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Authors

Martínez-Cerdeño, Verónica
García-Moreno, Fernando
Tosches, Maria Antonietta
et al.

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Update on forebrain evolution: From neurogenesis to thermogenesis

Verónica Martínez-Cerdeño[1], **Fernando García-Moreno**[2], **Maria Antonietta Tosches**[3], **András Csillag**[4], **Paul R. Manger**[5], and **Zoltán Molnár**[2]

[1]Department of Pathology and Laboratory Medicine, UC Davis; Institute for Pediatric Regenerative Medicine and Shriners Hospitals for Children Northern California; MIND Institute, UC Davis School of Medicine, California, USA

[2]Achucarro Basque Center for Neuroscience, Parque Científico UPV/EHU Edif. Sede, E-48940 Leioa, Spain

[3]Max Planck Institute for Brain Research, Frankfurt A.M., Germany

[4]Department of Anatomy, Histology and Embryology, Semmelweis University, Faculty of Medicine, Budapest, Hungary

[5]School of Anatomical Sciences, Faculty of Health Sciences, University of Witwatersrand, South Africa

Abstract

Comparative developmental studies provide growing understanding of vertebrate forebrain evolution. This short review directs the spotlight to some newly emerging aspects, including the evolutionary origin of the proliferative region known as the subventricular zone (SVZ) and of intermediate progenitor cells (IPCs) that populate the SVZ, neural circuits that originated within homologous regions across all amniotes, and the role of thermogenesis in the acquisition of an increased brain size. These data were presented at the 8th European Conference on Comparative Neurobiology.

Keywords

Radial glial cells; intermediate progenitor cells; cerebral cortex development; cerebral cortex evolution; neural circuits evolution; brain size; neurogenesis; thermogenesis; reptile; avian; mammal

Comparative developmental studies provide growing understanding of vertebrate forebrain evolution. This short review directs the spotlight to some newly emerging aspects, including the evolutionary origin of the proliferative region known as the subventricular zone (SVZ)

Corresponding authors: Zoltán Molnár: University of Oxford, UK, zoltan.molnar@dpag.ox.ac.uk, Verónica Martínez-Cerdeño: UC Davis School of Medicine, California, USA, vmartinezcerdeno@ucdavis.edu.

and of intermediate progenitor cells (IPCs) that populate the SVZ, neural circuits that originated within homologous regions across all amniotes, and the role of thermogenesis in the acquisition of an increased brain size. These data were presented at the 8th European Conference on Comparative Neurobiology.

1. Progenitor cells in the SVZ of the developing cerebral cortex

The great evolutionary expansion of the cerebral cortex underlies the unique cognitive functions of humans. Some of the most significant steps in the evolutionary expansion of the cerebral cortex include the radial expansion of the cortex from three to six layers, and the tangential cortical expansion from lyssencephalic to gyrencephalic [1–8]. This expansion is due in part by increased progenitor cell proliferation in the SVZ of the developing cortex. Cortical neurons are produced by progenitor cells that reside in the ventricular zone (VZ) directly adjacent to the ventricle, and the SVZ superficial to the VZ. Radial glia cells (RGCs), the primary progenitor cell of the cerebral cortex, reside in the VZ and express Pax6 [9], experience interkinetic nuclear movements [10–15], and have a bipolar morphology with a long pial process. Intermediate progenitor cells (IPCs), the secondary progenitor cell of the cerebral cortex, reside in the SVZ and express Tbr2 [16], undergo division away from the ventricle, lack interkinetic nuclear movement, and have a multipolar morphology [14, 17]. RGCs and IPCs have been identified in the developing cerebral cortex of all mammals investigated to date and other vertebrates including reptiles and birds [18]. A third progenitor cell, the translocating radial glia cells (also known as oRGCs) [19], detaches from the ventricle, migrate through the SVZ, and in the rat undergo divisions that generate non-neuronal daughter cells [14]. Work in human, macaque monkeys, and the other gyrencephalic mammals has shown that the SVZ is subdivided into distinct proliferative zones, the inner SVZ (iSVZ) and outer SVZ (oSVZ) [2, 5–8]. The oSVZ is also present in the rat during late gestation [2]. Early loss of IPCs in the SVZ leads to a decrease in cortical surface expansion and thickness with a neuronal reduction observed in all cortical layers. These findings suggest that IPC progeny contribute to the correct morphogenesis of each cortical layer (Sessa 08). These data highlight an evolutionary conserved role for Tbr2+ IPCs function in establishing correct cerebral cortex expansion and supporting correct development of specific brain structures [20]. Contrasting with these data, it has been suggested that distinct RGC lineages are specified to generate only upper-layer neurons, independently of niche and birthdate [21].

To understand the evolutionary origin of the SVZ and of the Tbr2+ IPCs in the developing cortex, we analyzed the presence and distribution of Tbr2+ cells and of the SVZ in the prenatal cortex of representative species of each of the amminote orders [1]. This study included mice, turtles, pigeons, doves, crocodiles, lizards, and snakes (Figure 1). We found that Tbr2+ cells are present in the proliferative zones of turtle dorsal cortex, and that in the turtle DVR Tbr2+ cells are organized into a tight SVZ band that is superficial to the ventricular zone (VZ) as in developing rodent forebrain. We also found that the developing (forebrain) telencephalon of chick and dove possess numerous Tbr2+ cells that are organized into a distinct SVZ in a manner that is indistinguishable from that of developing rodent telencephalon (forebrain). In addition, we showed that a small number of Tbr2+ cells are scattered throughout the VZ in the developing lizard and crocodile forebrain, but that a

distinct SVZ is not present. Interestingly, the developing snake cortex presented with a different structure than that of lizard and crocodiles, being more similar to that of mice where Tbr2+ cells were organized into a tight SVZ band that was superficial to the VZ. These data demonstrating that Tbr2+ cells and a distinct SVZ are present in mammals, birds, snakes, and some regions of the developing turtle brain, suggest that the principal cellular elements of the mammalian SVZ evolved prior to the appearance of modern day mammals and were likely present in the ancestor to both mammals and sauropsids. Based on our data, we propose that the presence of an SVZ increases the number of proliferative cells. We also propose that since turtle, the closest living ancestor to both reptiles and mammals, possesses a rudimentary SVZ, the SVZ and the proliferative cells it contains may be a factor that aided the radial expansion of from allocortex to isocortex. However, the fact that some mammalian species do not have an SVZ and that some reptile species do, suggests that the presence of the SVZ is not an essential requisite for the radial expansion of the cortex.

2. Intermediate progenitor derived cells contribute glutamatergic cells to all cortical layers

A new method (CLoNe) to study lineage in mammalian and avian brains was recently introduced [22]. This method was employed to assess the clonal size and dispersion of the neurons generated from the Tbr2+ IPCs in the mouse cerebral cortex. These studies revealed that IPC derived cells contribute glutamatergic cells to all cortical layers including the earliest generated subplate zone. All cortical layers receive contribution from the Tbr2+ IPCs, 20–40% of all layers is derived through Tbr2+ IPCs in S1. Layers 2–3 shows the highest (40.2%) while Layer 5 is the least (19.8%). However, no GABAergic interneurons or astrocytic cells are generated through these progenitor cells [23]. Moreover, the clonally related cells dispersed less than the clonally non-related neurons. Pair-generated cells in different layers cluster closer (142.1 ± 76.8 μm) than unrelated cells (294.9 ± 105.4 μm). The clonal dispersion from individual Tbr2+ IPCs contributed to increasing the cortical surface [23]. The distribution, compartmentalisation of the various progenitor cells in the germinal zones in various sauropsids and mammals has been linked to cortical thickness, cell numbers and brain folding. Previous studies of macaque and human cortices identified cytoarchitectonically distinct zones within the SVZ, the iSVZ and oSVZ that are separated by an inner fiber layer (IFL) [5]. The origins and destinations of the fibres in the IFL are not known, but Molnár and Clowry described selective immunoreactivity for SRGAP1 (ROBO1 receptor-associated protein) in the IFL in the developing cortex in 15PCW human [24]. However, it is clear that the cytoarchitectonic subdivisions of the SVZ to iSVZ and oSVZ is not a uniquely primate characteristics and it is not linked to gyrencephalic brains [2, 25]. Rather, cytoarchitectonic subdivisions of SVZ are an evolutionary trend and not a primate specific feature, and a large population of and oRGCs can be seen regardless of cortical folding. Garcia-Moreno and colleagues examined the Amazonian rodent agouti (*Dasyprocta agouti*) and the marmoset monkey (*Callithrix jacchus*) to further understand relationships among progenitor compartmentalization, proportions of various cortical progenitors, and degree of cortical folding. The proportions of RGCs, IPCs, and oRGCs populations were similar in midgestation lissencephalic marmoset as in gyrencephalic human or ferret. These

authors also identified a similar cytoarchitectonic distinction between the oSVZ and iSVZ at midgestation in marmoset and agouti as was described in rat, ferret and macaque [2].

The lineage and fate restrictions of the various cortical progenitors in various vertebrates are not fully understood. Studies on cortical radial progenitors demonstrated that a subset of early cortical progenitor cells show a delay in neuron generation (Figure 2). In the cortex, neurons are generated in a temporal sequence, however a subset of progenitor cells (RG cells) that lack neurogenic potential during the earliest phase of corticogenesis in mammals was recently described [21]. These early cortical progenitor cells that show a delay in neuron generation only generate upper-layer callosal neurons and glial cells [26]. It has previously been suggested that the temporal fate restriction of these cortical progenitor cells present in mammals do not occur in avian species [27]. It was tested whether progenitor cells that lack neurogenic potential during the earliest phase of corticogenesis and later selectively generate upper layer neurons are present in prenatal chicks. This study did not find evidence of the existence of neurogenic delay in progenitor cells of any avian pallial region [26]. It is known that heterochronic changes of developmental events have contributed to cortical variation within the mammalian taxon [28], but also to telencephalic divergences in the amniote radiation. These data strongly suggest that early during the mammalian radiation, a subset of cortical progenitor cells experienced a delay in their neurogenic period, which drove to the expansion of upper-layer like neurons and the genesis of an inter-pallial communication through the corpus callosum, two typical features of mammalian neocortex.

3. Transcriptome analysis uncovered an unexpected heterogeneity of both excitatory and inhibitory neurons in turtle and lizard

Amniote forebrains develop from conserved subdivisions of the pallium and subpallium [29]. But despite this, the forebrains of adult reptiles, birds and mammals show remarkable differences in neuroanatomy and function. For example, the telencephalon can be organized in three layers (reptilian cortex), six layers (mammalian isocortex) or nuclei (avian pallium). This diversity might be partially explained by differences at the level of the molecular identity, division behavior and fate of neural progenitors. However, it is not entirely clear whether cell types in amniote forebrains are still conserved at some level; in particular the evolutionary origin of the mammalian isocortical cell types remains obscure. Reconstructing forebrain evolution at the cell type level has key implications for our understanding of amniote cortical circuits. Numerous studies have noted similarities in connectivity and gene expression between the layers of the mammalian isocortex and the avian and reptilian pallia. However, these studies were limited to the analysis of few markers, leaving space to several conflicting hypotheses on neocortex evolution [30–32]. For example, recent studies have suggested the homology of mammalian layer 2/3, layer 4 and layer 5 cell types to adjacent regions in the avian telencephalon [27, 31, 33]. A recent comparative transcriptomic analysis on tissue microdissections allowed us to critically evaluate these results, overcoming the potential biases and ambiguities introduced by the analysis of few selected marker genes. Indeed, these homologies are not significantly supported when a global transcriptomic involvement is investigated (http://geserv.anat.ox.ac.uk) [34]. Furthermore, our transcriptomics analysis suggests there are genes that, if considered individually, could be

used to support many different relationships [33]. This is consistent with the observation that individual genes are typically expressed in multiple tissues, and that cell type and tissue identity are specified by combinatorial codes of transcription factors.

However, transcriptomics studies applied to dissected structures are not sufficient to identify cell type homologies, because they reflect the weighted average of all cells in a region and could be affected by cellular heterogeneity. To circumvent this problem, single cells rather than regions should have been studied and compared. Recent techniques, such as DropSeq [35], allow obtaining transcriptomes of individual cells, and these single cell transcriptomes can be used to reconstruct cell type evolution [36]. The application of these approaches to reptiles has yielded a new perspective on cortical evolution. In contrast to birds, the reptilian telencephalon harbors a simple three-layered cortex, which is likely similar to the ancestral amniote condition and it is easier to compare to the mammalian cortex [37]. In the turtle dorsal cortex, up to six different cell types had been previously identified on the basis of morphology and electrophysiological properties [38, 39]. Previous molecular studies, examining few markers, had suggested the existence of glutamatergic cells homologous to mammalian layer 2/3, layer 4 and layer 5 neurons, and located in adjacent regions of the turtle brain [31, 40]. Furthermore, the analysis of GABAergic interneuron markers had indicated the absence of several interneuron types, such as VIP and PV interneurons [32]. The single cell data from the turtle *Trachemys scripta* and the lizard *Pogona vitticeps* point to different conclusions. Markers of all mammalian GABAergic interneuron types are expressed also in reptilian interneurons, indicating that all the major classes of mammalian cortical interneurons are conserved in amniotes. Glutamatergic cells are also heterogeneous at the molecular level, and subsets of them express different mammalian layer markers. The exact position of these cell types in the pallium can be reconstructed with immunostainings and *in situ* hybridizations. This analysis reveals that cell types expressing different mammalian layer markers are intermingled in the reptilian dorsal cortex. Furthermore, these layer markers are expressed in combinations never observed in mammals: for example, classical markers of upper and lower layers are coexpressed in the same cells. Taken together, the unbiased analysis of single cell transcriptomes suggests that new neural types in the mammalian isocortex evolved from the complete rearrangement of ancestral regulatory programs determining cell fate. This conclusion is consistent with the differences observed in reptilian, avian and mammalian neurogenesis.

4. Specific regions of the ventrobasal forebrain and the arcopallial-subpallial pathway in chicken may be homologous to the nucleus accumbens and the amygdalar-striatum pathway, respectively, in mammals

Further studies on the evolution of pallial neural circuits were presented by Dr. András Csillag. Dr. Csillag investigated the evolutive origin of the ventrobasal forebrain (nucleus accumbens of the ventral striatum in mammals). The nucleus accumbens (Ac) receives projections from the pallial amygdala and regulates reward and motivation-related behaviors, also influenced by fear and anxiety. The nature and precise location of the Ac in avian species have been controversial until recently. Dr. Csillag investigated the possibility that the nucleus accumbens (in particular the shell) is part of extended amygdala (EA), a node of

social behavior network [41], in avian species, specifically in chicken. They performed choleratoxin B retrograde tracing and dextran anterograde tracing to demonstrate that fibers arising from the arcopallium (amygdalopiriform area, derivative of the lateral-ventral pallium, like the mammalian amygdala) project to the nucleus accumbens, along with other extended amygdalar nuclei, such as the bed nucleus of stria terminalis, lateral part (BSTL). Convergent amygdalar input to accumbens and EA likely transmits fear/anxiety signals to both viscerolimbic centers (EA) and to emotion- and goal-oriented movement centers (Ac). They also confirmed the excitatory nature of the arcopallial-accumbens pathway, based upon coexistence of L-glutamate and L-aspartate in asymmetric synaptic terminals of amygdalofugal axons in the accumbens core [42]. These data indicate that specific regions of the ventrobasal forebrain, partially coextensive with EA and the ‘subpallial organ’ [43] may be homologous to the nucleus accumbens, whereas the arcopallial-subpallial pathway in chicken corresponds to the amygdalar-striatum pathway of mammals [44], following long-conserved patterns [45] of vertebral evolution.

5. The large brain of cetaceans may be a evolutive response to rapid cooling of oceanic temperatures

In addition to the understanding of the developmental origin of the cerebral cortex, its different cell types and neural circuits, an important evolutionary trait of telencephalic evolution is the acquisition and function of an increased brain size. New research in some of the biggest mammalian brains, namely those of cetaceans, reveals novel ideas. Due to the large relative and absolute size of the cetacean brain and their cerebral cortex, it has long been thought that these animals, especially the readily trainable bottlenose dolphin, have a level of cognitive sophistication that distinguishes them from the other mammals and places them on a cognitive par with the great apes and humans. Despite this, the evidence from both behavioral and neuroanatomical data strongly questions this often over-stated conclusion [46, 47]. It was previously proposed that the large brain of cetaceans is a response to rapid cooling of oceanic temperatures during the evolution of the modern cetacean fauna [46], and the behavioral support for apparent cognitive sophistication in the cetaceans have been critically evaluated and found wanting [47]. The neuroanatomical [46, 48] and behavioral reasons [47] most salient to rejecting the complex cognitive proposal of cetacean brain evolution were described and an alternative path to evolving a large mammalian brain was outlined. While all the details of the thermogenetic proposal are not fully fleshed out, the concept that a large brain can evolve in response to selection pressures other than a *post-hoc* interpretation of a need for greater cognitive complexity represents a novel path for understanding brain evolution across vertebrate species as it breaks the ideological shackles of large brains evolving only to enhance cognitive abilities.

6. Conclusion

In conclusion: (1) The principal cellular elements of the mammalian SVZ evolved prior to the appearance of modern day mammals and were likely present in the ancestor to both mammals and sauropsids; (2) Early during the mammalian radiation, a subset of progenitor cells experienced, for the first time in evolution, a delay in their neurogenic period, which is

proposed to drive to the expansion of supragranular-like neurons and the genesis of substantive inter-pallial communication through the corpus callosum; (3) There is a great deal of heterogeneity of excitatory and inhibitory neuron types in turtle and lizard pallia; (4) Specific regions of the ventrobasal forebrain and the arcopallial-subpallial pathway in chicken may be homologous to the nucleus accumbens and the amygdalar-striatum pathway, respectively, in mammals; and (5) The large brains of cetaceans might be a response to rapid cooling of oceanic temperatures during the evolution of the modern cetacean fauna.

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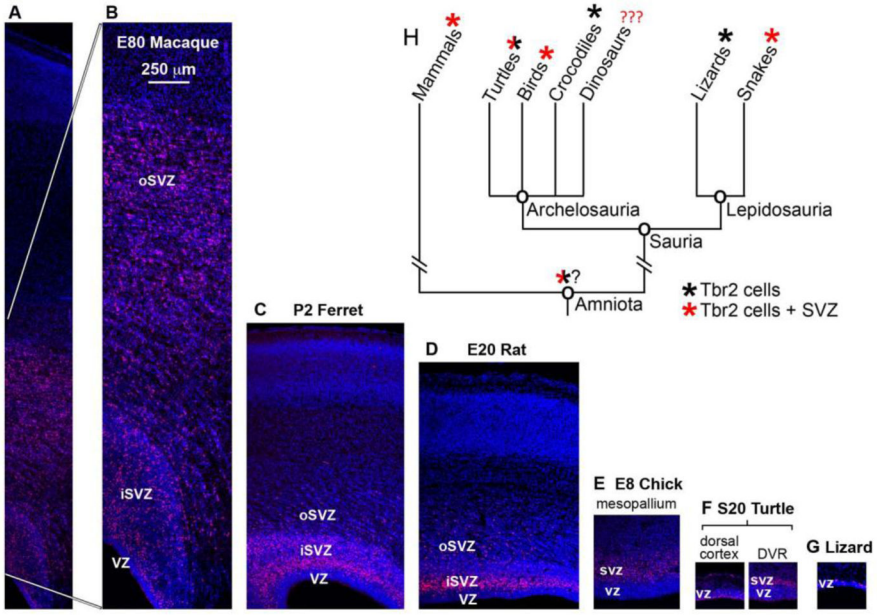

Figure 1.

The principal cellular elements of the mammalian SVZ evolved prior to the appearance of modern day mammals and were likely present in the ancestor to both mammal and sauria. (A–G) Position of Tbr2+ neural progenitor cells in the developing cortex of mammalian and non-mammalian species. Intermediate progenitor cells labeled with Tbr2 (red) are located within the cortical proliferative zones in a distinct fashion in each species. All cell nuclei are labeled with DAPI (blue). *(A)* Low power image of the primary sensory macaque cerebral cortex at gestation day 80. (B) Higher magnification image from panel (A). The outer SVZ (oSVZ) is substantially thicker, and the total number of Tbr2+ cells is greater in rhesus macaques neocortex compared to non-primate species. Images in panels *B–G* are shown at the same scale. (C) P2 developing ferret neocortex. (D) E20 developing rat neocortex. The thickness of the SVZ in the lissencephalic rat is similar to that present in the gyrencephalic ferret, but significantly thinner than in macaque. The number of Tbr2+ cells in a given section of brain tissue from the developing ferret (C) and rat (D) neocortex is similar. (E) E8 chick pallium. Tbr2+ cells are positioned within a robust SVZ. (F) In stage 20 turtle pallium Tbr2+ cells are dispersed throughout the VZ in the developing dorsal cortex. However, in the dorsal ventricular ridge (DVR) Tbr2+ cells are organized into a tight subventricular band. (G) Stage 11 lizard pallium. Tbr2+ cells in lizard are dispersed throughout the VZ. (H) Cladogram showing the relationship between mammals, turtles, birds, crocodiles, lizards, and snakes. Based on recent genetic analysis [49], turtles and birds have been placed in the recently proposed clade archelosauria, while lizards and other reptiles, such as snakes, are in the superorder lepidosauria. Mammals and birds possess an SVZ based on the distribution of Tbr2+ cells (red asterisks). Tbr2+ cells are present in both the dorsal cortex and dorsal ventricular ridge (DVR) of developing turtles but only the DVR shows evidence of an SVZ (red/black asterisk). Tbr2+ cells are also present in the developing lizard and crocodile forebrain, but there is no evidence of abventricular divisions or an anatomically defined SVZ (black asterisk). Interestingly, the developing snake cortex presented with a different

structure than that of reptiles and crocodiles, being more similar to that of mice where Tbr2+ cells were organized into a tight SVZ band that was superficial to the VZ. The presence of Tbr2+ cells and an SVZ in mammals and both reptilian clades, archelosauria and lepidosauria, supports the concept that the common ancestor for mammals and reptiles possessed Tbr2+ cells and an SVZ. Scale bar = 250 μm, and applies to B–G.

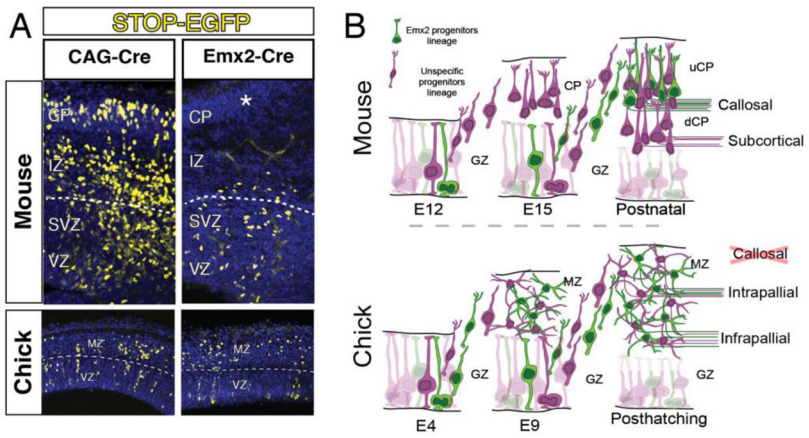

Figure 2.

Mammalian, but not avian, forebrain progenitor cells labelled through Emx2 promoter sequence show delayed neurogenesis (García-Moreno and Molnár, 2015). (A) Top row - Examples of the distribution of the cortical neurons in mouse E14, 2 days after electroporation with general and Emx2 promoter construct at E12. Bottom row - Chick cases at E6, 2 days after electroporation at E4. Left column - Labelled with EGFP (yellow), early progenitors of the forebrain generated neurons that migrated to the postmitotic areas (CP: cortical plate; MZ: mantle zone). Right column - Also labelled with EGFP, early progenitors selected by their expression of Emx2 did not generate neurons in mouse (observe the absence of yellow cells in CP) but were neurogenic in chick (green cells were present in MZ). (B) Schematic summary of the differences found in the development of the dorsal forebrain in mouse and chick. In mouse cortex there are progenitors that contribute to all layers and an early delayed progenitor subtype that mostly contributes to upper layers. In chick there is no such distinction between the differentially labelled progenitors.

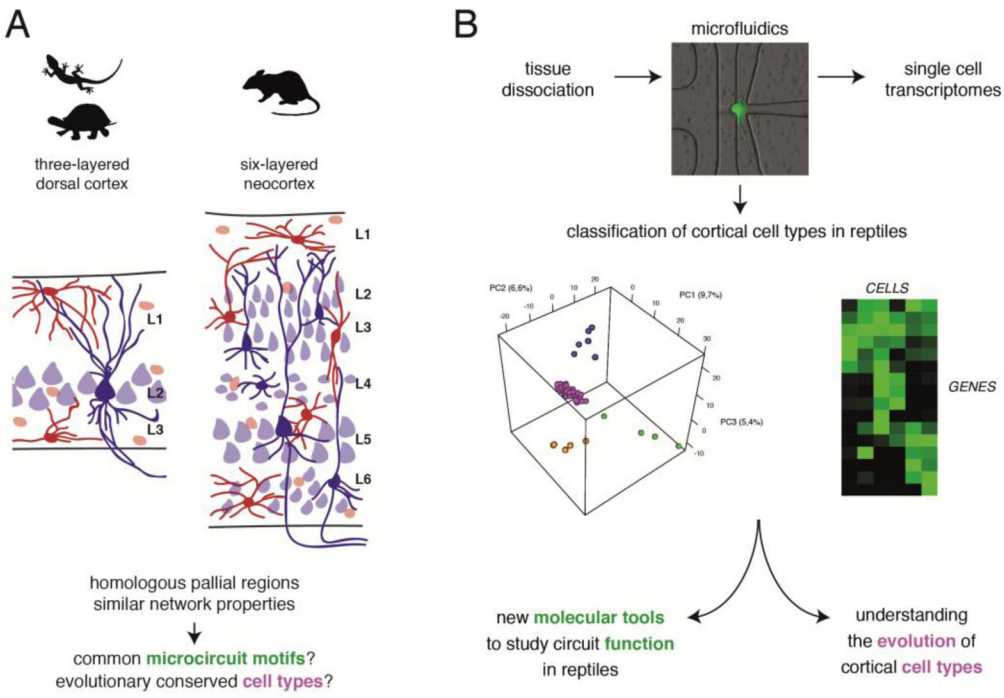

Figure 3.

Evolution of cortical circuits in amniotes. (A) The reptilian three-layered dorsal cortex and the mammalian six-layered neocortex develop from homologous pallial regions and display analogous properties at the functional level (blue and red: glutamatergic and GABAergic neurons). However, it is not yet clear how reptilian and mammalian cortices compare at the circuit level. Do they share evolutionarily conserved neuronal types? Do these cell types participate in common microcircuit motifs? (B) Tosches and colleagues are profiling reptilian cortical neurons using single cell transcriptomics. Microfluidics devices are used to capture individual cells from dissociated tissue and to obtain single-cell cDNAs for deep sequencing. Multivariate statistical approaches, like PCA and clustering, are used to identify molecularly defined neuronal types. These data contribute to our understanding of cortical evolution, and provide the foundation for building new molecular tools to study circuit function in reptiles.

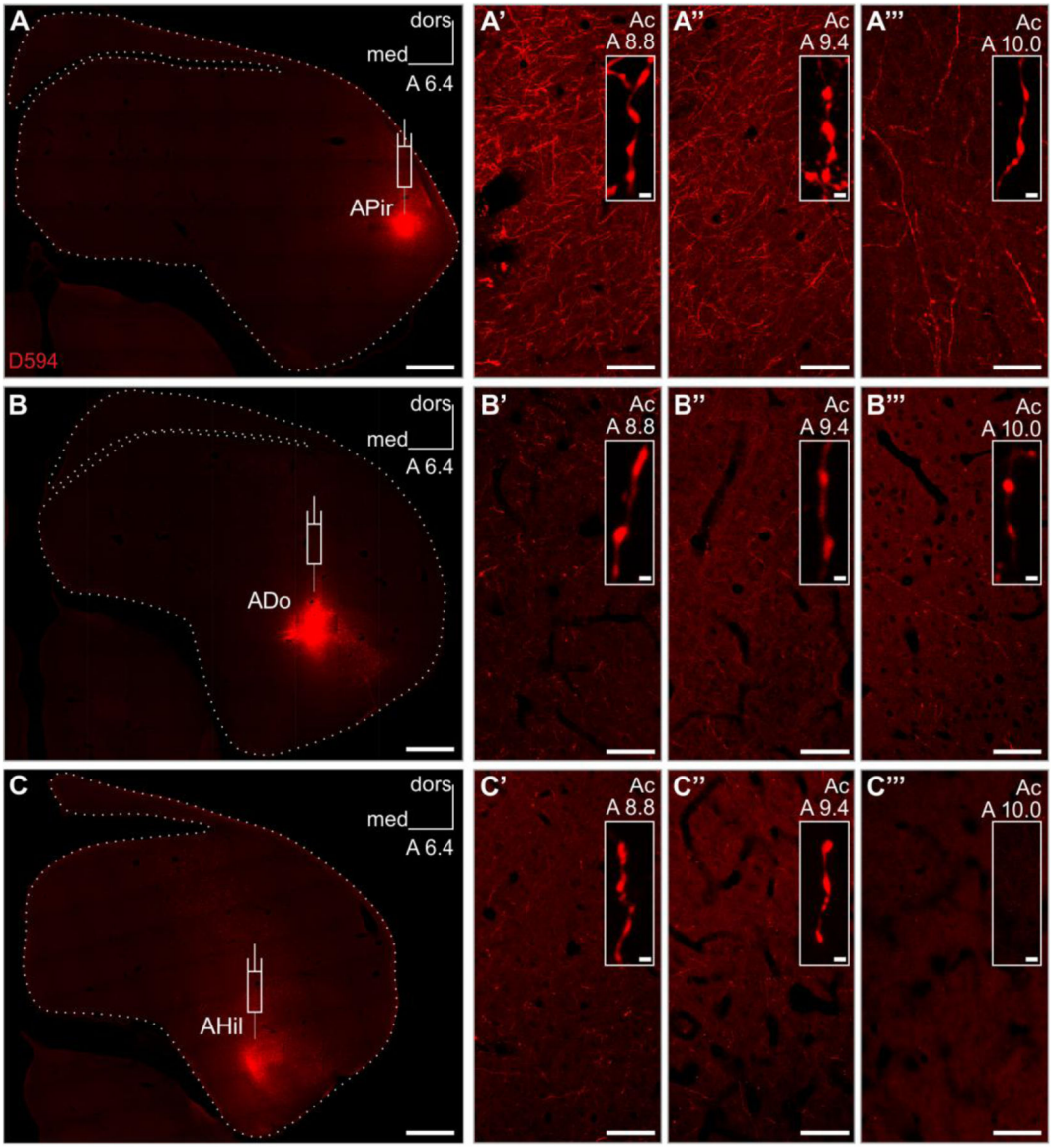

Figure 4.

The arcopallium projects domain-specifically onto the nucleus accumbens (also representative of other ventrobasal forebrain projections). (A–A‴) D594^{+} axons traced anterogradely from the amygdalopiriform area (APir) of the arcopallium, terminating in both the rostral, intermediate and caudal parts of the nucleus accumbens (Ac) in great density. Representative insets demonstrate varicose axons under high magnification. (B–B‴) Injection placed into the dorsal arcopallium (ADo) labeled fewer terminals throughout the Ac. Representative insets demonstrate varicose axons under high magnification, though such axons were rather sporadic in the rostralmost Ac. (C–C‴) The medial, hilar division of the arcopallium (AHil) gave rise to axons that terminated in the caudal and intermediate, but not the rostral, part of the Ac. Note the low density of terminal axons even in the intermediate and caudal parts of the Ac. Representative insets demonstrate varicose axons under high magnification. Here, due to an overall scarcity of varicose fibers, no such element could be

indicated in the rostralmost Ac. (A, B, C) Appropriate symbols indicate the injection sites. Due to low intensity of section images (optimized for the fluorescent signal of tracer deposit), the outlines of sections are indicated by doted lines. (A–C‴) Cranio-caudal levels of coronal sections are indicated as distance in millimeters AP according to Kuenzel and Masson (1988). Abbreviations: D594 Alexa Fluor® 594 conjugated high-molecular-weight (10kDa) dextran, dors dorsal, med medial. Scale bars: 1mm (A, B, C), 70μm (A′-A‴, B′-B‴, C′-C‴), 2μm (insets in A′-A‴, B′-B‴, C′-C‴). Reproduced with author's modification from Hanics et al, 2016 (Brain Structure and Function).

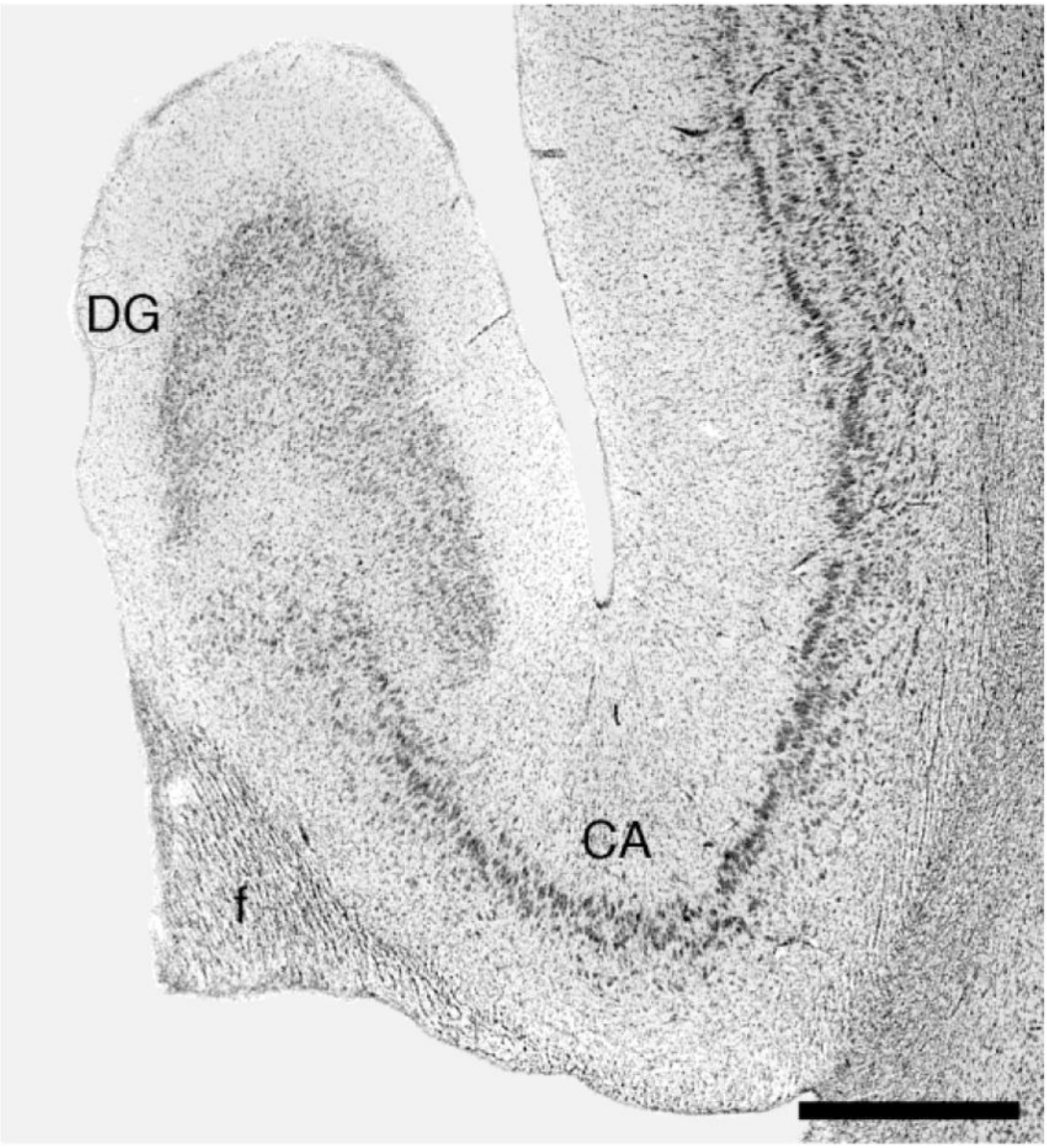

Figure 5.

Photomicrograph of the hippocampal formation in the 500 g brain of the harbour porpoise (*Phocoena phocoena*) showing the small size and loose architectural organization of this structure in the cetacean brain. The hippocampal formation of cetaceans is around 5 times smaller than one would expect for a mammal with the brain size of cetaceans (Patzke et al., 2015), and with either a very small or absent prefrontal cortex (Manger, 2006) it is unclear whether the cognitive functions associated with the hippocampus and prefrontal cortex in other mammals, such as the encoding, retrieval, long term storage and contextualization of memories, will be functionally relevant in the cetaceans. Scale bar = 1 mm. CA–cornu ammonis region; DG–dentate gyrus.