronal types, may derive from distinct, non-homologous developmental starting points. This type of process could generate the broad pattern of transcriptional similarity seen across distinct pallial sectors of the avian brain [Chen et al., 2013; Jarvis et al., 2013; Gedman et al., 2021].

However, the current data do not exclusively support this interpretation and additional analyses, employing high-resolution cellular transcriptomic atlases across...
more neural regions and species, would greatly clarify these issues surrounding regional and cellular homology. In particular, the current songbird analysis only includes the specialized neural circuitry of the song system, which has similar yet notably differentiated transcriptional, morphological, and physiological properties from adjacent tissue. It is possible that the transcriptional similarities seen between the song system and neocortex reflect the song system’s dedicated role in supporting the highly complex motor behavior that is birdsong (Fig. 1c). Key to resolving this issue would be separate single-cell RNA-seq datasets obtained from non-song-related regions in the nidopallium and arcopallium. Additionally, the apparent similarity between the ventral pallial song system and the neocortex could be due to the lack of high-resolution ventral pallial datasets in mammals. To date there has been a strong focus on generating thorough characterizations of the mammalian neocortex and hippocampus [Berg et al., 2021; Yao et al., 2021a, b]. Yet, similar cellular and spatial transcriptomics approaches to characterize the highly complex structures in the mammalian ventral pallium (in particular the various subdomains of the pallial amygdala and endopiriform nucleus) would be key comparators to understand the extent to which song system glutamatergic neurons are similar to glutamatergic neurons in the neocortex versus those in mammalian ventral pallium. One possibility is that glutamatergic neurons in the song motor pathway and the dorsal ventricular ridge generally are expansions of populations that were present in the last common amniote ancestor and are relatively less numerous and diversified in the mammalian ventral pallium (Fig. 1d). Finally, a more rigorous analysis of homology will require cell-resolved molecular characterizations of the brains of a range of representative species across phylogeny, including denser sampling of birds, non-avian reptiles, and mammals as well as anamniotes.

This framing has implications for how one should think about comparative analysis relating structures and neural circuits within common pallial regions across taxa. The key to resolving these issues will be an integrated, comparative understanding of how individual neural cell types and circuits develop in diverse brain regions and how phenotypic convergence and divergence are achieved at the molecular level. The arcopallium, a complex structure with its roles in both motor control and limbic function, could serve as a powerful model to understand the developmental gene regulatory mechanisms that connect the regional and functional identity of cell types and how modifications to these mechanisms may contribute to the evolution of neural circuit differences across species.

Motor Control in the Ventral Pallium and Relationships to the Amygdala

Avian Innovation in Pallial Motor Control

Beyond providing descending control of courtship song in songbirds, the arcopallium likely also serves as the primary pallial site for sensorimotor integration and motor control in all birds via a collection of subregions termed the “somatic” arcopallium. The largest descending fiber system in the avian brain, the occipitomesencephalic (OM) tract, stems from the arcopallium, with termini in a range of subcerebral targets that participate in motor control, including the medial spiriform nucleus (a pallial-cerebellar relay nucleus in the pretectum) [Gutiérrez-Ibáñez et al., 2018], midbrain and brainstem reticular formations, and cranial and trigeminal premotor nuclei [Zeier and Karten, 1971; Knudsen et al., 1995; Davies et al., 1997; Dubbeldam et al., 1997; Fernández et al., 2020]. Stimulation of the arcopallium in barn owls elicits head saccades [Knudsen et al., 1995; Winkowski and Knudsen, 2006], and lesions of the arcopallium or its descending tract produce akinesia in songbirds and disrupt pecking in pigeons [Levine and Zeigler, 1981; Mandelblat-Cerf et al., 2014]. Moreover, neural activity in the region that contributes most heavily to OM, the dorsal intermediate arcopallium (AId), is associated with voluntary motor actions, such as hopping and preening [Feenders et al., 2008; Yuan and Bottjer, 2020]. Notably, RA, the arcopallial song motor output nucleus, sits at the medial end of AId and has similar gene expression and connectivity as its neighbor, suggesting that RA is a specialized motor control region derived from ancestral motor circuitry [Feenders et al., 2008; Chakraborty and Jarvis, 2015; Jarvis, 2019; Nevue et al., 2020].

Combined, these data strongly suggest that a major source of descending motor control in the avian telencephalon is located in the ventral pallium (Fig. 2a). In mammals, similar sensorimotor circuitry in ventral pallial structures (e.g., pallial amygdala, piriform, and endopiriform nuclei) does not exist, suggesting that arcopallial motor control is a cellular/circuit innovation with no direct equivalent in the mammalian ventral pallium [Medina, 2007]. It is an open question, however, if there are cellular homologs in the mammalian ventral pallium to these arcopallial extratelecephalic neurons that are transcriptionally similar yet do not project to premotor regions. As noted above, a resolution to this issue would be aided by expanded cellular transcriptomic datasets from broader regions of the arcopallium, as well as higher-resolution cellular datasets from the...
mammalian ventral pallium, which has been poorly sampled to date.

In addition to this descending sensorimotor system in the avian ventral pallium, the most anterior and dorsal portion of the forebrain, the rostral hyperpallium or “Wulst,” also sends projections to extratelencephalic structures (Fig. 2a) [Karten et al., 1973; Reiner and Karten, 1983; Wild, 1992; Wild and Williams, 1999]. The hyperpallium is considered to be part of the dorsal pallium and homologous as a field to the neocortex of mammals, which has large extratelencephalic projection system from layer 5 pyramidal neurons via the pyramidal tract [Medina, 2007; Puelles et al., 2017]. Extratelencephalic projections also originate from a variety of pallial regions in lampreys [Ocaña et al., 2015], teleosts [Northcutt, 2006; Demski, 2013], and cartilaginous fish [Hofmann and Northcutt, 2012], which suggests that this class of projections is an ancestral trait among vertebrates.

These distinct extratelencephalic projection regions in birds, one from the ventral pallium and one from the dorsal pallium, potentially represent an example of phenotypic convergence across distinct neural domains. I propose that the gain of descending motor control in the ventral pallium occurred through the acquisition of an extratelencephalic cellular identity program utilized by ancestral neuronal populations located in other regions of the pallium. At the gene regulatory level, this acquisition could have occurred in two ways (Fig. 2b). Under one model, neurons in the arcopallium gained descending projections through the co-option of an ancestral gene regulatory network through cis-regulatory changes that allow regional transcription factors access to transcription factors specifying extratelencephalic-like cellular identity. Alternatively, regulatory networks that specify arcopallial regional identity could have directly gained access to effecter genes required for extratelencephalic projections (e.g., axon guidance ligand-receptor systems), again through evolution of cis-regulatory elements. These models can be tested using modern cell-resolved molecular assays to compare transcription factor expression and regulatory element accessibility in mature extratelencephalic projection neurons in the arcopallium and hyperpallium. These data could then be extended with datasets collected from the embryonic dorsal and ventral pallium to infer developmental trajectories of this neuronal type, using the mature molecular profiles as anchors, and to examine how early expressed patterning factors in each region intersect with regulatory networks specifying terminal neuronal identity.

Fig. 2. Developmental and evolutionary relationships between avian motor control regions and the pallial amygdala. a Schematic comparing pallial motor regions in birds and mammals. In birds, the arcopallium, a ventral pallial (VP) region, is a major center for descending motor control, containing glutamatergic neurons with transcriptional similarity to extratelencephalic (ET) neurons in the mammalian neocortex. The bird hyperpallium, a dorsal pallial (DP) region, also contains ET projection neurons; however, it is unclear if these play a role in motor control. The mammalian ventral pallium does not contain ET neurons, suggesting that their presence in this domain in birds is an evolutionary novelty. D, dorsal; P, posterior; L, lateral. b Two models of cell identity program acquisition across domains. Left: cis-regulatory changes give regional transcription factors access to ET selector transcription factor (TF) regulation, which in turn drives a conserved set of effecter genes that specify ET features. Right: different regional domains express distinct selector TFs that independently gain access to ET effecter genes through cis-regulatory evolution. c Two models of limbic and somatic neuron development in the arcopallium. Left: limbic and somatic neurons are generated by distinct radial units in the neuroepithelial progenitor zone. Right: each class of neuron shares a common progenitor zone and post-mitotic precursor neuron.
**Relationships between Somatic and Limbic Arcopallium**

A complementary issue to the convergence of cellular identity across different parts of the avian brain are the mechanisms that generate diverse neural circuits within a single region, and again the arcopallium serves as an interesting framework for this general question. The arcopallium is a highly heterogeneous structure, and the motor areas described above are surrounded by regions with limbic connectivity patterns and functional roles, consistent with topological and gene expression data that support the arcopallium as homologous to the pallial amygdala of mammals [Medina et al., 2017]. The developmental relationships between these motor and limbic neuronal populations are poorly understood. An analysis of neurogenesis during embryogenesis found that neurons in each region have distinct birth dates, such that somatic populations are born a day later than limbic populations [Tsai et al., 1981]. However, it is unknown if these different regions stem from distinct radial units, as has begun to be mapped for the different subdivisions of the mammalian pallial amygdala [Garcia-Calero et al., 2020], or if motor versus limbic diversification occurs at later developmental timepoints, as is seen in the specification of neocortical projection neuron subtypes [Di Bella et al., 2021] (Fig. 2c). As for understanding the regulatory relationships connecting hyperpallial and arcopallial neural circuits, cell-resolved assays of neurogenesis in the arcopallium, combined with lineage tracing using molecular barcoding [Kebschull and Zador, 2018], would be instrumental in resolving the developmental relationships connecting the distinct areas of this complex structure.

An intriguing idea is that the developmental and physical proximity of motor and limbic circuitry in birds confers distinct sensorimotor capabilities or restrictions that differ from those of mammals. In particular, many species of birds produce highly orchestrated courtship displays, such as birdsong and bird dance, at a level of complexity which is not seen in mammals. One particular question is whether the unique organization of the arcopallium enabled the evolution of such highly skilled social behaviors through the developmental and functional integration of sensorimotor and limbic circuitry.

**Outlook**

Many species of birds and mammals – classes separated by 320 million years of evolution – have independently evolved complex behavioral and cognitive abilities. This evolutionary distance is reflected in the markedly different pallial structures of these two taxa. Yet, clear similarities exist between the brains of birds and mammals, from cells to circuits. Many of these features, such as regional patterning factors and the specification of basic projection neuron classes and interneuron subtypes, likely derive from the last common ancestor of all amniotes. Other features, such as the presence of sensorimotor circuitry in distinct pallial regions in birds and mammals, may have arisen through a process of convergence. Further clarity will require expanding comparative cellular analyses to a broader range of species across the phylogenetic tree (to other amniotes and vertebrates more generally) and to a broader set of developmental stages to better infer developmental trajectories.

Finally, much energy has focused on understanding the ways in which the neural, behavioral, and cognitive features of birds and mammals overlap. However, an important future question is understanding to what extent these different evolutionary histories, and the distinct neural circuit architectures they engendered, have constrained or enabled taxa-specific features of behavior and cognition. Cell-resolved evo-devo approaches, combined with comparative connectomics and a careful accounting of behavioral differences, provide an exciting avenue to understand the mechanisms underlying the evolution and development of neural circuit and behavioral diversity.

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**Conflict of Interest Statement**

The author has no conflicts of interest to declare.

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**Author Contributions**

B.M. Colquitt wrote the manuscript.
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