

# Age-dependent alteration in the expression of oligodendrocyte-specific protein in the gerbil hippocampus

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**Abstract.** Oligodendrocytes are myelin-forming cells in the central nervous system. Research into the effects of aging on oligodendrocyte protein expression remains limited. The present study aimed to determine the alterations in oligodendrocyte-specific protein (OSP) expression in the gerbil hippocampus at 1, 2, 3, 4, 6 and 24 months of age with western blot and immunohistochemistry analyses. OSP expression levels in the hippocampus were highest at 6 months of age. OSP immunoreactivity was identified in numerous cell bodies at 1 month, although the number of OSP immunoreactive cells was different according to hippocampal subregion. The number of OSP immunoreactive cells significantly decreased at 2 months and, thereafter, numbers decreased gradually. The

detection of OSP immunoreactive fibers was negligible in all layers in the hippocampal subregions until 4 months. OSP immunoreactive fibers were abundant at 6 and 24 months, although the fiber distribution patterns in the CA1-3 areas and dentate gyrus were different. The results demonstrated that OSP expression in the gerbil hippocampus was age-dependent. The detection of OSP immunoreactive cell bodies and fibers was significantly different according to the layers of hippocampal subregions, indicating that myelination may be continuously altered in the hippocampus during normal aging.

## Introduction

Aging is accompanied by cognitive decline that is associated with morphological and functional alterations in hippocampal neurons, which have an important role in learning and memory (1-3). Glial cells additionally influence the function of hippocampal neurons during aging; decreased long-term potentiation (LTP) is linked to age-associated microglial activation in perforant path-granule cells in the hippocampus (4). Exogenous astrocyte-derived glial cell derived neurotrophic factor and D-serine in the hippocampal CA1 area are able to reverse age-induced cognitive deficits by increasing neurotransmitter synthesis, and enhancing N-methyl-D-aspartic acid receptor-dependent LTP, respectively (5,6). Furthermore, epigenetic memory stored in the chromatin of mature oligodendrocytes is reduced in the corpus callosum, due to alterations in gene expression (7).

Oligodendrocytes form myelin, which is involved in the fast saltatory conduction of nerve impulses in the central nervous system (CNS) (8); 20-30% of the proteins of which myelin is

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composed are specific to myelin and oligodendrocytes (9). Myelin basic protein and proteolipid protein account for ~80% of the total myelin protein, and oligodendrocyte-specific protein (OSP) is the 3rd most abundant protein, accounting for ~7% of the total myelin protein (8,10). OSP is primarily expressed in oligodendrocytes, and it exhibits channel functions and oligodendrocyte growth regulation in the CNS (10).

Age-associated alterations in oligodendrocyte protein expression have been studied in various brain regions, including the cortex and corpus callosum of monkeys (11,12), the corpus callosum of rats (13) and the hippocampus of mice (14,15). Additionally, increased numbers of newly-generated oligodendrocytes in the hippocampus (14) and spinal cord (16) during the aging process have been reported. However, to date, limited studies have reported the distribution of OSP in the hippocampus at various ages. Therefore, the objective of the present study was to determine the age-dependent alterations in OSP expression, an oligodendrocyte marker, in the gerbil hippocampus as a good model of aging (17,18).

## Materials and methods

**Experimental animals.** Male gerbils (*Meriones unguiculatus*; n=84) were supplied by the Experimental Animal Center, Kangwon National University (Chuncheon, South Korea) and used at post-natal month (PM) 1 (young), PM 2, PM 3, PM 4, PM 6 (adult) and PM 24 (aged). The gerbils were housed at 23±3°C and 55±5% relative humidity in a 12-h light/dark cycle and were allowed free access to food and water. Gerbils (n=14 in each group) were handled following the National Institutes of Health (Bethesda, MD, USA) Guide for the Care and Use of Laboratory Animals. The experimental protocol of the present study was approved (approval no. KW-160802-2) by the Institutional Animal Care and Use Committee of Kangwon National University (Chuncheon, South Korea).

**Western blot analysis.** Animals (n=7 in each group) were used to examine alterations in OSP expression levels. Western blotting was performed according to a previously published method (19). Briefly, following sacrifice of the animals, the hippocampus was removed and the hippocampal tissues were homogenized and centrifuged, and the supernatants were subjected to western blot analysis. The membranes were incubated with Rabbit anti-OSP (cat. no. ab53041; 1:1,000; Abcam, Cambridge, MA, USA) and mouse anti-β actin (cat. no. A5441; 1:5,000; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) overnight at 4°C. Following washing 3 times with PBST (each for 10 min; Sigma-Aldrich; Merck KGaA), the membrane was incubated with peroxidase-conjugated donkey anti-rabbit IgG or goat anti-mouse IgG (cat. no. sc-2305 or cat. no. sc-2031; 1:1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) for 1 h at room temperature, and an ECL kit (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The results of the western blot analysis were scanned, and densitometric analysis was applied for quantification of the bands as relative optical density (ROD) using ImageJ software (version 1.46; National Institutes of Health). The ROD ratio was calibrated as the percentage expression compared with PM 1 gerbils, which was designated as 100% following normalization to each β-actin band.

**Immunohistochemistry.** To examine the age-dependent alterations in OSP immunoreactivity in the hippocampus during normal aging, immunohistochemical staining and subsequent quantitative analysis was performed, according to a previously published protocol (20). Gerbils (n=7 in each group) were intraperitoneally anesthetized with pentobarbital sodium (40 mg/kg) and transcardially perfused with 4% paraformaldehyde. Brain tissues were cut into 25-μm thick sections at -20°C. Rabbit anti-OSP (1:500; Abcam) was used as the primary antibody and incubated overnight at 4°C. Following washing three times for with PBS (each for 10 min; Sigma-Aldrich), the brain tissues were incubated with biotinylated goat anti-rabbit (cat. no. BA-1000; 1:200; Vector Laboratories Inc., Burlingame, CA, USA) for 2 h at room temperature and streptavidin peroxidase complex (cat. no. SA-5004; 1:200; Vector Laboratories Inc.) for 45 min at room temperature. A negative control test was performed using pre-immune serum instead of the primary antibody to establish specificity of the immunostaining. The negative control resulted in no immunoreactivity in the stained sections.

To quantitatively analyze OSP immunoreactivity, digital images of hippocampal sections were captured from six sections per gerbil using a AxioM1 light microscope at 20x magnification (Zeiss AG, Oberkochen, Germany) equipped with a digital camera (Axiocam; Zeiss AG). OSP immunoreactive neurons were counted in a 400x400-μm square area at the center of the CA1 area, CA2/3 areas and the dentate gyrus using Optimas image analysis software (version 6.5; CyberMetrics Corporation, Phoenix, AZ, USA). Cell numbers were determined by calculating the mean total cell number obtained from the 7 sections per gerbil.

**Statistical analysis.** The experiments were repeats three times. Data are expressed as the mean ± standard error of the mean. Statistical analysis was performed using one-way analysis of variance with a post hoc Tukey's test for multiple comparisons by GraphPad Instat version 3.05 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

## Results

**OSP protein levels.** Significant alterations in OSP protein expression were detected between groups via western blot analysis [F(5,36)=23.493; P<0.0001]. OSP protein levels in the gerbil hippocampus were significantly altered during normal aging. The lowest OSP expression was detected in the PM 3 group and the highest in the PM 6 group (Fig. 1). OSP levels in the PM 24 group were significantly decreased compared with the PM 6 group, although they were higher compared with those detected in the PM 1 group (Fig. 1).

**OSP immunoreactivity.** Patterns of OSP immunoreactivity were different according to gerbil age and hippocampal subregions during normal aging (Figs. 2-4). OSP immunoreactivity was detected in cells in the PM 1-4 groups and in fibers in the PM 6 and PM 24 groups.

**CA1 area.** Significant alterations in OSP-positive cell number were detected [F(5,36)=176.98; P<0.0001]. In the PM 1 group,

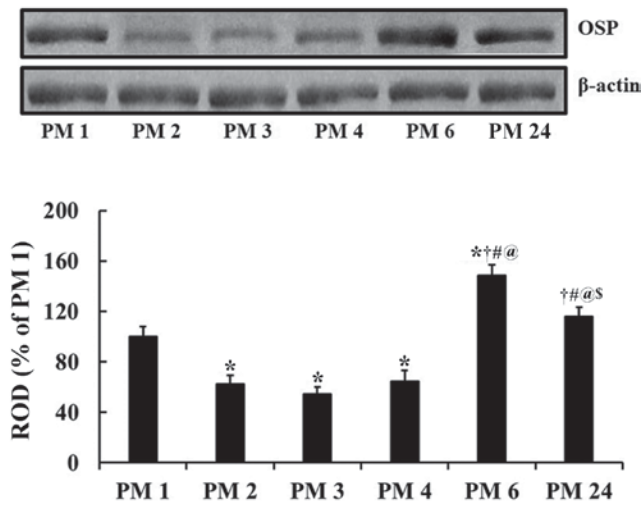


Figure 1. Western blot analysis of OSP expression in the gerbil hippocampus at various ages. ROD is presented as a percentage of the PM 1 immunoblot band. n=7 per group. \*P<0.05 vs. PM 1, †P<0.05 vs. PM 2, #P<0.05 vs. PM 3, @P<0.05 vs. PM 4, S P<0.05 vs. PM 6. Bars indicate the mean  $\pm$  standard error of the mean. OSP, oligodendrocyte-specific protein; ROD, relative optical density; PM, post-natal month.

several OSP immunoreactive cell bodies were observed in the stratum oriens and radiatum. Negligible numbers of OSP immunoreactive fibers were detected in the CA1 area (Fig. 2A). In the PM 2 group, a significant decrease in the number of OSP immunoreactive cell bodies was observed in the CA1 area (Fig. 2G), and OSP immunoreactive fibers were uncommon (Fig. 2B). However, pyramidal cells of the stratum pyramidale, or pyramidal neurons, had weak OSP immunoreactivity (Fig. 2B). In the PM 3 and PM 4 groups, OSP immunoreactive cell body numbers (Fig. 2G), OSP immunoreactive fiber distribution and OSP immunoreactivity in pyramidal neurons were similar to those in the PM2 group (Fig. 2C, D). In the PM 6 group, OSP immunoreactive cell bodies had shrunk in size and significantly reduced in numbers compared with the PM 4 group (Fig. 2G); however, OSP immunoreactive fibers were increased in the stratum oriens of the CA1 area (Fig. 2E). In the PM 24 group, OSP immunoreactive cell body numbers were not significantly different compared with the PM 6 group (Fig. 2G), and OSP immunoreactive fibers were increased in the stratum oriens and pyramidale of the CA1 area (Fig. 2F). Additionally, OSP immunoreactivity in pyramidal neuron was negligible (Fig. 2F).

**CA2/3 region.** Significant alterations in the number of OSP-positive cells were detected [F(5,36)=542.50; P<0.0001]. In the PM 1 group, numerous OSP immunoreactive cell bodies were found in all layers, although OSP immunoreactive fibers were rarely detected (Fig. 3A). In the PM 2 group, the number of OSP immunoreactive cell bodies significantly decreased (Fig. 3G) compared to that in the PM1 group and few OSP immunoreactive fibers were observed (Fig. 3B). However, pyramidal neurons of the stratum pyramidale exhibited OSP immunoreactivity (Fig. 3B). In the PM 3 and 4 groups, numbers of OSP immunoreactive cell bodies had decreased compared with the PM 2 group (Fig. 3G) and OSP immunoreactivity in pyramidal neuron gradually decreased with age (Fig. 3C, D).

In the PM 6 group, a few small OSP immunoreactive cell bodies were observed (Fig. 3G); however, OSP immunoreactive fibers had significantly increased in all layers, particularly in the stratum radiatum (Fig. 3E). In the PM 24 group, the number of OSP immunoreactive cell bodies was similar to the PM 6 group (Fig. 3G); however, OSP immunoreactive fibers were evenly distributed in the stratum oriens and lucidum (Fig. 3F). OSP immunoreactivity in pyramidal neuron in the CA2/3 region was negligible (Fig. 3F).

**Dentate gyrus.** Significant alterations in the number of OSP positive cells were detected in the dentate gyrus [F(5,36)=87.037; P<0.0001]. In the PM 1 group, OSP immunoreactive cell bodies were primarily detected in the polymorphic layer; the detection of OSP immunoreactive fibers in the dentate gyrus was uncommon (Fig. 4A). In the PM 2 group, the number of OSP immunoreactive cell bodies significantly decreased in the polymorphic layer (Fig. 4G) and few OSP immunoreactive fibers were detected (Fig. 4B). OSP immunoreactivity was also detected in cells of the granule cell layer, which are neurons (Fig. 4B). In the PM 3 and 4 groups, OSP immunoreactive cell bodies gradually decreased with age (Fig. 4G) and OSP immunoreactivity in granule cells was very weak (Fig. 4C and D). In the PM 6 group, the number of OSP immunoreactive cell bodies had decreased (Fig. 4G) and OSP immunoreactive fibers had marginally increased in the polymorphic layer (Fig. 4E). OSP immunoreactivity in granule cells was not observed (Fig. 4F). In the PM 24 group, the number of OSP immunoreactive cell bodies was similar to the PM 6 group (Fig. 4G); however, OSP immunoreactive fibers had marginally increased in the polymorphic layer (Fig. 4F).

## Discussion

Myelin serves an important role in the function of nervous tissue, and alterations in myelin-specific proteins causes a several neurological disorders (21,22). However, little is known about how OSP expression is affected by aging in the hippocampus. In the present study, alterations in OSP levels and immunoreactive structures were investigated in gerbil hippocampi at 1, 2, 3, 4, 6 and 24 months with western blot analysis and immunohistochemistry. It was demonstrated that OSP levels and immunoreactive structures were significantly altered with age.

In the present study, OSP immunoreactive cell bodies were observed in the gerbil hippocampus at 1-4 months. At these ages, the detection of OSP immunoreactive fibers was uncommon. However, abundant OSP immunoreactive fibers were detected from 6 months. It was additionally revealed that the distribution of OSP immunoreactive cell bodies and fibers was markedly different according to the layers of the hippocampal subregions. Distributions of other oligodendrocyte proteins in rodent brains has been studied previously using various antibodies. For example, Yamada and Jinno (14) reported that the immunoreactivity of oligodendrocyte transcription factor (Olig2), a basic helix-loop-helix transcription factor encoded by Olig2 gene, is detected in cell bodies in the hippocampus of the C57BL/6J mouse between 2 and 12 months of age. Additionally, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), a myelin-associated enzyme that makes up 4% of the total CNS myelin protein, is

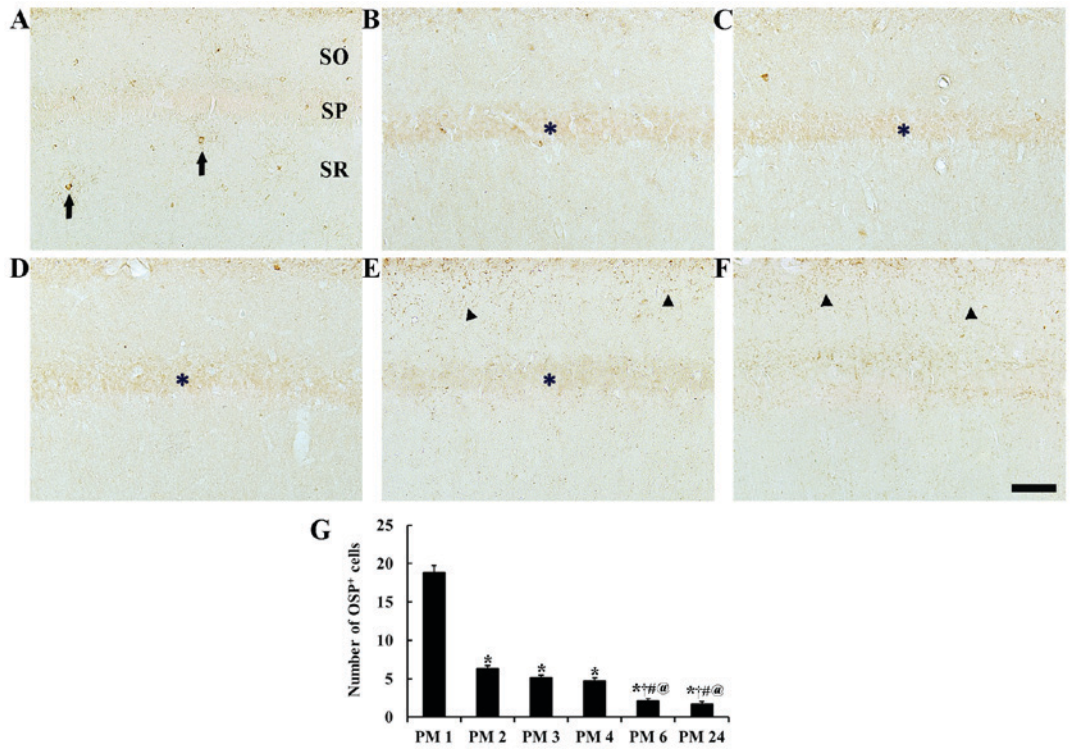


Figure 2. OSP immunohistochemistry in the hippocampal CA1 area of the (A) PM 1, (B) PM 2, (C) PM 3, (D) PM 4, (E) PM 6 and (F) PM 24 groups. OSP immunoreactive cell bodies are indicated by the arrows and were mainly detected in the SR at PM 1, with numbers gradually decreasing with age. OSP immunoreactive fibers are indicated by arrowheads and were markedly increased in the SO at PM 6 and 24. OSP immunoreactivity was detected in the SP at PM 2-6 and is indicated by the asterisk. Scale bar, 100  $\mu$ m. (G) Mean number of OSP immunoreactive cell bodies in the CA1 area. n=7 per group. \*P<0.05 vs. PM 1, †P<0.05 vs. PM 2, ‡P<0.05 vs. PM 3, @P<0.05 vs. PM 4. Bars indicate the mean  $\pm$  standard error of the mean. OSP, oligodendrocyte-specific protein; PM, post-natal month; SR, stratum radiatum; SO, stratum oriens; SP, stratum pyramidale.

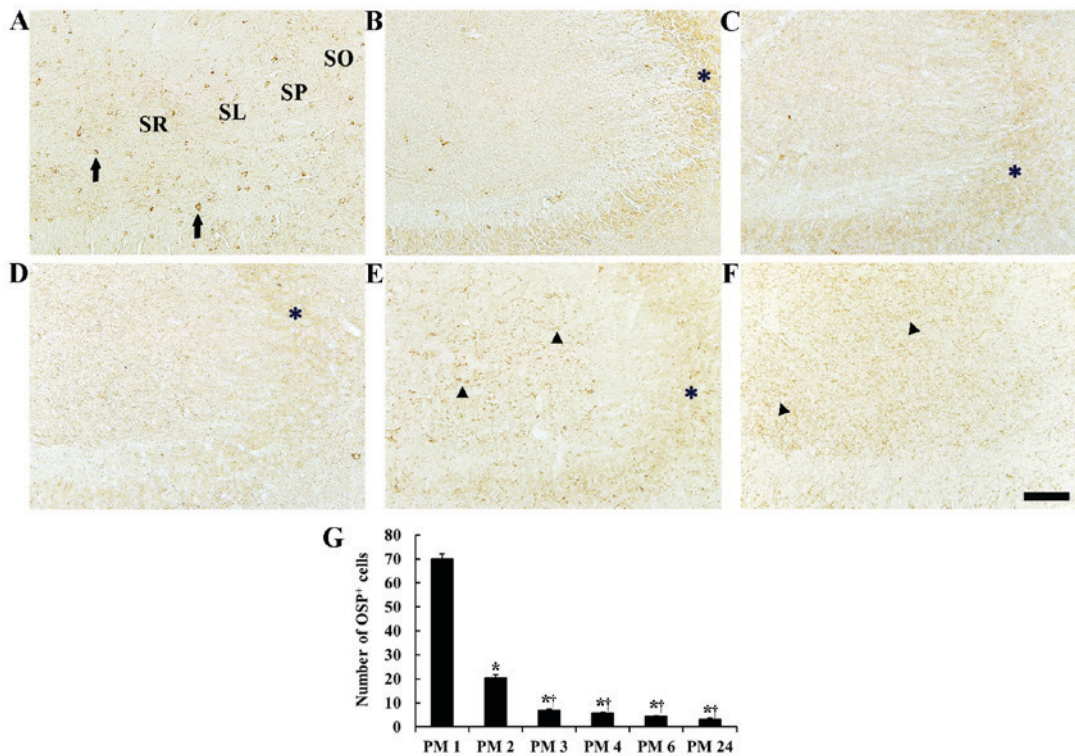


Figure 3. OSP immunohistochemistry in the hippocampal CA2/3 area of the (A) PM 1, (B) PM 2, (C) PM 3, (D) PM 4, (E) PM 6 and (F) PM 24 groups. OSP immunoreactive cell bodies are indicated by the arrows and were detected abundantly throughout all layers. OSP+ cell body numbers significantly reduced from PM 2. The numerous OSP immunoreactive fibers are indicated by arrowheads and were abundantly detected in the SR at PM 6 and 24. OSP immunoreactivity is indicated by the asterisk and was observed in the SP at PM 2-6. Scale bar, 100  $\mu$ m. (G) Mean number of OSP immunoreactive cell bodies in the CA2/3 area. n=7 per group. \*P<0.05 vs. PM 1, †P<0.05 vs. PM 2. Bars indicate the mean  $\pm$  standard error of the mean. OSP, oligodendrocyte-specific protein; PM, post-natal month; SR, stratum radiatum; SO, stratum oriens; SL, stratum lucidum; SP, stratum pyramidale.

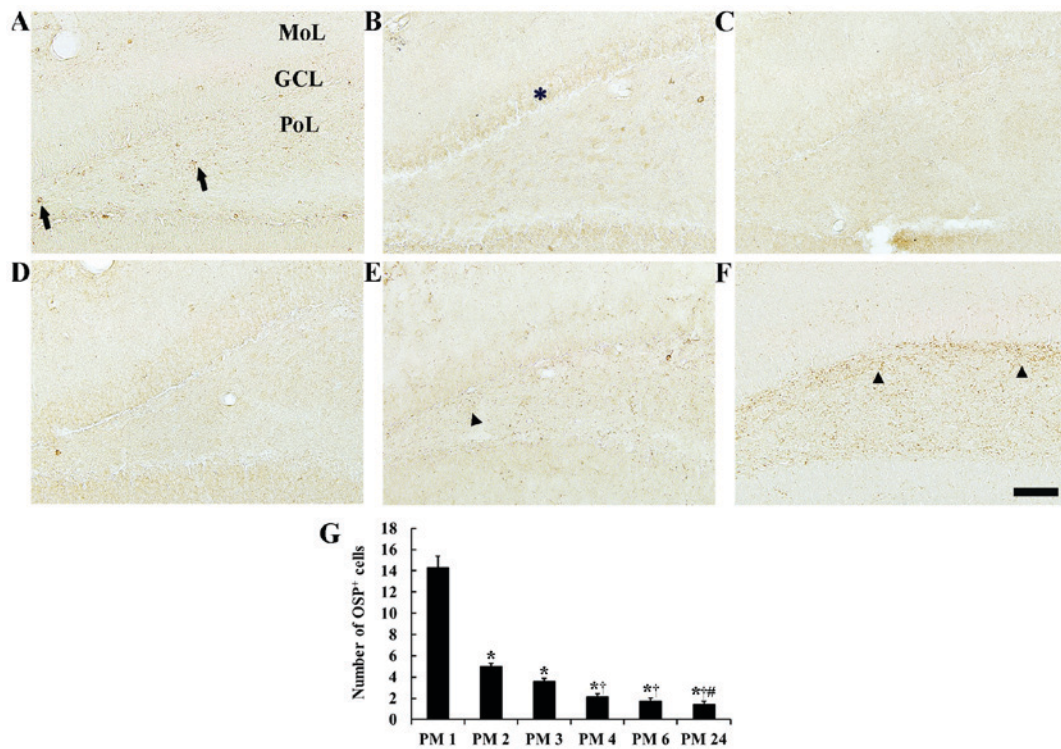


Figure 4. OSP immunohistochemistry in the dentate gyrus of the (A) PM 1, (B) PM 2, (C) PM 3, (D) PM 4, (E) PM 6 and (F) PM 24 groups. OSP immunoreactive cell bodies are indicated by the arrows. Many cell bodies were detected in the PoL until PM 2, where numbers decreased. OSP immunoreactive fibers are indicated by arrowheads and detection markedly increased in the PoL at PM 6 and PM 24. OSP immunoreactivity was observed in the GCL at PM 2, as indicated by the asterisk. Scale bar, 100  $\mu$ m. (G) Mean number of OSP immunoreactive cell bodies in the dentate gyrus. n=7 per group. \*P<0.05 vs. PM 1, †P<0.05 vs. PM 2, #P<0.05 vs. PM 3 group. Bars indicate the mean  $\pm$  standard error of the mean. OSP, oligodendrocyte-specific protein; PM, post-natal month; PoL, polymorphic layer; GCL, granule cell layer; MoL, molecular cell layer.

detected in the fibers in the hippocampus of ICR mice between 2 and 59 weeks of age (23). Xie *et al* (24) reported that the levels of myelin oligodendrocyte glycoprotein in the rat brain significantly decrease from 5 months of age and are progressively downregulated until 26 months. Similarly, the present study demonstrated that levels of OSP protein in the gerbil hippocampus were highest in the PM 6 group, and decreased in the PM 24 group. Based on the results of previous research and the current study, expression patterns of proteins in the myelin- or oligodendrocyte may be different according to the kind of antibodies used.

In the present study, the number of OSP immunoreactive cell bodies in the gerbil hippocampus abruptly decreased at 2 months. Subsequently, the numbers gradually decreased with increasing age. A small number of OSP immunoreactive cell bodies were observed at 6 and 24 months; however, no significant difference in numbers was detected between the groups. In the C57BL/6J mouse, it has been reported that Olig2 immunoreactive cells are distributed in all hippocampal subregions between 2 and 10 months of age, with no significant difference in the numbers of Olig2 immunoreactive cells between mouse hippocampal subregions (14). It has additionally been demonstrated that the age-associated decrease in remyelination efficiency is due to the impairment of oligodendrocyte progenitor recruitment and differentiation (25). Based on previous research and the current study, OSP and Olig2 immunoreactive cell numbers may be significantly decreased from an early age, which may be associated with dysfunction of myelination.

OSP immunoreactive fiber density was significantly increased in the CA1-3 areas and the dentate gyrus at 6 months in the present study. The density was increased in the polymorphic layer of the dentate gyrus at 24 months. Regarding age-dependent alterations in myelin- or oligodendrocyte-associated proteins in the CNS, it has been reported that CNPase immunoreactive fiber density significantly decreases in the hippocampal CA1 region from 10 months in normal (23) and senescence-accelerated mice (15). Therefore, the expression of myelin-associated proteins in fibers may be differentially altered in various brain regions during normal aging.

In conclusion, the present study demonstrated that the expression pattern of OSP immunoreactivity in the gerbil hippocampus was significantly different according to hippocampal subregion and the layers in the subregions. OSP was detected in cell bodies prior to adult age, and in fibers from adult gerbils. The present results suggested that OSP expression alterations may be part of the normal aging process.

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