GFAP Expression Related to Brain Development of Postnatal Mice

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Abstract

Glial fibrillary acidic protein (GFAP) is an intermediate filament (IF) class III protein which also includes vimentin and nestin. This class is important for the brain to accommodate neural activities and changes during development. The present study examined the changes of GFAP protein expression in the postnatal development of the mouse brain tissue. Mouse brains were sampled on postnatal day 3 (P3), 5 (P5) and 7 (P7). GFAP immunoreactivity was localized in the subventricular zone (SVZ) and subgranular zone (SGZ). Both in SVZ and SGZ, GFAP expression was acutely increased in the early phase of postnatal development then subsequently decreased from P3, P5, to P7. These opposing changes of GFAP were related to the increasing cortical thickness, brain and body weights. However, in SGZ of adult mice (mother mice, M), very high GFAP immunoreactivity was shown in the processes and cytoplasm of ramified shapes of the cells which revealed satellite and radial shapes. In conclusion, the increase in GFAP-positive astrocytes of both SVZ and SGZ was observed within 1 week of postnatal mice. Therefore, these findings provide valuable information on the developmental processes, and studies on GFAP regulation are useful to understand not only brain physiology but also brain developmental defect in offspring mice.

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Introduction

Intermediate filament (IF) proteins, one of the cytoskeleton, have an average diameter of 10 nm, which is between that of actin (microfilament) and microtubule. IFs are regulated developmentally and tissue-specifically. These proteins give mechanical stability to cells and tissues, and play an important role in mechanotransduction and neurogenesis.[1] Changes in IF expression occur sequentially, coincidentally with changes in cellular differentiation states. IFs are subcategorized into six classes based on similarities in amino acid sequence and protein structure.[2] IF gene expression changes occur during key steps in the differentiation of cell types in the mammalian central nervous system (CNS).[3,4] GFAP is a key IF class III protein, which also includes vimentin and nestin. GFAP is found as the cytoskeleton structure of glial cells, such as astrocytes,[5] and other cell types, such as Leydig cells in the testis and satellite cells in the liver.[6] It is functionally involved in many cellular processes and communications, and the blood-brain barrier.[7] Previous neuroanatomical studies showed that glial cells also play a key role in the development, proliferation, differentiation and morphogenesis of hippocampal neurons and have been indicated as neuron-glia interactions.[8,9]

Neurons and glia in the CNS originate from neuronal stem cells (NSCs), in a process called neuro/gliogenesis. NSCs are present not only in the developing brain but also in the mature brain. It is because in mammals, neuro/gliogenesis occurs throughout life in two germinative regions,[10] the rostral subventricular zone (SVZ) of the lateral ventricles[11] and the subgranular zone (SGZ) of the dentate gyrus (DG).[12] Neurons that arise in the SVZ travel via the rostral migratory stream to the olfactory bulb[13] and also enter association neocortex.[14] and new neurons leaving the SGZ migrate into the adjacent DG granule cell layer. After CNS injury, reactive change of glial cells, particularly astrocytes, called astrogliosis, and formation of a glial scar also result from upregulation of IFs.[15,16] Moreover, it has been reported that the most vulnerable brain region (especially multipotent progenitor cells in the DG) in hippocampus show neuro/gliogenesis and its proliferation capability for cell replacement follows cell death induced by various effectors.[17,18] Previous reports have also shown the difference of GFAP expression in various animal species, and many brain regions at various ages. In addition, because of genetically manipulated development, information on astrogliogenesis in embryonic mice would certainly serve an important purpose. Therefore, the present study aimed to examine the changes of GFAP protein

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expression in the postnatal development of the mouse brain tissue, particularly in SVZ and SGZ, because these two regions are the multipotent progenitor cells in developing and injured brains.

Materials and Methods

Experimental animals

Mother ICR mice (M), 12-week-old (National Laboratory Animal Center Mahidol University, Nakhon Pathom, Thailand) were maintained in a laboratory at the Faculty of Veterinary Science, Mahidol University. The 3-, 5-, and 7-day postnatal mice were included from two or three litters. The animals were housed in a controlled environment, 25°C, 50-60 % humidity and allowed food and tap water ad libitum. The room lights were on between 7:00 and 19:00 hours. All experiments were performed in compliance with international ethical standards and have been approved by Ethical Committee on Animal Care and Use of the Faculty of Veterinary Science, Mahidol University, FVS-ACUC, Protocol No. MUVS-2016-09-35.

Immunohistochemistry determination

The mice were anesthetized intramuscularly by the combination of ketamine (35 mg/kg) and xylazine (2.4 mg/kg) at 3, 5, and 7 days of age. Three mice at each time point were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), then followed with a normal saline flush. Their brains were removed after perfusion and immersed in the same fixative at 4°C for 1 h, then processed and embedded in paraffin. For immunoperoxidase staining, brain sections (5 µm thick) were stained using anti-glial fibrillary acidic protein antibody (1:100 GFAP, Chemicon International Inc., CA, USA) for 1 h and with EnVision Kit (K8002-40, Dako). Adjacent sections were counterstained with Harris hematoxylin for 30 s. Stained sections were examined with a light microscope at a magnification of 40x. Negative control was stained by omission of primary antibody, which showed no notable staining.

Image analysis

The GFAP immunoreactivity in SVZ and SGZ of mouse brains were compared. As shown in Figure 1, the cortical layer thickness in coronal section of SVZ was also measured and compared in relation to brain and body weights. The cortical thickness is defined as the distance from the surface of cerebral cortex to upper border of corpus callosum.

For all measurements in this study, slides were blindly analyzed with image analysis software (ImageJ 1.46r, National Institutes of Health, Bethesda, Maryland, USA). Initial settings of the software were applied to measure distance (µm), area (µm^2), integrated intensity and area fraction. Global scale of the image analysis was set to 1,560 pixels = 1 mm, in a pixel ratio of 1. A maximum of 3 images from the main representative areas was used. In the analysis of immunoreactivity, area fraction (% area) in thresholded images is the percentage of pixels or staining in the image that has been highlighted in red after using Image, Adjust then Threshold of 8-bit image in the program.[19]

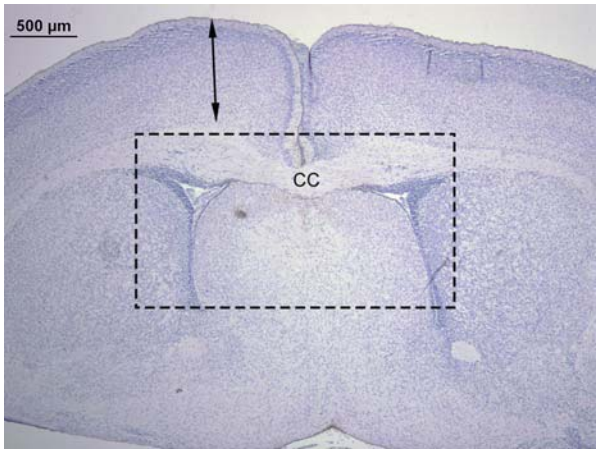

Figure 1 A coronal section at SVZ; the cortical layer thickness (arrow) was measured and compared in relation to brain and body weights. The rectangle indicates SVZ. CC, corpus callosum.

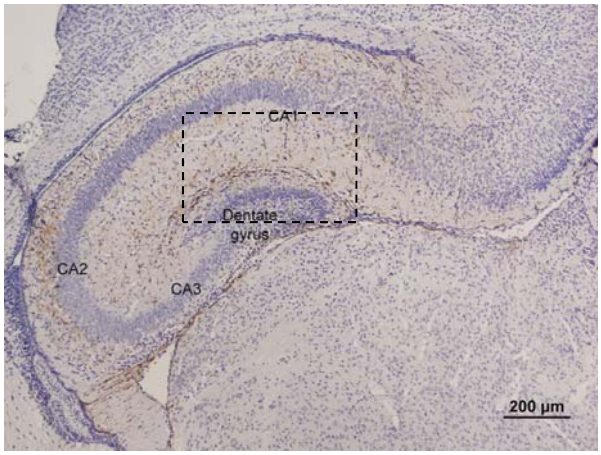

Figure 2 A coronal section of hippocampus, showing the CA1, CA2, CA3 and dentate gyrus. The rectangle indicates SGZ.

Results

The changes of GFAP protein expression have been studied in relation to the postnatal development of the mouse brain tissue. In Table 1, the cortical thickness, brain and body weights, expressed as mean ± SD, and GFAP immunoreactivity in SVZ and SGZ, expressed as integrated intensity (% area), were compared among P3, P5 and P7 (n = 3 each). There were age-related increases in cortical thickness, brain weight and body weight, but an opposite decrease in GFAP immunoreactivity in SVZ and SGZ.

A representative photograph of GFAP immuno-staining in the right side of the hippocampus, including CA1, CA2, CA3 and dentate gyrus, was shown in Figure 2; SGZ was indicated by the rectan-

gle. As compared in Figure 3, the GFAP immunoperoxidase activity of SVZ increased in the early phase of postnatal development (P3) then subsequently decreased from P3, P5 to P7.

In Figure 4, the GFAP immunoperoxidase activity of SGZ in the right side of the hippocampus was also compared, showing an increase in the early phase of postnatal development (P3), then subsequently

Table 1 Cortical thickness, brain and body weights, and GFAP immunoreactivity in SVZ and SGZ, comparing among P3, P5 and P7.

		P3	P5	P7
Cortical thickness (μm)		816.13 ± 22.49	838.70 ± 30.02	1068.25 ± 24.08$^{***,\#\#\#}$
GFAP expression (%area)	- SVZ	5.29 ± 0.61	1.53 ± 0.27***	0.14 ± 0.05$^{***,\#\#}$
	- SGZ	3.73 ± 0.70	2.07 ± 0.37*	1.10 ± 0.62**
Brain weight (g)		0.09 ± 0.01	0.14 ± 0.02*	0.19 ± 0.03**
Body weight (g)		1.27 ± 0.15	2.33 ± 0.06***	3.73 ± 0.23$^{***,\#\#\#}$

Data are mean ± SD; n =3 each. SGZ, subgranular zone; SVZ, subventricular zone; $^{*,**,***}P$ < 0.05, 0.01, 0.001, compared to P3; $^{\#,\#\#,\#\#\#}P$ < 0.05, 0.01, 0.001, compared to P5; one-way ANOVA with *post hoc* Tukey's multiple comparison test.

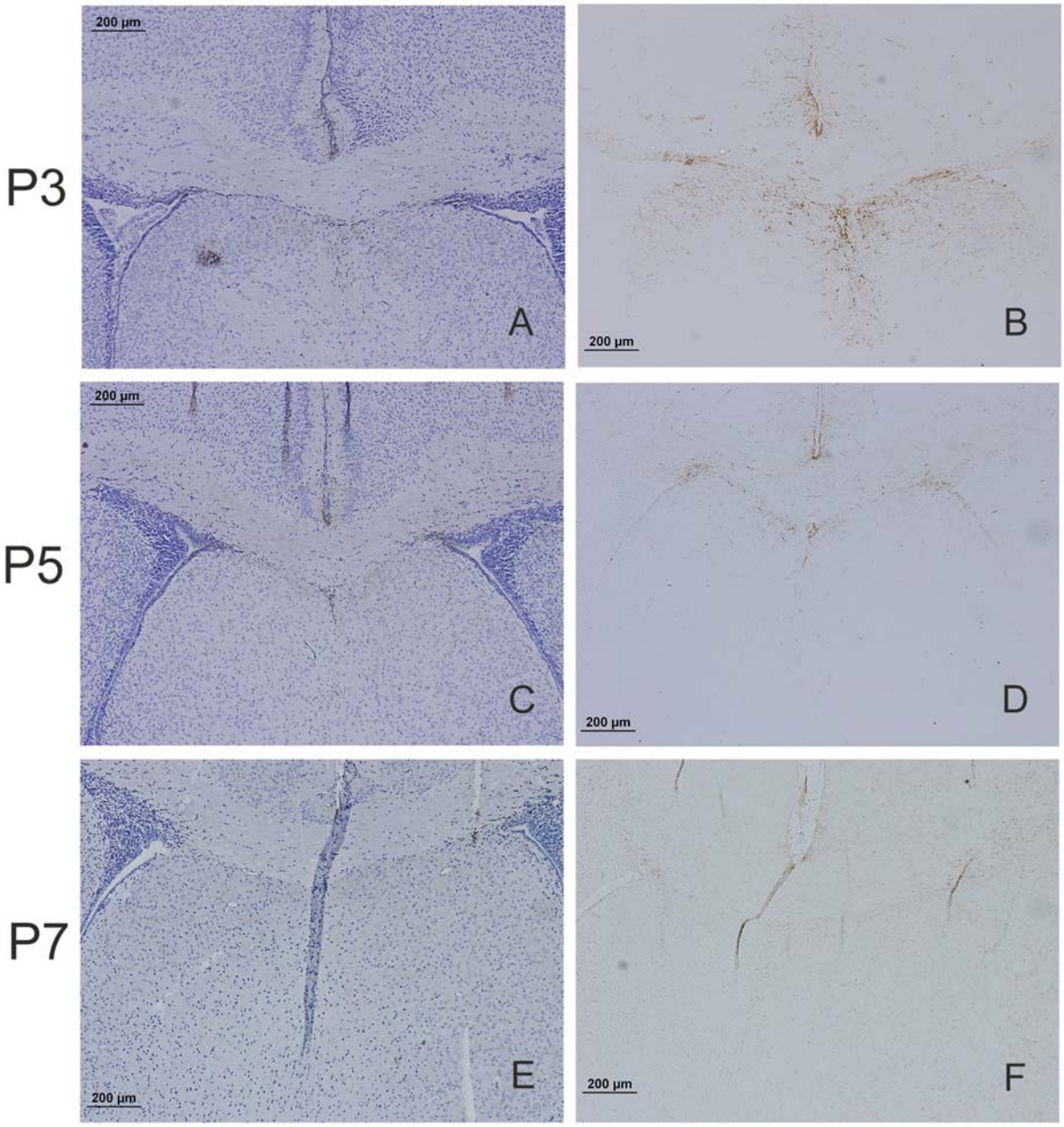

Figure 3 The GFAP immunoperoxidase activity of SVZ (as indicated by the rectangle in Figure 1) decreased gradually from P3, P5 to P7. The coronal brain sections were shown as counterstained (A, C, E) and non-counterstained sections (B, D, F).

decreasing from P3, P5 to P7. Few GFAP-positive elements were detected at P7 in the hippocampus, although GFAP immunoreactivity was found in the margin cells of the dentate gyrus.

In Figure 5, the GFAP immunoperoxidase activity of SGZ was compared between the P3 and mother mice (M). The positive GFAP staining of P3 was less than M and no staining in the hilus of dentate gyrus in P3. There were differences in the number and shape of the GFAP-positive cells, which were more satellite-shaped in M. The higher magnification of dentate gyrus in mother mice in Figure 5E and 5F showed many GFAP-positive cells with the appearance of typical mature astrocytes and were satellite, radial, or ramified in shape. There were intense staining in the processes and cytoplasm of ramified-shape astrocytes. In Figure 5E, the astrocytic foot processes were shown to extend to a capillary and adjacent neurons.

Discussion

As described previously, there is a great diversity of astrocytes revealed by more detailed morphological and biochemical analyses. The old classification of two major subpopulations of astrocytes, i.e., fibrous astrocytes in the white matter and protoplasmic astrocytes in the gray matter,[20] is still unclear. Fibrous astrocytes have long, thin processes, yielding a star-like appearance. Protoplasmic astrocytes have many

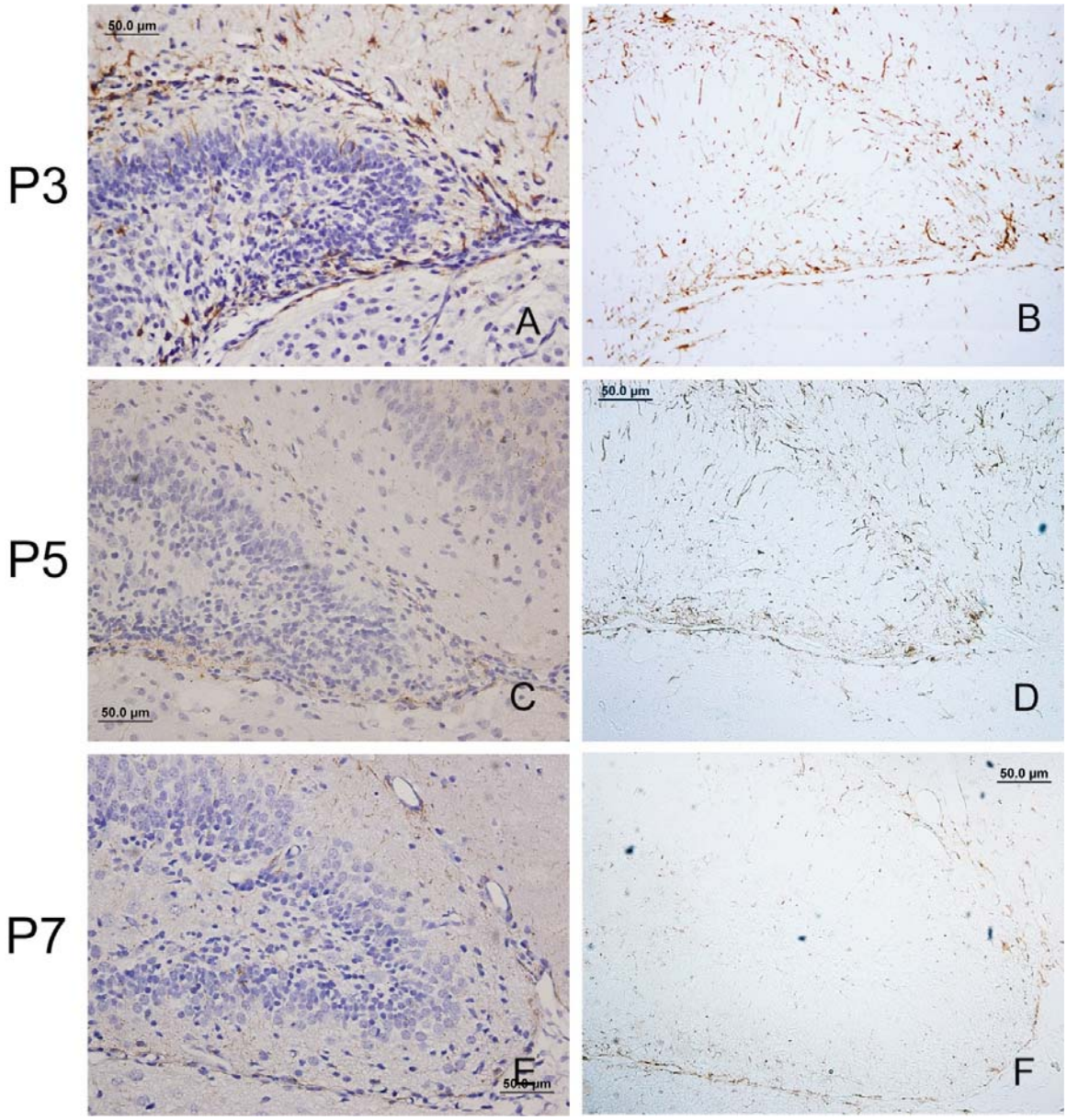

Figure 4 The GFAP immunoperoxidase activity of SGZ (as indicated by the rectangle in Figure 2) decreased gradually from P3, P5 to P7. The coronal brain sections were shown as counterstained (A, C, E) and non-counterstained sections (B, D, F).

branching processes, which contact and ensheath synapses, and usually have one or two processes in contact with blood vessels. Recently Yuasa, 2001,[21] mentioned that the distribution and morphology of astrocytes vary according to each cortical structure; these heterogeneous processes depend on the site of cell origin and the stratification of cortical structures. Most astrocytes exhibit a stellate morphology, and some astrocytes undergo specialized morphological differentiation, such as the astrocytes with unipolar radial processes that are seen in the cerebellar cortex and hippocampus.[22] For CNS injury, reactive morphological changes in astrocytes and formation of a glial scar result from the upregulation of Ifs, also indicated as the astrogliosis.[15,16] Previous reports have also shown the difference of GFAP expression between animal species. Sievers et al.[9] showed that in neuro/gliogenesis a strong GFAP immunoreactivity indicated the astrogliogenesis appears in the early developmental stage of the hamster dentate gyrus. Yuasa[21] also mentioned that very little is known about the early stage of astroglial precursors in the developing hippocampus of the mouse because the expression of GFAP occurs at a much later stage as compared with hamster. Rakic et al.[23] reported that the GFAP immunoreactivity in radial glia in rodents

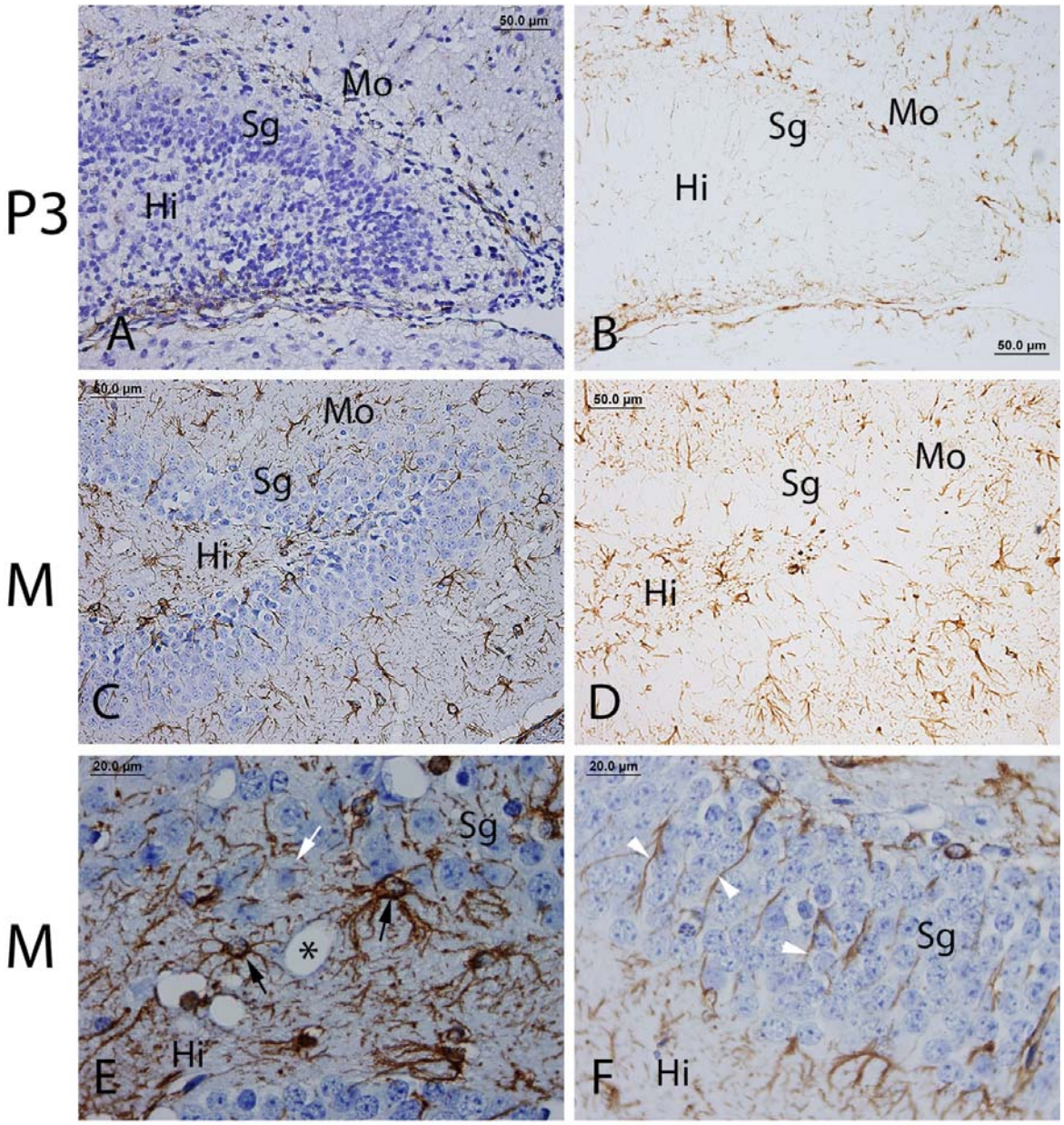

Figure 5 GFAP immunoperoxidase activity of SGZ of postnatal P3 (A & B) was less than mother mice (M) in C and D. The coronal brain sections were shown as counterstained sections (A, C, E and F) and non-counterstained (B and D). E and F were the higher magnification of dentate gyrus in mother mice showing GFAP positive staining in the processes and cytoplasm of ramified-shape astrocytes which revealed satellite (black arrows) and radial (arrow head) shape cells. In E, the astrocytic foot processes extended to capillary (asterisk) and adjacent neurons (white arrow). Hi, hilus; Mo, molecular layer; Sg, dentate granule cell layer.

can be observed only after birth. In addition, the information on astrogliogenesis in embryonic mice would certainly serve an important purpose in genetically manipulated development, therefore, the present study examined the GFAP expression to indicate the development of astrocytes in the mouse postnatal brain tissue. Special attention was paid to the differences in astroglial development in rostal SVZ and SGZ in the dentate gyrus. Previously, Kimoto et al.[24] described that GFAP expression is also specifically found in radial glia. Radial glia are a type of precursor cell located in the SVZ and the hippocampus. They are a heterogeneous population of cells that are able to self-renew and produce neurons as well as glia. Neuron-glia interactions play a key role in the development and functional activity of brain including differentiation of neurons.[24,25] The patterns of GFAP expression in mouse hippocampi progressively changes during postnatal development. Kim et al.[26] reported that GFAP immunoreactivity sharply increased in the early phase of postnatal development. Kimoto et al.[24] also reported that increase in the number of GFAP-positive astrocytes was observed in the hippocampus of 1-week-old mice as compared with 8-week-old animals. In this study, both in SVZ and SGZ, GFAP expression was acutely increased in the early phase of postnatal development then subsequently decreased from P3, P5 to P7. Interestingly, previous reports have also suggested that all pyramidal cells of the hippocampus are formed before birth, whereas 80–90% of the dentate granule cells are formed postnatally.[27,28] Therefore, the increase in GFAP-positive astrocytes of both SVZ and SGZ was observed within 1 week of postnatal mice. These results show that glial cells may play some roles in the maintenance and neuronal functions of granule cells of dentate gyrus during postnatal development and this may be related to the growth of the dentate granule cells or may play an important role in maturation. An increased body of evidence suggested that astrocytes perform a wide range of adaptive functions in the mammalian nervous system, including neurotransmitter uptake,[29] synthesis and secretion of trophic factors,[30] aiding in wound repair and regeneration,[31] and the regulation of synaptic density.[32] However, Kimoto et al.[24] reported that in the dentate gyrus, 1-week-old ICR mice showed no significant changes in the number of GFAP-positive astrocytes. It was similar to the decrease in GFAP immunoreactivity of P7 in the present study. Furthermore, they also reported that the total number of GFAP-positive astrocytes was unchanged in dentate gyrus from 2 to 8 weeks of birth. On the other hand, in 10-week-old mother mice of this study, very high GFAP immunoreactivity of SGZ was observed in the processes and cytoplasm of ramified-shape cells which revealed satellite and radial shape. These opposing changes of GFAP immunoactivity in postnatal development was related to the increased cortical thickness, brain and body weights.

Conclusion

The present study shows a progressive decrease of GFAP expression in the SVZ and SGZ of mice during postnatal development, suggesting that the GFAP expression during the early stage of postnatal development is important for neuro/gliogenesis or neural development in SVZ and dentate gyrus of SGZ. However, many questions concerning the functional role and transcriptional regulation of GFAP expression in various cells and tissues remain unanswered. Therefore, further studies should be performed to investigate the precise mechanisms responsible for our findings.

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Conflict of Interest

None to declare.

References

1. Bennett GS. Changes in intermediate filament composition during neurogenesis. Curr Top Dev Biol. 1987; 21: 151-83.
2. Lendahl U, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. Cell. 1990; 60(4): 585-95.
3. Menet V, Gimenez YRM, Sandillon F, Privat A. GFAP null astrocytes are a favorable substrate for neuronal survival and neurite growth. Glia. 2000; 31(3): 267-72.
4. Zimmerman L, Parr B, Lendahl U, Cunningham M, McKay R, Gavin B, et al. Independent regulatory elements in the nestin gene direct transgene expression to neural stem cells or muscle precursors. Neuron. 1994; 12(1): 11-24.
5. Tardy M, Fages C, Le Prince G, Rolland B, Nunez J. Regulation of the glial fibrillary acidic protein (GFAP) and of its encoding mRNA in the developing brain and in cultured astrocytes. Adv Exp Med Biol. 1990; 265: 41-52.
6. Davidoff MS, Middendorff R, Kofuncu E, Muller D, Jezek D, Holstein AF. Leydig cells of the human testis possess astrocyte and oligodendrocyte marker molecules. Acta Histochem. 2002; 104(1): 39-49.
7. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). Neurochem Res. 2000; 25(9-10): 1439-51.
8. Rickmann M, Amaral DG, Cowan WM. Organization of radial glial cells during the development of the rat dentate gyrus. J Comp Neurol. 1987;264(4):449-79.

9. Sievers J, Hartmann D, Pehlemann FW, Berry M. Development of astroglial cells in the proliferative matrices, the granule cell layer, and the hippocampal fissure of the hamster dentate gyrus. J Comp Neurol. 1992; 320(1): 1-32.
10. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell. 2008; 132(4): 645-60.
11. Luskin MB, Parnavelas JG, Barfield JA. Neurons, astrocytes, and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: an ultrastructural analysis of clonally related cells. J Neurosci. 1993; 13(4): 1730-50.
12. Kaplan MS, Hinds JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science. 1977; 197(4308):1092-4.
13. Lois C, Garcia-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. Science. 1996; 271(5251): 978-81.
14. Gould E, Reeves AJ, Graziano MS, Gross CG. Neurogenesis in the neocortex of adult primates. Science. 1999; 286(5439): 548-52.
15. Jin JK, Jeong BH, Na YJ, Kim YS, Carp RI, Wie MB, et al. Increased expression of the embryonic intermediate filament, nestin, in the brains of scrapie-infected mice. Neurosci Lett. 2004; 367(2): 254-8.
16. Moon C, Ahn M, Kim S, Jin JK, Sim KB, Kim HM, et al. Temporal patterns of the embryonic intermediate filaments nestin and vimentin expression in the cerebral cortex of adult rats after cryoinjury. Brain Res. 2004; 1028(2): 238-42.
17. Seo HS, Yang M, Song MS, Kim JS, Kim SH, Kim JC, et al. Toluene inhibits hippocampal neurogenesis in adult mice. Pharmacol Biochem Behav. 2010; 94(4): 588-94.
18. Yang M, Kim JS, Song MS, Kim SH, Kang SS, Bae CS, et al. Cyclophosphamide impairs hippocampus-dependent learning and memory in adult mice: Possible involvement of hippocampal neurogenesis in chemotherapy-induced memory deficits. Neurobiol Learn Mem. 2010; 93(4): 487-94.
19. Fuhrich DG, Lessey BA, Savaris RF. Comparison of HSCORE assessment of endometrial beta3 integrin subunit expression with digital HSCORE using computerized image analysis (ImageJ). Anal Quant Cytopathol Histpathol. 2013; 35(4): 210-6.
20. Miller RH, Raff MC. Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. J Neurosci. 1984; 4(2): 585-92.
21. Yuasa S. Development of astrocytes in the mouse embryonic cerebrum tracked by tenascin-C gene expression. Arch Histol Cytol. 2001; 64(1):119-26.
22. Kosaka T, Hama K. Three-dimensional structure of astrocytes in the rat dentate gyrus. J Comp Neurol. 1986; 249(2): 242-60.
23. Rakic P. Developmental and evolutionary adaptations of cortical radial glia. Cereb Cortex. 2003; 13(6): 541-9.
24. Kimoto H, Eto R, Abe M, Kato H, Araki T. Alterations of glial cells in the mouse hippocampus during postnatal development. Cell Mol Neurobiol. 2009; 29(8): 1181-9.
25. Mamber C, Kamphuis W, Haring NL, Peprah N, Middeldorp J, Hol EM. GFAPdelta expression in glia of the developmental and adolescent mouse brain. PLoS One. 2012; 7(12): e52659.
26. Kim JS, Kim J, Kim Y, Yang M, Jang H, Kang S, et al. Differential patterns of nestin and glial fibrillary acidic protein expression in mouse hippocampus during postnatal development. J Vet Sci. 2011; 12(1): 1-6.
27. Dalmau I, Finsen B, Zimmer J, Gonzalez B, Castellano B. Development of microglia in the postnatal rat hippocampus. Hippocampus. 1998; 8(5): 458-74.
28. Schlessinger AR, Cowan WM, Gottlieb DI. An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. J Comp Neurol. 1975; 159(2): 149-75.
29. Diamond JS, Jahr CE. Transporters buffer synaptically released glutamate on a submillisecond time scale. J Neurosci. 1997; 17(12): 4672-87.
30. Liesi P, Silver J. Is astrocyte laminin involved in axon guidance in the mammalian CNS? Dev Biol. 1988; 130(2): 774-85.
31. Rudge P. Clinical neurology. A review. J R Coll Physicians Lond. 1989; 23(2): 83-91.
32. Meshul CK, Seil FJ, Herndon RM. Astrocytes play a role in regulation of synaptic density. Brain Res. 1987; 402(1): 139-45.