FULL PAPER *Anatomy*

Distribution of Astroglial Lineage Cells in Developing Chicken Telencephalon from Embryo to Young Chick

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ABSTRACT. The largest area of the avian telencephalon (Tc) is the subpallium [basal ganglia (BG)], and the pallium (cortex) is a narrow area located at the surface of the Tc. However, recent studies have proposed that most of the area of the avian Tc is the pallium, which corresponds to the cerebral cortex of mammals. This theory is based on neuronal elements with little regard to glial cells, which play important roles in neurogenesis. In the present study, we observed the distribution of glial cells using immunohistochemistry during maturation and discuss the division of the Tc by glial elements. In the early stage, the distribution and morphology of vimentin-positive radial glial cells were different between dorsal and ventral areas when they began to spread their processes toward the pia matter. During the development stage, vimentin-positive long processes divide the pallium and BG by the lamina pallio-subpallialis. Moreover, the pallium was divided into four regions by vimentin and glial fibrillary acidic protein-positive elements in the later stage.
KEY WORDS: avian telencephalon, basal ganglia, glial distribution, pallium, radial glia.

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Astrocytes comprise the majority of glial cells in the central nervous system and have numerous functions. Astrocyte lineage cells in vertebrates possess vimentin, an intermediate filament, at an early stage [8], and glial fibrillary acidic protein (GFAP), an intermediate filament, at the mature stage [5]. Vimentin is gradually replaced by GFAP during maturation in mammals, while in lower vertebrates, radial glia persists to express vimentin throughout their entire life cycle [10, 15].

Radial glia are transformed from neuroepithelial cells in the ventricular zone (VZ) and are recognized as neuronal stem cells [16, 21, 23], neuronal precursors [18, 24], glial cells, and glial precursors. During the stage of embryonic neurogenesis, the radial processes of the radial glial cells become scaffolds for migrating neurons [22, 31], and then radial glial cells retract their processes and differentiate into astrocytes [30] or oligodendrocytes. The projective neurons in both the pallium and subpallium migrate radially with scaffolds of radial glia, while interneurons derived from the subpallium migrate tangentially to their final position in the pallium or subpallium in mammals [1, 17, 25, 32] and birds [4].

The cerebrum in mammals is composed of the basal ganglia (BG) located in the ventral region of the lateral ventricle (LV) and the cortex wraps around the BG. The cerebral cortex, especially the neocortex, is associated with intelligence, while the BG is associated with instinct. It has been thought that the intelligence of the birds is lower than mammals and some structural differences have been reported. The LV locates on the surface of the telencephalon (Tc) and the entire Tc is composed of nucleated structures in a similar manner to mammalian BG. Therefore, the classical theory describes that the pallium in the avian Tc is confined only to the surface, and most of the avian Tc is encompassed by the BG and contain "-striatum" in their names [2, 7, 13] (Fig. 1a). However, recent studies based on neurochemistry, hodology, functionality, and neurogenesis suggest that most of the area of the avian Tc, the region thought to be the BG in the classical theory, is homologous to the mammalian cerebral cortex [12, 26, 28] (Fig. 1b). Especially, distribution of tyrosine hydroxylase (TH), which indicates dopaminergic neurons and the fibers, clearly shows the BG area of the new theory in birds [6] as well as in mammals [11, 20]. Therefore, the Avian Brain Nomenclature Forum has recommended to change the name in most areas of the Tc [9, 29], that is, most areas named with "-striatum" are renamed with "-pallium".

This new theory is based on neuronal elements. However, as mentioned above, radial glia is involved in the generation and migration of neurons in mammals. So, we hypothesize that the glial elements play a role in the avian Tc compartmentalization. In the present study, we introduce that distribution of glial cells or fibers compartmentalize avian Tc.

MATERIALS AND METHODS

Animals and tissue preparation: Fertilized chicken eggs (White Leghorn) obtained from local suppliers were incubated at 37.5°C and 60% relative humidity in a forced draft incubator. Three embryos of each of the incubation days 4 (E4), E5, E6, E8, E10, E12, E14, E16, E18, E20 and post-

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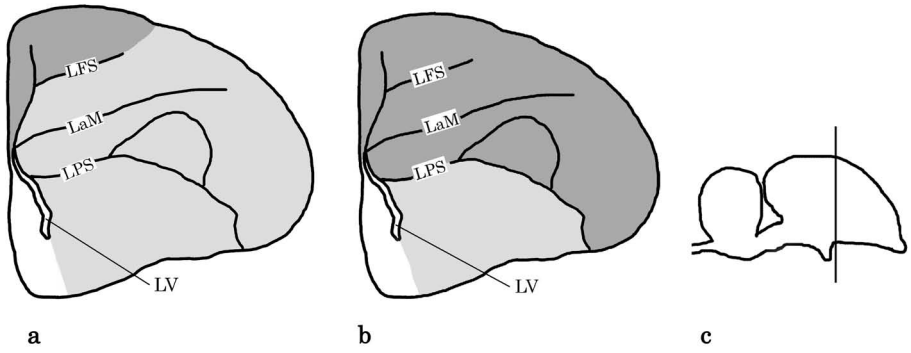

Fig. 1. Schematic drawings of adult chicken Tc. The dark grey vertical stripe region indicates the pallium and the light grey region shows the basal ganglia. a: classical theory; almost all of the Tc area corresponds to the basal ganglia. b: new theory; the majority of the region thought to be the basal ganglia corresponds to the pallium. c: The line indicates transverse cut-level in this paper. LaM: lamina mesopallialis; LFS: lamina frontalis superior; LPS: lamina pallio-subpallialis; LV, lateral ventricle.

hatched animals on days 1 (P1), P5, P10, P15 and P30 were used. All experiments were conducted in compliance with the guidelines of the Animal Care and Use Committee of Tottori University and the AVAA Guidelines on Euthanasia.

Embryos were decapitated and the post-hatch chickens were deeply anaesthetized with diethyl ether and perfused transcardially with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.6) at 4°C. After decapitation or perfusion, the Tc was removed and fixed by immersion in the same solution overnight at 4°C. Then, the tissues were dehydrated with a series of ethanol solutions and embedded in paraffin. Transverse and saggital sections (10 μm thick) were cut on a sliding microtome. The cut level of transverse section is shown in Fig. 1c. These sections were processed for immunohistochemistry against GFAP, vimentin or TH, or for thionine staining according to Nissl.

Immunohistochemistry: Sections were rinsed twice in 0.01 M phosphate buffered saline (pH 7.4) with 0.03% Triton-X (PBS-T) for 10 min, and were pretreated with 1 mM EDTA (pH 8.0) for 3 min in an autoclave to remove the effect of aldehydes on the antigen-determinant groups. This step was followed by incubation in 1% normal rabbit serum for 30 min at room temperature to avoid nonspecific background staining. These and the following steps included a rinse with PBS-T between changes of reagents. The primary antibodies were mouse monoclonal antibodies against GFAP (1:400, Sigma, St. Louis, MO, U.S.A.), vimentin (VIM3B4, 1:100, Progen, Heidelberg, Germany) or TH (1:100, Millipore, Billerica, MA, U.S.A.). Sections were incubated with the primary antibodies overnight at 4°C. Rabbit anti-mouse IgG (1:100, Jackson, West Grove, PA, U.S.A.) was used as a secondary antibody (for 60 min at room temperature), followed by mouse peroxidase-anti-peroxidase complex (1:100, Jackson) for 90 min at room temperature. The immunocomplexes were visualized using 3,3'-diaminobenzidine with 0.006% H_2O_2. Then, the sections were rinsed in distilled water, mounted, and observed under a light microscope. All incubations were performed in a moist chamber, and all antibodies were diluted in PBS-T. For identification and nomenclature of chicken Tc regions, the stereotaxic atlas of Kuenzel and Masson (1988) [14] was used.

RESULTS

At E4, the Tc consisted of a dilated ventricle (neural tube) and the VZ, which was densely packed with small proliferating cells. The vimentin-positive (Vim^+) cells, located in the ventricular surface, extended radial processes to the pial surface (Fig. 2a). At E5–E6, the dorsomedial area of the Tc (the roof plate) was caved in the ventral direction and the septal wall and LV were formed (Fig. 2b). In the ventral area, Vim^+ cells extended radial processes in the VZ and the processes became ramified leading to the development of the reticular formation out of the VZ (Fig. 2e), while in the dorsal area, Vim^+ cells extended radial processes to the pial surface (Fig. 2d). By E8, in the dorsal area, the VZ became thin, but Vim^+ cells were observed as well as at E5 (Fig. 2f). In the ventral area, the VZ remained thick (Fig. 2c), and Vim^+ long radial processes were observed out of the VZ to the pial surface (Fig. 2g).

At E10–E12, the LV became narrow and was located at a more medial site of the Tc. The cell-free lamina, named lamina pallio-subpallialis (LPS), was observed in the boundary between the dorsal and ventral areas in the early stage. Vim^+ processes were observed in the LPS (Fig. 3a). GFAP-positive processes appeared near pia surface and TH-positive nerve endings or fibers appeared in the ventral area first at E12. By E14, two other cell-free laminas, the lamina frontalis superior (LFS) and lamina mesopallialis (LaM), were distinguished in the dorsal area of LPS. Although Vim^+ reactions gradually disappeared at this time, Vim^+ processes were observed in the LPS, in the LFS, under the LFS and in the hippocampus (Hp) (Fig. 3b, 3d and 3e). By E14, TH-positive nerve endings or fibers were restricted in

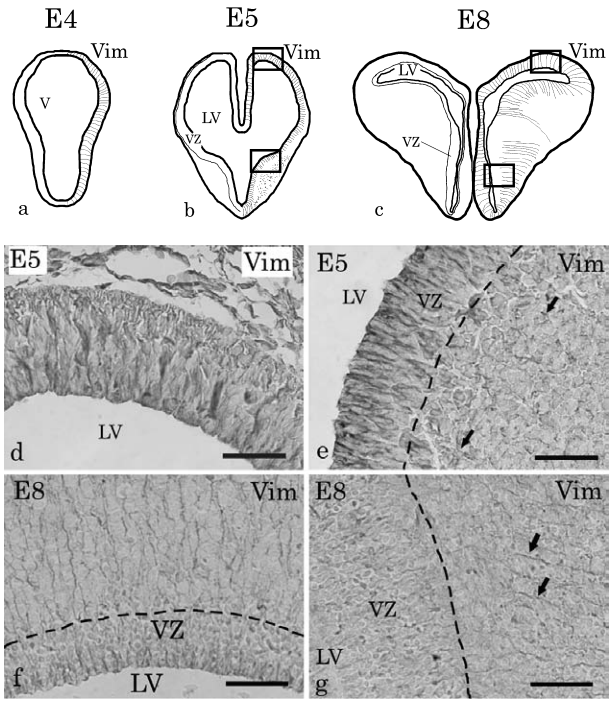

Fig. 2. Schematic drawings of Tc (a–c) and vimentin immunostaining (Vim) (d–g) at E4, 5, 8. a, b, c: Schematic drawings of chicken Tc and Vim-positive processes at E4 (a), E5 (b) and E8 (c). The left side shows the shape of the developing Tc, and the right side shows Vim-positive processes. d: The immunostaining of the same area of the upper square in (b); Vim-positive cells extend radial processes to pial surface (scale=50 μm). e: The immunostaining of the same area of the lower square in (b); Vim-positive cells stretch radial processes only in the VZ, and their processes out of the VZ are reticular (arrows). Broken line indicates the bounder between VZ and out of the VZ (scale=50 μm). f: The immunostaining of the same area of the upper square in (c). Vim-positive cells in the VZ extend radial processes to pial surface. Broken line indicates the bounder between VZ and out of the VZ (scale=50 μm). g: The immunostaining of the same area of the lower square in (c). Vim-positive radial processes are observed out of VZ (arrows). Broken line indicates the bounder between VZ and out of the VZ (scale=50 μm). LV, lateral ventricle; V, ventricle; VZ, ventricular zone.

the bottom of the ventral area (Fig. 3c), and by E16, the TH-positive nerve endings were observed throughout the entire ventral area of the LPS (Fig. 3f). By E16, GFAP-positive cells appeared in the subpallium and they increased rapidly by hatching.

Vim^+ processes were observed after hatching even at P30 in the LPS, in the LFS, under the LFS and in the Hp (Fig. 4a and 4c). After hatching, GFAP-positive cells increased more in the BG, entopallium, LPS, LFS and Hp (Fig. 4b). On saggital section, Vim^+ processes and GFAP-positive cells lined dense along the LFS and LPS (Fig. 4d).

DISCUSSION

Recent studies have shown new compartmentalization of avian Tc by the aspect of distribution of the neural elements [12, 26, 28] (Fig. 1). We guessed that glial elements were important for compartmentalization of Tc as well as neural elements, so we examined the expression of the Vim and GFAP in the chicken Tc. In neurogenesis studies, projective neurons, derived from the pallium (cortex) and subpallium (BG), migrate radially with scaffolds of radial glia, while interneurons derived from the subpallium migrate tangentially [1, 4, 17, 25, 32]. In the present study, there were differences between the dorsal Tc and the ventral Tc at the points of the radial processes of Vim^+ cells and area of the VZ in the early developing stage. In the dorsal area, Vim^+ cells always extended radial processes from the VZ to the pial surface, and this may be involved in radial migration of projective neurons. In contrast, in the ventral area, Vim^+ cells formed reticular network out of the VZ. Therefore, the Vim^+ processes in the ventral Tc in early days may be involved in tangential migration of interneurons like in mammalian subpallium.

In the developing stage, the cell-free lamina named LPS was observed by E12. This lamina consisted of Vim^+ long processes and appeared in the border between the dorsal and ventral areas of the Tc, which was observed in the early days. This border corresponded to the border of TH-positive areas in later days. TH-positive area was small at E12 and the border of the TH-positive area corresponded to the LPS by E16. TH-positive area indicates BG in the new theory [9, 29]. It is reasonable to surmise that the LPS is the border between the pallium and the BG formed by glial elements in an earlier stage than the stage of TH-positive cell maturation. Therefore, the new border between the pallium and the BG in birds may be divided by the distribution of glial elements during maturation.

In the pallium, the other cell-free laminas LFS and LaM appeared by E14. Vim^+ long processes were observed in the LFS and under the LFS even at P30. The neostriatum [new name: nidopallium (N)] and hyperstriatum ventrale [new name: mesopallium (M)] have been reported to form a single ontogenetical unit [10, 27]. However, in the present study, in the later embryonic stage and the postnatal stage, Vim^+ long processes were found in the LFS and under the LFS, although few Vim^+ long processes were found in the LaM, a border between N and M. Moreover, in the postnatal stage, there were many GFAP-positive cells along and above the Vim^+ long processes in the M and LFS, although there were few GFAP-positive cells in the N except for the entopallium, the primary visual cortex in N. Therefore, the data suggests that N and M have different distributions of glial elements. Similarly, the dorsal area of LFS, called hyperpallium (H), and ventral area of LFS (i.e., M) were observed the different distribution of glial elements as well as the difference between M and N. Moreover, there were differences between the medial and lateral upper areas of the dorsal edge of the LV. Many Vim^+ processes and GFAP-

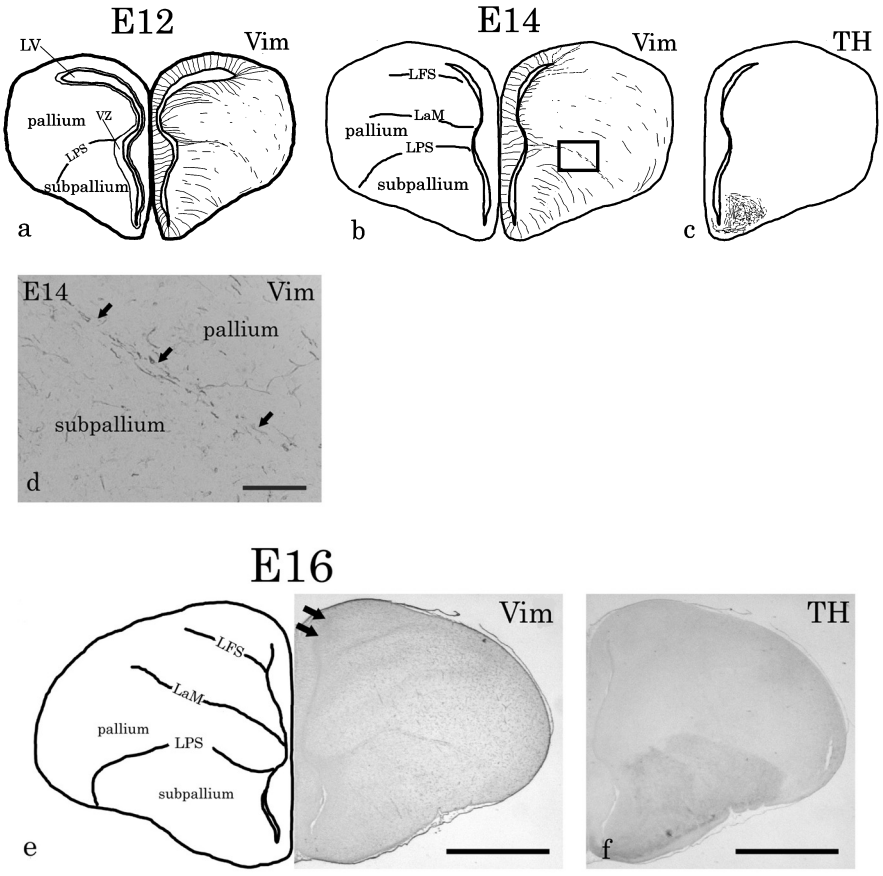

Fig. 3. Schematic drawings of Tc (a–c) and immunostaining of TH and vimentin (Vim) at E12, 14, 16. a, b: Shematic drawings of shape the Tc and the distribution of Vim-positive reaction at E12 and E14. c: Schematic drawings of TH immunostaining at E14. TH-positive elements locate in the ventral area of subpallium. At E14, the dorsal edge of TH-positive area is lower than the bounder between pallium and subpallium. d: The immunostaining of the same area of the square in (b). LPS is consisted of Vim-positive processes (arrows) (scale=100 μm). e, f: Schematic drawings of Tc and immunostaining of vimentin and TH at E16 (scale=2 mm). Vim-positive processes are observed in LPS, LFS, M, and upper area of the dorsal edge of LV (arrows). TH-positive staining restrict in subpallium. BG, basal ganglia; H, hyperpallium; LaM, lamina mesopallialis; LFS, lamina frontalis superior; LPS, lamina pallio-subpallialis; M, mesopallium; N, nidpallium; VZ, ventricular zone.

positive cells were observed in the Hp, while few Vim$^+$ processes and GFAP-positive cells were observed in the H. Mammalian pallium (developing cortex) consists of four subdivisions: dorsal pallium (DP), medial pallium (MP), lateral pallium (LP), and ventral pallium (VP). In birds, it has been reported that the DP gives rise to the H, the MP gives rise to the Hp, the LP gives rise to the M, and the VP gives rise to the N and arcopallium [3, 19]. In the present study, in the pallium, the distribution of glial elements corresponded to these four regions. This may support the fact that the avian pallium is divided in the same manner as the mammalian pallium.

Because of the remarkable differences of the neuroepithelial proliferation and the distribution of radial processes, it is reasonable to surmise that the dorsal and ventral areas of the LPS are different areas corresponding to the pallium and BG of mammals, respectively. Moreover, the pallium was divided into four regions (N, M, H, and Hp) by the distribution of glial elements in the later embryonic days or postnatal days. These results suggest that avian Tc is compartmentalized by the distribution of glial elements as well as neural elements.

REFERENCES

1. Anderson, S. A., Eisenstat, D. D., Shi, L. and Rubenstein, J. L. R. 1997. Interneuron migration from basal forbrain to neocortex: dependence on Dlx genes. *Science* **278**: 474–476.
2. Ariëns-Kappers, C. U., Huber, G. C. and Corsby, E. 1936. The Comparative Anatomy of Nervous System of Vertebrates, Including Man, Hafner, New York.

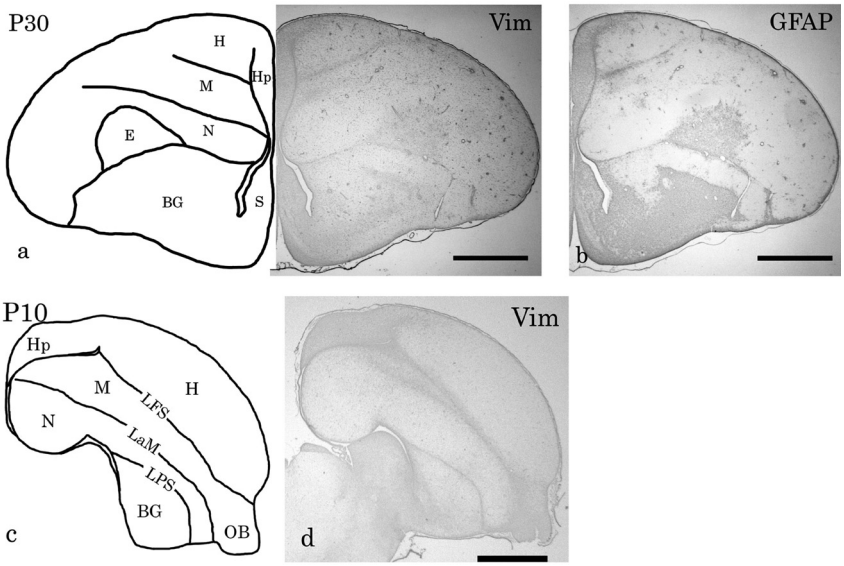

Fig. 4. Immunostaining of vimentin (Vim) and GFAP (GFAP) after hatching (Scales=2 mm). a: The left side shows compartment of Tc, and right side shows vimentin immunostaining at P30. Vim-positive processes exist in the LPS, M, LFS, HP, and upper area of the dorsal edge of LV. b: GFAP immmunostaining at P30. GFAP-positive cells exist in the BG, E, LPS, M, LFS, HP, and upper area of the dorsal edge of LV. c, d: Schematic drawing (c) and Vimentin immunostaining (d) on saggital section at P10. In LFS and LPS, Vim-positive processes are observed at any levels. BG, basal ganglia; E, entopallium; H, hyperpallium; Hp, hippocampus; LFS, lamina frontalis superior; LPS, lamina pallio-subpallialis; M, mesopallium; N, nidpallium; OB, olfactory bulbs; S, septum.

3. Butler, A. B. 1993. The evolution of dorsal pallium in the telencephalon of amniotis: cladistic analysis and new hypothesis. *Brain Res. Rev.* **19**: 66–101.
4. Cobos, I., Puelles, L. and Martinez, S. 2001. The avian telencephalic subpallium originates inhibitory neurons that invade tangentially the pallium (dorsal ventricular ridge and cortical areas). *Dev. Biol.* **239**: 30–45.
5. Dahl, D. and Bignami, A. 1985. Intermediate filament in nervous tissue. pp. 75–96. *In*: Cell and Muscle Motility, 6th ed. (Shay, J. W. ed.), Plenum Press, New York.
6. Durstewitz, D., Kröner, S. and Güntürkün, O. 1999. The dorpaminergic innervation of avian telencephalon. *Progress Neurobiol.* **59**: 161–195.
7. Edinger, L. 1908. The relations of comparative anatomy to comparative psychology. *J. Comp. Neurol. Psychol.* **18**: 437–457.
8. Elmquist, J. K., Swanson, J. J., Sakaguchi, D. S., Ross, L. R. and Jacobson, C. D. 1994. Developmental distribution of GFAP and vimentin in Brazilian opossum brain. *J. Comp. Neurol.* **344**: 283–296.
9. Jarvis, E. D., Güntürkü, O., Bruce, L. L., Csillag , A., Karten, H. J., Kuenzel, W., Medina, L., Paxinos, G., Perkel, D. J., Shimizu, T., Strieditер, G. F., Wild, J. M., Ball, G. F., Dugas-Ford, J., Durand, S., Hough, G., Husband, S., Kubikova, L., Lee, D. W., Mello, C. V., Powers, A., Siang, C., Smulders, T. V., Wada, K., White, S. A., Yamamoto, K., Yu, J., Reiner, A. and Butler, A. B. 2005. Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* **6**: 151–159.
10. Kálmán, M., Székely, A. D. and Csillag, A. 1998. Distribution of glial fibrillary acidic protein and vimentin-immunopositive elements in the developing chicken brain from hatch to adulthood. *Anat. Embryol.* **198**: 213–235.
11. Karle, E. J., Anderson, K. D., Medina, L. and Reiner, A. 1996. Light and electron microscopic immunohistochemical study of dopaminergic terminals in the striatal portion of the pigeon basal ganglia using antisera against tyrosine hydroxylase and dopamine. *J. Comp. Neurol.* **369**: 109–124.
12. Karten, H. J. 1969. The organization of the avian telencephalon and some speculations on the phylogeny of the amnito telencephalon. pp. 146–179 *In*: Comparative and Evolutionary Aspects of the Vertebrate Central Nervous System (Noback, C. and Petras, J. eds.), Ann. N.Y. Acad. Sci., New York.
13. Karten, H. J. and Hodos, W. 1967. A stereotaxic atlas of the brain of the pigeon, *Columba livia*, The Johns Hopkins University Press, Baltimore.
14. Kuenzel, W. J. and Masson, M. 1988. A stereotaxic atlas of the brain of the chick (*Gallus domesticus*), The Johns Hopkins University Press, Baltimore.
15. Lazzari, M. and Franceschini, V. 2001. Glial fibrillary acidic protein and vimentin immunoreactivity of astroglial cells in the central nervous system of adult *Podarcis sicula* (Squamata, Lacertidae). *J. Anat.* **198**: 67–75.
16. Malatesta, P., Hartfuss, E. and Götz, M. 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* **127**: 5253–5263.
17. Marin, O. and Rubenstein, J. L. 2001. A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev. Neurosci.* **2**: 780–790.
18. Martínez-Cerdeño, V., Noctor, S. C. and Kriegestein, A. R. 2006. The role of intermediate progenitor cells in the evolu-

tionary expansion of the cerebral cortex. *Cereb. Cortex* **16**: i152–161.
19. Medina, L. and Abellán, A. 2009. Development and evolution of the pallium. *Sem. Cell Dev. Biol.* **20**: 698–711.
20. Metzger, M., Jiang, S., Wang, J. and Braun, K. 1996. Organization of the dopaminergic innervation of forebrain areas relevant to learning: a combined immunohistochemical/retrograde tracing study in the domestic chicken. *J. Comp. Neurol.* **376**: 1–27.
21. Miyata, T., Kawaguchi, A., Okano, H. and Ogawa, M. 2001. Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron* **31**: 727–741.
22. Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O. L. and Pearlman, A. L. 2001. Two modes of radial migration in early development of cerebral cortex. *Nat. Neurosci.* **4**: 143–150.
23. Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. and Kriegstein, A. R. 2001. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**: 714–720.
24. Noctor, S. C., Martínez-Cerdeño, V., Ivic, L. and Kriegestein, A. R. 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* **7**: 136–144.
25. O'Rourke, N. A. 1996. Neuronal chain gangs: homotypic contacts support migration into the olfactory bulb. *Neuron* **16**: 1061–1064.
26. Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S. and Rubenstein, J. L. 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *J. Comp. Neurol.* **424**: 409–438.
27. Rehkämper, G. and Zilles, K. 1991. Parallel evolution in mammalian and avian brains: comparative cytoarchitectonic and cytochemical analysis. *Cell Tissue Res.* **263**: 3–28.
28. Reiner, A., Medina, L. and Veenman, C. L. 1998. Structural and functional evolution of the basal ganglia in vertebrates. *Brain Res. Brain Res. Rev.* **28**: 235–285.
29. Reiner, A., Perkel, D. J., Bruce, L. L., Butler, A. B., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striediter, G. F., Wild, M., Ball, G. F., Durand, S., Gütürkün, O., Lee, D. W., Mello, C. V., Powers, A., White, S. A., Hough, G., Kubikova, L., Smulders, T. V., Wada, K., Dugas-Ford, J., Husband, S., Yamamoto, K., Yu, J., Siang, C. and Jarvis, E. D. 2004. Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J. Comp. Neurol.* **473**: 377–414.
30. Schmechel, D. E. and Rakic, P. 1979. A Golgi study of radial glial cells in developing monkey telencephalon: morphogenesis and transformation into astrocytes. *Anat. Embryol. (Berl.)* **156**: 115–152.
31. Tabata, H. and Nakajima, K. 2003. Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. *J. Neurosci.* **23**: 9996–10001.
32. Tamamaki, N., Fujimori, K. E. and Takauji, R. 1997. Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *J. Neurosci.* **17:** 8313–8323.