Extensive Proliferation of Oligodendrocyte Precursors in the Parenchyma of the Embryonic Chick Central Nervous System

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The proliferation of oligodendrocyte lineage cells in the chick embryo central nervous system (CNS) was examined by double-immunolabeling with a lineage marker monoclonal antibody (mAb) O4 or mAb O1 and 5-bromo-3'-deoxyuridine (BrdU). In all regions examined, the first O4-positive (O4⁺) cells appeared in restricted regions of the ventricular zone (VZ), regarded as a site of oligodendrocyte origin. Within the O4⁺ focus, less than 20% of the O4⁺ cells incorporated BrdU. In contrast, O4⁺ cells in the parenchyma were mitotically active; for example, 40–50% of early O4⁺ cells were labeled with BrdU. Some of these were unipolar in shape, indicative of migratory precursor cells. The frequency of O4⁺/BrdU⁺ cell appearance decreased to less than 20% with further development. O1⁺ oligodendrocytes were largely mitotically inactive, with only approximately 5% of O1⁺ cells incorporating BrdU. These results clearly demonstrated that the VZ generates relatively few precursor cells and that these oligodendrocyte precursors actively generate their cohort in the parenchyma of the CNS.

Key Words: BrdU; oligodendrocyte; migration; O4; O1; spinal cord; metencephalon; optic nerve; retina.

INTRODUCTION

Oligodendrocytes are myelin-forming cells in the vertebrate central nervous system (CNS). During CNS development, oligodendrocytes or their precursors appear to be generated in restricted regions of the ventricular zone (VZ). For example, oligodendrocyte precursors in the spinal cord first appear in the ventral VZ adjacent to the floor plate and then disperse throughout the spinal cord. These cells can be identified through expression of the alpha subunit of the receptor for platelet-derived growth factor (PDGFRα; Pringle and Richardson, 1993) and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (Yu et al., 1994). Gradual and oriented dispersal of PDGFRα-positive (PDGFRα⁺) cells in the rat optic nerve may also reflect precursor cell migration (Pringle et al., 1992). In the embryonic chick CNS, oligodendrocyte development may be followed by in situ hybridization for PLP (proteolipid protein)/DM20 mRNA or labeling with a monoclonal antibody (mAb) O4. PLP/DM20 expression demarcates possible sites of oligodendrocyte origin in the VZ in the very early embryo around embryonic day 2 (E2) (Perez Villegas et al., 1999). The earliest O4⁺ cells also show focal spots in the VZ (Ono et al., 1995, 1997a,b). O4⁺ cells then migrate radially and tangentially to populate the CNS parenchyma and subsequently express galactocerebroside (GaC) and myelin basic protein (MBP), markers for differentiated or mature oligodendrocytes (Miller and Ono, 1998; Ono et al., 1998).

The timing and extent of oligodendrocyte precursor proliferation has been extensively studied with rat optic nerve oligodendrocytes (Skoff et al., 1976a,b). In vitro identification of bipotential glial progenitor cells, termed O-2A progenitor cells, by binding of the mAb A2B5 enabled identification of the molecular regulation of oligodendrocyte precursor proliferation (Raff et al., 1983; Raff, 1989). The major mitogen for A2B5⁺ cells is PDGF-AA mediated through the PDGFRα (Fok-Seang and Miller, 1994; Hall et al., 1996; Nishiyama et al., 1996a,b). As immature A2B5⁺ cells mature, they begin to express O4 antigens and become mitotically less active. Upon differentiation, oligodendrocytes express GaC and MBP and no longer appear to
proliferate (Bansal et al., 1992; Gard and Pfeiffer, 1989). These data suggest that rat oligodendrocyte lineage cells undergo proliferation predominantly during the immature O-2A progenitor stage.

We used chick embryos to demonstrate the focal origin and subsequent dispersal of oligodendrocyte lineage cells by labeling with mAb O4 in the spinal cord (Ono et al., 1995), metencephalon (Ono et al., 1997b), and optic nerve and retina (Ono et al., 1997a, 1998). After O4+ cells leave the ventricular focus and invade the parenchyma, they rapidly spread throughout the CNS parenchyma. For example, the first O4+ cells in the metencephalon are detected in the VZ at E5, and the labeled cells widely disperse throughout the whole area of the metencephalon, including the cerebellar anlage, by E8. Active cell migration apparently contributes to the rapid dispersal of O4+ cells (Ono et al., 1997a).

Glial cells and their precursors divide not only in the VZ, but also in the CNS parenchyma. It is unclear, however, whether O4+ oligodendrocyte precursor cells proliferate predominantly in the VZ or in the parenchymal area after leaving the VZ, or both. It has been proposed that the major expansion of cells in the oligodendrocyte population occurs in the white matter of the newborn rat spinal cord (Miller et al., 1997). In the present study, we defined the temporal and spatial patterns of oligodendrocyte lineage cell division of the embryonic chick CNS. Cells were double-labeled with a mAb O4 or mAb O1 to define the oligodendrocyte lineage and with 5-bromo-3′-deoxyuridine (BrdU) to demonstrate S-phase cells, indicative of multiplying cells. The results clearly demonstrated that O4+ cells proliferated extensively in the parenchyma of the CNS both during and after migration. By contrast, in the VZ, O4+ cells appeared to undergo only limited proliferation. These data suggest that the majority of expansion of the oligodendrocyte lineage occurs in the CNS parenchyma, rather than at the focal points of origin.

MATERIALS AND METHODS

Animals

Fertilized chicken eggs purchased locally were incubated at 38°C. The stages of the chick embryos were determined according to Hamburger and Hamilton (1951). All procedures were approved by the Institutional Animal Care Ethics Committee of Shimane Medical University.

Double-immunolabeling with BrdU and a Lineage Marker

Eggs were windowed at the desired stages. Embryos received an injection of BrdU (100–300 µl of 5 mg/ml in PBS; Sigma Chemical Co., St. Louis, MO) into the egg yolk. After BrdU injection, windows were sealed, and the embryos were incubated for an additional 5–6 h. To localize O4+ and O1+ cells, CNS tissues including the spinal cord, metencephalon includingpons and cerebellum, the optic nerve, and retina were fixed by immersion in 4% paraformaldehyde in a phosphate buffer (PB; pH 7.2, 0.1 M) at 4°C for 2–5 h and stored overnight in PB containing 30% sucrose. Embryos were examined between stages 26 (fifth day of incubation, E5) and 40 (E14). The CNS tissues were isolated from the surrounding tissues, embedded in 5% agar, and 70-µm-thick sections were cut on a Vibratome. The free-floating sections were sequentially incubated with 10% normal goat serum (NGS) for 2–10 h, a primary antibody (O4, culture supernatant, 1:10 in PB + 10% NGS; or O1, culture supernatant, 1:50 in PB + 10% NGS; Sommer and Schachner, 1981) overnight, and finally with goat anti-mouse IgM conjugated to rhodamine (1:400; Cappel, Durham, NC) for 60 min. Sections were washed with PB after each antibody incubation. To visualize BrdU + cells, sections were treated with 1.2 N HCl for 5 h and subsequently incubated with an anti-BrdU antibody (1:500 in PB + 10% NGS + 0.1% Triton X-100; Pharmingen, San Diego, CA) overnight and with a goat anti-mouse Fc conjugated to FITC. Following final washing, sections were mounted onto gelatin-coated glass slides, coverslipped with PBS-glycerol, and observed under an epifluorescent microscope (ECLIPS; Nikon, Japan).

Cell Counting

Labeled cells were examined in at least three to five randomly chosen sections or areas of each brain region, and depending on the region and stages, 50 to 400 O4+ cells were examined in each experiment. Cells were scored either BrdU + or BrdU—. At least three independent experiments were carried out for every stage. The examined results are shown as a percentage of BrdU+ cells in the oligodendrocyte lineage (labeling index).

RESULTS

In all regions examined, the foci of O4+ cells in the VZ, which was regarded as the origin site of oligodendrocyte lineage cells, contained few O4+ or BrdU+ cells. For example, in no cases were greater than 20% of O4+ cells labeled with BrdU in the VZ. In contrast, within the parenchyma, greater than 40% of early O4+ cells in all regions were labeled with BrdU in young animals. This proportion gradually decreased as the embryos developed. The time courses of the labeling index (double-labeled cell frequency) in each region are summarized in Fig. 1 and Table 1. Details of individual areas are described below.

Spinal Cord

A small focus of O4+ cells in the ventral VZ adjacent to the floor plate was observed mainly at E6 and E7. At these ages, the cells in the dorsal half of the VZ actively incorporated BrdU, while the ventral half contained fewer BrdU+ cells (Figs. 2A and 2B). The bilateral foci in each section were composed of several O4+ cells. Within each focus, approximately 20% of O4+ cells incorporated BrdU during a 5–6 h pulse (Table 1 and Figs. 1, 2C, and 2D). Outside the VZ, O4+ cells were observed in both the mantle layer and marginal zone. At E7 and E8, about 40% of O4+ cells incorporated BrdU following a 5–6 h pulse (Table 1 and Figs. 2E–2J). Some of these O4+/BrdU+ cells were unipolar or asymmetrical bipolar in shape and oriented either dorsoventrally or rostrocaudally in the spinal cord (Figs. 2E–2H, 4A, and 4B). This
morphology suggests that these O4+/BrdU+ cells were migratory precursor cells (Kakita and Goldman, 1999). The first GalC+/O1+ oligodendrocytes appeared around E9 in the spinal cord (Ono et al., 1995). The appearance of O1+ cells correlated with a decreased frequency of O4+/BrdU+ cells among the O1+ oligodendrocytes; mean ± SEM.

TABLE 1
Percentage of BrdU-Positive Cells in the Oligodendrocyte Lineage

<table>
<thead>
<tr>
<th>Focus in VZ (O4/BrdU)</th>
<th>Parenchyma (O4/BrdU)</th>
<th>O1/BrdU</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>E5</td>
<td>E6</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>18.0 ± 5.87 (E6-E7)</td>
<td>—</td>
</tr>
<tr>
<td>Pons</td>
<td>18.8 ± 3.85 (E6-E7)</td>
<td>44.4 ± 5.76</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Optic nerve</td>
<td>17.3 ± 1.38 (E5-E7)</td>
<td>—</td>
</tr>
<tr>
<td>Retina</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Numbers are mean ± SEM. N.D., not determined.
FIG. 2. Distribution of $O4^+$ cells and BrdU$^+$ cells in the developing spinal cord. (A–D) E6. (E–J) E8. (A) $O4^+$ cell focus (arrows) in the ventral ventricular zone. A coronal section. (B) BrdU$^+$ cells in the ventricular zone. The same field as A. The dorsal ventricular zone contains more labeled cells than the ventral part. (C) Higher magnification of the ventricular $O4^+$ cell focus in A. (D) BrdU$^+$ cells in the ventral ventricular zone. The same field as C. Arrows in C and D indicate $O4^+$/BrdU$^+$ cells in the focus. Note that no, or few, $O4^+$ cells in the right side focus incorporate BrdU. (E) A longitudinal section of the ventral marginal zone labeled with $O4$. (F) The same field as E, labeled with BrdU. Arrows in E and F indicate $O4^+$/BrdU$^+$ cells. (G and H) A dorsally orienting $O4^+$/BrdU$^+$ cell (arrows in G and H) in the gray matter. (I) Distribution of $O4^+$ cells in the ventral half of the E8 spinal cord. (J) Distribution of BrdU$^+$ cells in the ventral half of the E8 spinal cord. The same field as I. Arrows in I and J indicate some $O4^+$/BrdU$^+$ cells. Note that both $O4^+$ cells and BrdU$^+$ cells are preferentially located in the marginal part of the spinal cord. Bars in A and B = 100 μm, in D–H = 20 μm, and in I and J = 50 μm.
TABLE 2

Frequency of BrdU+ Cells among O4+ Cells in the Spinal Cord

<table>
<thead>
<tr>
<th></th>
<th>Gray matter</th>
<th>White matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>31.3 ± 6.29</td>
<td>48.0 ± 4.98*</td>
</tr>
<tr>
<td>E8</td>
<td>25.1 ± 1.35</td>
<td>46.4 ± 2.44*</td>
</tr>
<tr>
<td>E10</td>
<td>18.8 ± 2.49</td>
<td>28.4 ± 2.89</td>
</tr>
</tbody>
</table>

*The difference in O4+/BrdU+ frequency in the gray matter and in white matter was significant at P < 0.05. (t test). Numbers are mean ± SEM.

also BrdU+, while in the E12 and E14 spinal cord, less than 20% of O4+ cells incorporated BrdU during the same pulse period (Fig. 1). The majority of O1+ oligodendrocytes in the E10 and older spinal cord were BrdU− (about 3% were O1+/BrdU+; Table 1 and Fig. 1), suggesting that these cells are nonproliferative.

The distribution of O4+/BrdU+ cells was not uniform throughout the spinal cord. In the E7, E8, and E10 spinal cord, both O4+ cells and BrdU+ cells were preferentially observed in the marginal zone (Figs. 2I and 2J). A comparison of the proportion of double-labeled cells in the mantle layer (immature gray matter) with that in the marginal zone (immature white matter) showed that the frequency of O4+/BrdU+ cells was approximately 1.5 times higher in the marginal zone than in the mantle layer (Table 2). These data suggest that O4+ cells preferentially proliferate in immature white matter, rather than immature gray matter, in the spinal cord.

Metencephalon (Pons and Cerebellum)

A focus of O4+ cells was first observed at E5, located in the VZ adjacent to the floor plate (Ono et al., 1997b). Proliferating cells were distributed throughout the VZ, with the exception of the floor plate at E5. The focus of O4+ cells contained both BrdU+ and BrdU− cells, suggesting that some cells were actively proliferating in the region (Figs. 3A and 3B). One day later at E6, the BrdU+ cells in the VZ tended to separate from O4+ cell foci with partial overlapping (Fig. 3C) and localized in the inner part of the VZ, not directly at the ventricular surface (Fig. 3D). Less than 20% of O4+ cells were labeled with BrdU in the ventral VZ at E5 and E6 (Fig. 1A–1D and Table 1). Even in E5, when the ventricular O4+ cell focus contained some BrdU+ cell nuclei, the majority of O4+ cells did not incorporate BrdU (Figs. 3A and 3B). Contrary to the low level of BrdU incorporation in the VZ, O4+ cells in the brainstem parenchyma frequently incorporated BrdU. For example, approximately 40–50% of O4+ cells were BrdU+ at E5–E7 (Fig. 4C). Some of the double-labeled cells were irregularly shaped, while others were more unipolar. From E8 onward, approximately 20% of O4+ cells were labeled with BrdU in the brainstem. O1+ cells appeared at E8, and only a limited number of O1+ cells were labeled with BrdU (Fig. 1 and Table 1).

The first O4+ cells in the cerebellar anlage were detected at E7 (Figs. 4D and 4E; Ono et al., 1997b). The frequency of double-labeled cells was approximately 50% at this stage (Fig. 4E). While about 40% of O4+ cells were labeled with BrdU in the E8 cerebellum, the proportion of double-labeled cells reduced during further development, and by E10, approximately 20% of O4+ cells incorporated BrdU. O1+ oligodendrocytes were first observed at E12 in the cerebel- lum, and less than 5% of these incorporated BrdU in the E12 and E14 cerebellum. These data suggest that, as in the spinal cord, the majority of oligodendrocyte precursor proliferation occurs in the parenchyma of the brainstem and cerebellum.

Optic Nerve and Retina

Oligodendrocytes in the optic nerve were reported to originate from the O4+ focus in the floor of the third ventricle (Ono et al., 1997a). Less than 20% of O4+ cells incorporated BrdU within the focus at E5–E7 (Figs. 3E and 3F). The optic nerve at E7 or older contained O4+ cells both in the retinal and chiasmal regions (Ono et al., 1997a). Approximately 40% of O4+ cells incorporated BrdU in the optic nerve at E7–E10 (Figs. 4F and 4G). Most of the double-labeled cells in these stages were unipolar or asymmetrically bipolar in shape, arranged along the long axis of the optic nerve (Fig. 4G), while a lesser number were oval shaped, some of which were at the metaphase of the cell cycle (Fig. 4F). In the E12 optic nerve, more than 30% of O4+ cells were still BrdU−, suggesting a moderate level of proliferation. This proliferation index decreased during further development such that less than 20% of O4+ cells incorporated BrdU in the E14 optic nerve. The first O1+ oligodendrocytes appeared by E12 (Miller and Ono, 1998). As in the spinal cord and metencephalon, less than 5% of these cells incorporated BrdU.

While most mammalian retinae, except for the rabbit retina, are devoid of oligodendrocyte or myelin, avian retina contains oligodendrocytes and myelinated axons in the optic nerve layer (Nakazawa et al., 1993). The first O4+ oligodendrocyte precursors appeared in the retina at E10 (Ono et al., 1998), and only the area close to the optic fissure contained labeled cells. At this stage, approximately 40% of O4+ cells were labeled with BrdU (Figs. 1 and 4H). The frequency of double-labeled cells gradually decreased as the embryo developed, about 25% in E12 and 20% in E14 retina.

DISCUSSION

Extensive O4+ Cell Proliferation in the CNS Parenchyma, but Not in the VZ

The present double-labeling experiment clearly demonstrated the timing, extent, and location of rapidly prolifer-
ating oligodendrocyte precursors in the chick embryo CNS. Within the sites of oligodendrocyte origin in the VZ, only a limited number of O4+ cells incorporated BrdU. The low frequency of O4+/BrdU+ cell appearance could be a reflection of the slow division of precursor cells in the VZ. After leaving the VZ, O4+ cells in the parenchyma showed a

**FIG. 3.** O4+ cells (red) and BrdU+ cells (green) in the ventricular zone. (A) E5 metencephalon. Only a limited number of O4+ cells are observed in the medial part (arrow) adjacent to the floor plate (FP). (B) Higher magnification of O4+ cells in A. Note that the O4+ cell indicated by an arrow is not labeled with BrdU (BrdU−). (C) E6 metencephalon. O4+ cells are observed both in the ventricular focus (arrow) and in the parenchyma. (D) Higher magnification of the O4+ cell focus in C. Note that the BrdU+ cell population is separated from the O4+ cell focus at the border indicated by a larger arrow, and the majority of O4+ cells in the focus (smaller arrows) are BrdU−. (E) E7 optic nerve (ON) and optic chiasm (OC). Arrow indicates a O4+ cell focus in the chiasmal region. F, Higher magnification of the chiasmal O4+ cell focus. Arrows indicate O4+/BrdU− cells in the focus. Bar in A, C, E = 100 μm; in B, C, F = 20 μm.
higher proliferative (labeling) index; 40–50% of O4+ cells incorporated BrdU during a 5–6 h pulse, which was indicative of rapid proliferation in the parenchymal area. These data suggest that the majority of oligodendrocyte precursor expansion occurs in the parenchyma, but not in the VZ. In addition, extensive proliferation of oligodendrocyte precursors...
sors occurred during a defined period of development. For example, while about 20% of double-labeled cells were seen in the brainstem VZ at E5–E6, this proportion increased dramatically in the parenchyma until E8. Two days later the proportion of double-labeled cells in the brainstem decreased, most likely as a result of differentiation of cells to O1+ oligodendrocytes.

It is unclear whether the current approaches detected all O4+ and BrdU+ cells within the tissue. Since the proportion of O4+ cells that incorporated BrdU was relatively constant across different preparations in a particular region at a specific stage, however, these data do provide an accurate comparison of the proportion of proliferative O4+ cells in different regions of the CNS during development.

It is not known why O4+/BrdU+ cells are frequently observed in the CNS parenchyma, but not in the VZ. One possibility is that proliferative oligodendrocyte precursor cells are more influenced by trophic factors in the parenchyma than in the VZ. O-2A progenitor cells in vitro have been reported to migrate toward the source of PDGF (Arms\-trong et al., 1990), and the developing CNS parenchyma expresses PDGF and other factors for oligodendrocyte proliferation. It is plausible that oligodendrocyte precursor cells leave the VZ seeking trophic factors and start rapid proliferation after acquiring enough of these factors in the parenchyma (discussed below).

Miller and colleagues suggested that relatively few oligodendrocyte precursors invade developing white matter and that these cells then undergo extensive local proliferation (Miller et al., 1997, 1999). Our present results provided direct evidence for this; O4+ cells were highly proliferative in the parenchymal area, whereas those in the VZ were less mitotic. The unipolar shape of O4+/BrdU+ cells and their oval morphology at the metaphase (Fig. 4) suggest that BrdU may be incorporated by migrating O4+ cells and that these cells temporally cease relocation during cell division (metaphase). In addition, within the spinal cord, immature white matter (marginal zone) contained O4+/BrdU+ cells more frequently than immature gray matter (mantle layer) in young animals. Therefore, even after migration from the VZ to the white matter, O4+ cells retain characteristics of precursor cells and may be mitotically very active.

The local proliferation of O4+ cells may be regulated by local expression of growth factors (Barres and Raff, 1994). PDGF-AA is a strong mitogen for oligodendrocyte precursors in vitro (Fok-Seang and Miller, 1994; Nishiyama et al., 1996a,b) and delays oligodendrocyte differentiation (Butt et al., 1997). This growth factor is expressed and secreted by neurons and astrocytes in vivo (Yeh et al., 1991). Genetic deletion of PDGF reduces the number of oligodendrocytes and induces severe hypomyelination in the spinal cord (Fruttiger et al., 1999). One source of growth factors may be axons which have been reported to regulate oligodendrocyte cell division in an activity-dependent manner that can be mimicked by application of exogenous PDGF-AA (Barres and Raff, 1993). In addition, the GRO-1 chemokine expressed by astroglia also promotes oligodendrocyte precursor proliferation (Wu et al., 2000). In the present study, O4+ oligodendrocyte precursors proliferated mainly in the parenchyma, including the white matter, in which the precursors are surrounded by neurons, axons, and probably immature astrocytes. It seems likely that the high levels of O4+ precursor cell proliferation in these regions reflect local signals from their surroundings.

Both neurons and oligodendrocytes develop from precursor cells in the VZ and in the subventricular zone (SVZ). The mechanisms of cell fate commitment, at least in part, are common to both cell lineages (Miller et al., 1999; Richardson et al., 2000). However, primary sites of oligodendrocyte lineage proliferation are different from those of neurons; oligodendrocyte lineage cells proliferate predominantly in the CNS parenchyma, while neuronal cells do so exclusively in the VZ and SVZ. It is probable that the mechanisms of neuronal cell lineage proliferation differ greatly from those of oligodendrocyte lineage. Compared to neuronal proliferation, oligodendrocyte lineage cell proliferation may be more influenced by environmental factors such as trophic factors and neuronal activity.

Comparison with Rodent Oligodendrocyte Precursor Cells

O4+ cells in the chick embryo CNS, especially those in the early stages, are apparently different from rodent O4+ cells. In both species, mAb O4 recognized sulfatide and uncharacterized glycolipids termed POA (pro-oligodendroglial antigen; Bansal et al., 1992; Ono et al., 1995). O4+ cells in the rat have a limited capacity to migrate when grafted into a hypomyelinated brain (Warrington et al., 1993). Hardy and Friedrich (1996) demonstrated that O4+ cells did not divide in the fetal mouse brain. In the newborn rat optic nerve, unipolar-shaped NG2+ glial progenitors proliferate actively (Ueda et al., 1999) and demonstrated a similar morphology to O4+/BrdU+ cells in the present study. It is highly likely that bipolar NG2+ cells in the developing rodent CNS correspond to bipolar O4+ cells in the early chick embryo CNS; both cells are highly motile and proliferative oligodendrocyte precursors. Gard and Pfeiffer (1989) demonstrated that GalC+ cells in the rat CNS rarely incorporated BrdU. The current data are consistent with this; a limited proportion of O1+ cells were BrdU+ and less than 5% of O1+ oligodendrocytes incorporated BrdU in the developing chick CNS. Both rodent and chick GalC+ (or O1+) cells are mature, nonproliferative oligodendrocytes.

In summary, the present and previous findings strongly indicate that, during oligodendrocyte development, a limited number of precursors are generated in the ventricular focus. They migrate into the gray and white matter in which oligodendrocyte precursors expand their populations through a defined period of rapid proliferation. During later development, the proliferation of oligodendrocyte lineage cells is reduced. This reduction may reflect local reduction in growth factor expression and maturation of precursor.
cells into more differentiated, nonproliferative oligodendrocytes that express mature oligodendrocyte antigens.

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